Avian Sex Ratio And Sex-Specific Traits In Offspring

Razón de sexos y atributos sexuales de la descendencia en aves

María Martínez Benito

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de la descendencia en aves

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PhD
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Memoria presentada por
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Els petits canvis són poderosos
— Capitá Enciam


O. Aldeguer

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Contents

General Introduction

Introduction & Overview

> *I formerly thought that when a tendency to produce the two sexes in equal numbers was advantageous to the species it would follow from natural selection, but now I see that the whole problem is so intricate that it is safer to leave its solution to the future.*
>
> — C. Darwin, 1874
> The Descendent of Man and Selection in Relation to Sex

> *Darwin's sentiments have, I suspect, rung a chord in virtually every researcher who has tried to make sense of the exasperating literature concerning the adaptiveness for parents of biasing sex ratios towards either sons or daughters.*
>
> — I. C. Hardy, 1987

Background

Historically, the study of sex ratio began with the observation that, in most species of common animals with separate sexes, about as many males as females were born. Although Darwin initiated the discussion of possible explanations (even sketched an evolutionary argument, Darwin 1871), soon he realized that the question was problematic for his theory of natural selection (Darwin 1874).

The root of this interest, however, lies in a previous question: given that there is sex, how are the distinct 'sexes' characterized? Males and females are often different in both morphology and behaviour, being the essential difference the anisogamy, i.e. the size and motility of their gametes. Females produce large immobile gametes, eggs, while males produce smaller mobile gametes, sperm, which are different in production costs. In general, this means that the reproductive success of a male is basically limited by the number of females he can mate with, while a female's reproductive success is limited by her fecundity. In this context, males compete among themselves to mate with as many females as possible, whereas females should choose a male that will father the fittest offspring (Williams 1966; Trivers 1972). The resulting different strength of sexual selection in each sex has important evolutionary consequences and explains many of the differences between the sexes. Sexual differences and their variance are the material base for differences in the relative reproductive success of males and females, which among other aspects, determines sex allocation (SA; see Box 1 for basic concepts).

Box 1. CONCEPTS

Sex allocation (SA): proportion of resources allocated to male and female reproduction. More specifically, the division of reproductive effort of parents into the production of sons and daughters.

Sex ratio (SR): relative number of males and females in a population. Calculated as the number of males divided between the total number of males and females of the population, and usually expressed as proportion of males in percentage:

$$\text{SR} = ♂ \, / \, (♀ + ♂) \; [\text{x}\,100]$$

E.g. A population of 620 individuals,

$\text{SR} = 0.50$ (or 50%) $\rightarrow$ ♂ (310) = ♀ (310) – exact parity
$\text{SR} = 0.65$ (or 65%) $\rightarrow$ ♂ (403) > ♀ (217) – male biased ratio ($\text{SR} > 0.5$)
$\text{SR} = 0.43$ (or 43%) $\rightarrow$ ♂ (267) < ♀ (353) – female biased ratio ($\text{SR} < 0.5$)

According to the life stage at which the sex ratio is measured:
Primary sex ratio: in a strict sense, the proportion of males at the time of conception (determined in embryos). Almost equivalent in many cases and used in this thesis is the
Hatching sex ratio (HSR): ratio at the time of birth, determined (in avian species) in newly hatched chicks.
Secondary or ***Fledging sex ratio (FSR)***: determined (in avian species) in fledgling chicks or those about to fledge (many authors, however, consider Secondary the sex ratio at time of hatching, to distinguish it from that of conception).
Tertiary or ***Operational sex ratio (OSR)***: the ratio of mature (ready to breed) organisms.

These ratios will differ if the sexes differ in their mortality rates at various stages in their development. For example, if mortality rates are greater for males, then an unbiased primary sex ratio would become a female biased secondary sex ratio (see Box 1b, *Example 2*). This introduces the concept of **Sex-specific mortality** or **Differential mortality (DM)**. Differential mortality during the growing period is expressed as the difference between sex ratio at hatching and sex ratio at fledging, and calculated as: FSR−HSR.

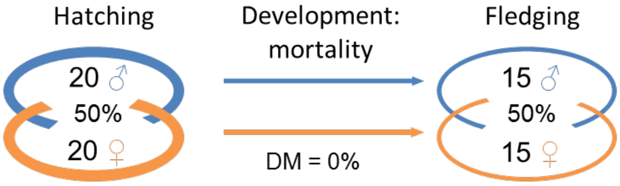
Reproductive value of an individual: its expected genetic contribution to the future population. It measures the long-term contribution to the gene pool. In some instances, it is equivalent to a short-term measure of success, such as recruitment, but usually such a simple measure is not enough.

(Annual/Lifetime) Reproductive success of an individual: its relative production of fertile offspring (in a year/throughout the life). In practice, this is often a calculation of the number of offspring produced by the individual.

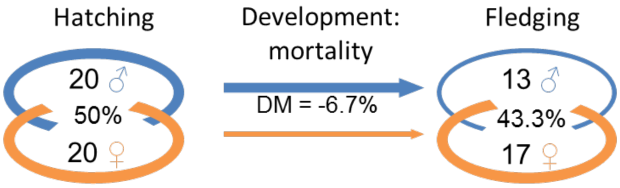
Box 1b. GETTING USED TO SEX RATIOS

Some examples may help to clarify concepts and get used to some possible scenarios:

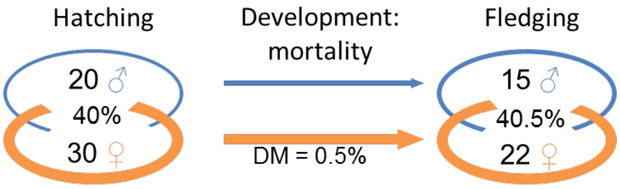
Example 1. Imagine a population where 20 males and 20 females are born (unbiased HSR = 50%). The same quantities for each sex (5 males, 5 females) die during growth. At fledging time, we would have FSR = 50% again. Though there was obviously some mortality (10 individuals out of 40, 25%), there was no differential mortality: DM = 50 − 50 = 0%.



Example 2. Population where 20 males and 20 females are born (unbiased HSR = 50%). During development, 7 males and 3 females die. At fledging, FSR = 13/30 = 43.3% (female-biased). Note that whereas mortality among the offspring was 25% (10 individuals out of 40), differential mortality was DM = 43.3 − 50 = -6.7%. The absolute value indicates how much, in percentage, one sex dies respect to the other, and the negative sign indicates higher male mortality.



Example 3. Population with female-bias at hatching, where 20 males and 30 females are born (HSR = 40%). During development, 5 males and 8 females die, so FSR = 40.5%. Differential mortality = 0.5 (positive sign indicates higher female mortality). Note that, in this case, even if more females than males died, the FSR is still female-biased (but in another case it could become male-biased).



Sex allocation theory is one of the great successes of evolutionary biology. Used to examine a wide range of questions, it has attracted the most attention as the area of research that

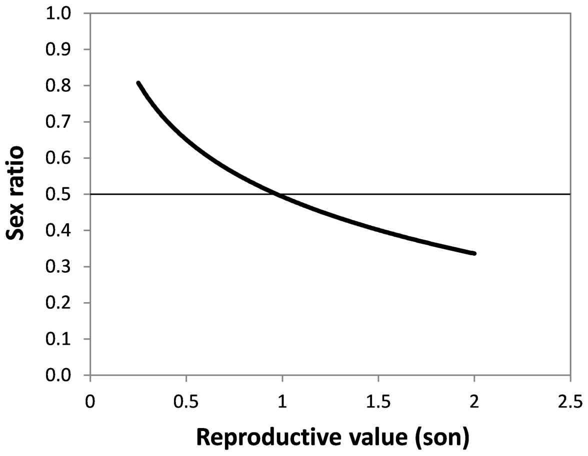


Figure 1: Fisher's classic result. Population SR for situations where the reproductive value of sons is lower, equal or higher than that of daughters. The mean reproductive value of females is assumed to be 1.0, and the mean reproductive value of males is given by the average number of matings they will obtain multiplied by the mean reproductive value of females, which is given by [(nr. females / nr. males) x 1.0]. E.g. if there are 100 sons and 100 daughters, the reproductive value for sons = 1 and for daughters =1 and thus the SR would be equal (re-drawn from West 2009).

considers how, in species with separate sexes, the division of resources between sons and daughters occurs. The first consequence of this division is immediately noticeable in the numbers of each sex that are produced, as Darwin stated. This is termed sex ratio (SR, see Box 1). Due to the difficulties of measuring sex allocation in practice (how to deal with the multidimensional nature of resources? how to include costs of future reproductions? what and how to measure in the field or the lab?), the sex ratio is used very often to study some aspects of sex allocation, as a related or even equivalent concept in some cases (Frank 1990).

These two topics (sex ratio/sex allocation, with sexual differences as their basis) are thus the focus of this thesis work.

Theory of sex ratio and sex allocation

Sex allocation in a population

The basic principle that explains why balanced sex ratios evolve so often, after being tentatively described by Darwin (Darwin 1871), was first mathematically developed by Düsing (Düsing 1883, 1884), and then summarized by Sir Donald A. Fisher (Fisher 1930).

Fisher's principle, one of the "most celebrated arguments in evolutionary biology" (Edwards 1998), shows that, everything else being equal, natural selection favours equal parental investment on both sexes. The idea is described in economic metaphor: parents allocate portions of their limited reproductive resources to sons and daughters, and for each sex they get certain returns measured as genetic contribution to future generations. In a population with a biased SR, offspring of the rarer sex have better mating prospects, and parents with a genetic tendency to produce more of the rarer sex achieve a higher than average number of grandchildren. The tendency to produce the rare sex becomes more widespread in the population, and thus the sex ratio bias decreases. Hence, frequency dependent selection returns sex ratio biases to equality, and the argument holds true regardless of which is the rarer sex.

When the benefits and costs (invested resources) associated to each sex are equal, parents invest equally and SR over a population is at equilibrium, which implies an evolutionary stable strategy (ESS, Maynard Smith and G. R. Price 1973). When the benefits and costs associated to each sex are different, the ESS is still to invest equal overall amounts of resources into male and female offspring. This implies that a population SR will be biased towards the sex that incurs the less amount of investment or "cheaper" sex. For instance, if sons are twice as costly to produce as daughters, the daughters will be produced twice as numerous ($\mathrm{SR} = 0.33$), to equalize overall investment in the sexes (Figure 1).

The argument, later formalized mathematically (Shaw and Mohler 1953), comprises three key aspects: (1) total male and total female fitness is equal (each offspring has a mother and father, and so males and females make equal genetic contributions to the next generation); (2) the right measure of fitness is the relative number of grandchildren and, (3) over population as a whole equal investment in the two sexes, and not necessarily equal numbers, is expected. Fisher's prediction applies to secondary sex ratio in species with post-natal care, considering total investment until end of parental care, but in case of differential mortality during development, it also applies to primary sex ratio (see page 10).

Fisher's scenario is based on a number of implicit assumptions that subsequent sex ratio research has clarified and relaxed (e.g. Williams and Williams 1979; Maynard Smith 1980; Frank 1990; Godfray 1994). These new models arose mostly from empirical observations that could not be explained by Fisher's theory. Modern SR research began with Hamilton (Hamilton 1967), who observed strong female-biased sex ratios in insects. His hypothesis, the *Local Mate Competition* (LMC), shows that when brothers compete directly among themselves for the limited number of mates available in a local group, parents are favoured to invest more resources in daughters. A similar idea (Clark 1978) defined the *Local Resource Competition* (LRC) hypothesis after observation of male-biased ratios in primates: when one sex is phylopatric and the other one disperses before mating, competition within the phylopatric sex and with parents for limited local resources may favour parents to invest more in the dispersive sex. In general, the important contribution of these models to SA theory was the idea that sex-specific kin competition may affect selection on the SR. Selection favours a sex ratio biased towards the sex that competes less or conversely, towards the philopatric sex when it improves conditions for the relatives (*Local Resource Enhancement* LRE, Clark 1978; e.g. avian helpers-at-the-nest).

Sex allocation among families

Fisher's theory applies to total population investment in the sexes and predicts the population's mean SR, but does not explain how individuals are expected to allocate their resources (Williams and Williams 1979). For example, each family could allocate equally to the sexes, or one half of the families could allocate only to sons while the other half allocates only to daughters. Kolman (Kolman 1960) showed that this distribution of sex ratios within families (= sex ratio variance) in a population at fisherian equilibrium is not affected by natural selection since fitness depends only in the family's total allocation, not how the total is divided. However, the same as Fishers' model, this theory rests on one essential assumption: the relationship between costs and benefits is linear for both sexes (double the investment, double the benefit).

On the contrary, changes in investment may not be (and often are not in many species) directly proportional to the changes in benefits (non-linear returns on investment). A series of models predict adaptive SA in response to sexually differential returns from investment in offspring (e.g. Trivers 1972; Maynard Smith 1980; Charnov 1982; Frank 1990). The starting point was the hypothesis of "maternal dominance" of Trivers and Willard, who suggested that if

i food availability varies and affects maternal condition (there are mothers in relatively better conditions than others)

ii maternal condition is correlated with offspring condition (mothers in better condition produce higher quality offspring, which become higher quality adults)

iii sons gain a greater fitness benefit from more resources and quality than daughters,

then mothers in relatively good conditions should overproduce sons, and those in relatively poor conditions, daughters (Trivers and Willard 1973).

The classic studies of Clutton-Brock *et al.* (Clutton-Brock *et al.* 1981, 1986) in red deer *(Cervus elaphus)* provided the first compelling support for the hypothesis, which was initially developed for polygynous species with litter or clutch size of one. Two main assumptions underlie: (1) the functions that describe the relationship between investment and returns are non-linear and different for each sex; and (2) families vary on the amount of available resources they can allocate.

In his essential book *The theory of sex allocation*, Eric L. Charnov developed the Trivers-Willard work by showing how the same principle could be applied to a wide range of taxa and in general more widely: apart from parental conditions, any socio-environmental parameters that might predictably affect the fitness of sons and daughters unequally, would favour parents to invest more in the sex that is expected to have the highest fitness (Charnov 1982). Currently, the idea that selection favours conditional SA if environmental conditions differentially influence the fitness of males and females is broadly termed the *Trivers-Willard hypothesis* (Figure 2).

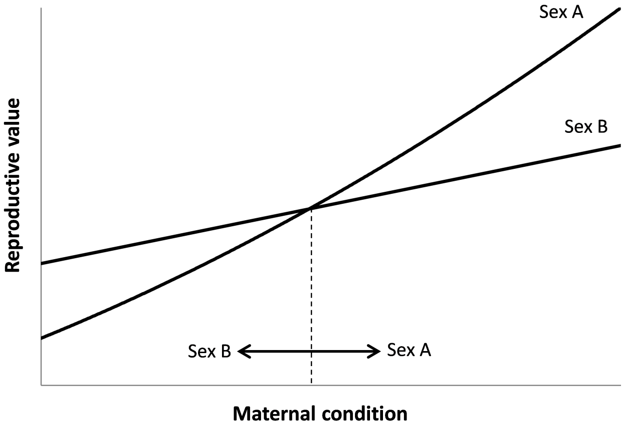


Figure 2: The Trivers-Willard hypothesis. Conditional allocation when the fitness consequences of variation in maternal condition differ between the sexes. Specifically, the sex that gains the greater fitness from being produced with better maternal condition (sex A) should be produced under relatively good conditions, and the other sex (sex B) under relatively poor conditions. Currently, it is applied to a variety of indices besides maternal condition (adapted from West 2009).

Another major step was the understanding of the population-level consequences of individual-level adjustment. Charnov's model showed that frequency-dependence is still a key feature of sex allocation, but whenever there are nonlinearities in the returns of sons and daughters, an equal allocation at the population level is not expected. This means that even if the Trivers-Willard hypothesis concerns family-level SR adjustment, it has consequences for the population level: it leads to bias in the population sex ratio, although its direction and magnitude may be difficult to predict (Frank 1990, 1998). Besides, there is currently no theory that can tell us how often the prediction of Fisher's theory is qualitatively incorrect if the assumptions of Trivers and Willard hold (Komdeur and Pen 2002).

In any case, these models greatly promoted subsequent empirical testing, which has helped to make the Trivers-Willard hypothesis and its various extensions one of the most productive areas of SA study.

Mechanisms for adjusting sex allocation

Sex ratio variation in response to the parent's ability to invest resources differentially is achieved by one of these two mechanisms (or both):

1. Manipulation of primary SR (before birth)

In opposition to Trivers and Willard (Trivers and Willard 1973), who suggested greater post-natal mortality of one sex as a mechanism to generate variation, Myers proposed that parents might be able to adjust primary sex ratio (J. H. Myers 1978). Her model emphasized that sex ratio composition of families may be adjusted to maximize the number of successful offspring rather than the average reproductive potential of each offspring. If we assume, as all the previous models do, that sex allocation is controlled by autosomal genes (not sexual chromosomes), then parental control over the sex of offspring becomes a highly valuable tool for adaptive adjustment.

Do animals possess mechanisms which allow them this control? The capacity to adjust sex depends on the sex determination system. In some reptiles and fishes, with environmental sex determination (ESD; e.g. pH, incubation temperature), mothers can exert some control through their egg laying behaviour (incubation temperature has been also found to influence bird sex ratios by means of differential mortality; Eiby *et al.* 2008; Göth and Booth 2005; see Box 2). In haplodiploid organisms, such as insects, fertilised (diploid) eggs develop into females and unfertilised (haploid) eggs into males, which gives the mother very precise control over the sex of each offspring (Bull 1983).

Genetic or chromosomal sex determination (CSD) of organisms like birds and mammals, on the contrary, was traditionally assumed to constraint severely this control (Williams and Williams 1979; Bull and Charnov 1988; Frank 1990). The random process of Mendelian segregation leads to an expectancy of primary sex ratios close to parity, approximately as described by a binomial distribution. However, although both Fisher's theory and CSD both provide explanations for unbiased SR, Fisher does not predict binomial sex ratio variance.

In the last decade this conception has been overturned (but see Uller, Pen, *et al.* 2007), thanks to studies (especially in birds) that have reported repeatable patterns of SR adjustment and significant evidence of offspring sex control (Komdeur, Daan, *et al.* 1997; West and Sheldon 2002; West 2009). Caution is still needed before assuming that uneven sex ratios imply adaptive control (there may be non-adaptive explanations too), but it seems now clear that CSD constraints may be overcome and vertebrates could possess mechanisms of sex manipulation, although they remain partially elusive (see Box 2). Active manipulation of the primary SR would be the most cost-efficient way for the mother to adjust offspring sex ratio since it minimizes investment in the undesired sex (Alonso-Álvarez 2006).

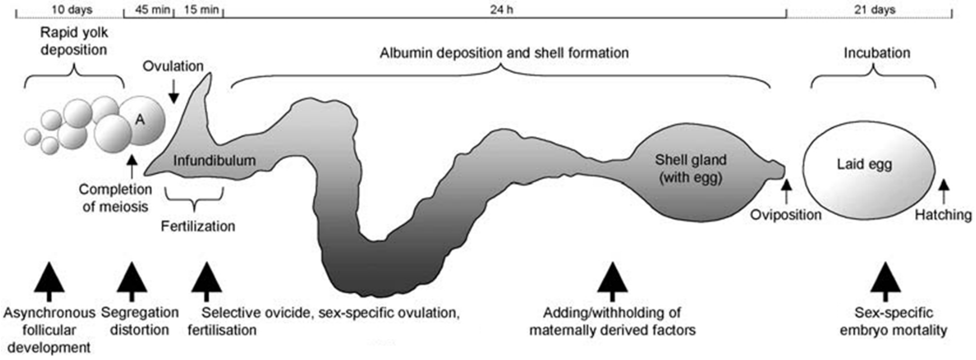
2. Manipulation of secondary SR (after birth)

In species with parental care, much sex-biased investment can occur after birth, since sex allocation and juvenile mortality are potentially controlled by parents. Parental strategies may include selective killing and sex-biased defense, shelter, or differential provisioning of food (Bortolotti 1986; Lessells 2002; Leech, Rowe, *et al.* 2006; Cameron-MacMillan *et al.* 2007; Mainwaring *et al.* 2011) that may lead to differential mortality or reproductive success.

Sex-specific mortality occurring during the period of parental investment selects for biased sex ratio in terms of Fisher's principle (Seger and Stubblefield 2002; West 2009). If sons and daughters are equally costly to produce but sons are more likely to die, the average cost of a son is lower than that of a daughter. This favours a sex ratio bias towards sons in the primary SR (to equalize investment). The higher mortality of sons, however, may lead the secondary SR to be biased toward daughters by the end of parental care period.

Box 2. POTENTIAL MECHANISMS OF AVIAN SEX MANIPULATION

The combination of CSD and female heterogamety in avian species provides an especial potential for maternal influence on offspring sex, although the precise mechanisms are still poorly understood. Females, the heterogametic sex (ZW) produce Z ('male') and W ('female') gametes while the males (homogametic sex, ZZ) contribute Z sperm. There are good reviews of the topic and the available evidence (Pike and Petrie 2003; Alonso-Álvarez 2006; Rutkowska and Badyaev 2008); here only the most likely mechanisms are addressed:



(Figure from Pike and Petrie 2003)

Phase of rapid follicular development

1. **Asynchronous follicular development:** differential growth rate of ♂ and ♀ follicles could alter the ovulation order and allow the control of SR in relation to laying sequence.

2. **Sex-specific atresia:** process of degeneration and re-absorption of pre-meiotic follicles before a state of maturity.

Phase of meiosis

3. **Segregation distortion or Meiotic drive:** selection of the sex chromosome at the first meiotic division; the unwanted one would be banished to the polar body, while the preferred one would be assigned to the ovum.

Phase of pre-ovulation and ovulation

4. **Selective ovicide or re-absorption:** abortion and subsequent re-absorption of post-meiotic ova with the unwanted sex-chromosome instead of being ovulated.

5. **Sex-specific ovulation:** plasma levels of a number of hormones a few hours before ovulation could induce a non-random detachment of ovas with Z or W-chromosome.

Phase of fertilization

6. **Sex-specific fertilization:** regulation by the mother of fertilization probabilities of ♂ and ♀ oocytes, via different mechanisms (selectively-released spermatozoa, limitation of motility or fecundity of released sperm, inhibition for sperm to penetrate the ova membrane or inhibition of zygote development...)

Right before or shortly after oviposition

7. **Sex-specific embryo mortality:** via alteration of egg composition (sex-differential allocation of substances in the yolk) or via direct manipulation of incubation temperature.

Two common factors to the preceding mechanisms are: (1) they are thought to be directly mediated by circulating maternal hormones and (2) the breeding female needs to accurately recognize the sex of the ova. Positive evidences exist for both factors, though still much is unknown (Groothuis and Schwabl 2008). Recently, Uller and Badyaev 2009 have suggested the existence in birds of systems that combine CSD with environmental pathways that can acquire a sex-determining function.

Under non-fisherian principles, even if sex ratio at conception/birth is fixed, there may be huge variation in SA based on behavioral and physiological mechanisms which may themselves have been shaped by selection. Thus, differential mortality should always be considered as a possible explanation for adaptative biases in the secondary SR of a given population. However, the underlying mechanisms may simply come about by sex-specific sensitivity to hormones, parasites, food shortage or because offspring show differential skills at extracting resources from parents (Clutton-Brock 1991). In practice, many times it is difficult to distinguish between explanations. It has also been proposed that the heterogametic sex is more susceptible to mortality because of the hemizygous state of its sex chromosome (*Heterogamety hypothesis*; J. H. Myers 1978). Mortality under stress would be then, for instance, male biased in mammals and female biased in birds. However, general mortality patterns appear to be male-biased in both birds and mammals (Clutton-Brock *et al.* 1985), suggesting either that sex-biased parental investment or sex-biased response to poor conditions are more likely explanations than heterogamety.

Manipulating the survival of offspring after birth is argued to be costly in energetic terms, since it entails a loss of already invested resources (J. H. Myers 1978; Maynard Smith 1980). However, the costs of this control may be limited if the costs of offspring production are low (Clutton-Brock 1991), or at least lower than a certain threshold below which it pays to bias the sex ratio. For instance, under certain assumptions some mathematical models have calculated in 17% of total resources the maximal waste that a parent could sustain (Pen and Weissing 2002).

Why studying avian sex ratio?

The phenomenon of offspring sex ratios deviating from the expected equal proportions of sons and daughters has fascinated researchers for many decades. Today, various taxa across the animal kingdom have yielded correlative and experimental evidence for parental influence if not even control (insects: Flanders 1965; Hardy 1994; mammals: Cameron 2004; reptiles: Radder 2007; birds: Sheldon *et al.* 1999; Nager, Monaghan, Griffiths, *et al.* 1999; Pike 2005; fishes: Conover and Voorhees 1990). However, while sex ratio shifts in invertebrates can be extraordinary and their behaviour has provided some of the strongest tests of SA theory (Ode and Hunter 2002), in birds (and mammals) the shifts are usually small and their sex allocation much more difficult to understand (Komdeur and Pen 2002; Cockburn *et al.* 2002; West 2009).

Birds are characterized by life histories that clearly do not fit with a number of assumptions of the classic models. Apart from the CSD mechanism, they possess long lives, low fecundity, mostly biparental care (in 81% of species, although with different degrees and means of control; Cockburn 2006), complex mechanisms for adjusting parental investment and trade-offs between current and future reproduction events. Until not many years ago, determining the sex of morphologically indistinguishable embryos/hatchlings was another obstacle to the study of avian sex ratios, but it has now largely disappeared with the advent of reliable molecular sexing techniques (Griffiths *et al.* 1998; Fridolfsson and Ellegren 1999). Still, avian SR literature is unevenly distributed across species, and the allocation patterns reported are known for their inconsistency (Palmer 2000; Krackow 2002; Ewen *et al.* 2004).

Despite these difficulties, there are several reasons for interest in the study of avian sex ratios. First, empirical studies have revealed strong patterns of allocation that make clear that the constraint of Mendelian segregation can be overcome. Second, complex avian societies overrule the assumptions of random mating and non-overlapping generations that lay in the root of models predicting equal investment in offspring (Fisher 1930). Third, rearing a brood involves a very heavy investment for a relatively small number of young, which makes the choice between sons and daughters particularly sensitive. Thus, from the theoretical point of view, SR research contacts many aspects of avian biology, being able to provide insights into behavioural and evolutionary ecology features, e.g., the strength of sexual selection forces.

From the view of applied sciences, further knowledge about the factors that influence SR in a predicted direction is also of great value. Conservation programs could benefit from the manipulation of individual condition or environmental cues to alter offspring sex ratios in order to help population growth (Lenz *et al.* 2007; Lawrence *et al.* 2008), for example inducing mothers to overproduce daughters (Wedekind 2002).

The kakapo (*Strigops habroptilus*), a critically endangered species, exemplifies the importance of SA studies for practical conservation (Clout *et al.* 2002). This parrot species, lek-polygynous and highly size dimorphic (heavier males), shows a highly male-biased SR in adults. Part of the conservation efforts included the provision of supplementary food to encourage breeding. However, and as predicted by the Trivers-Willard hypothesis, this provoked the undesirable effect of supplemented females overproducing the sex with higher fitness returns, males (Clout *et al.* 2002). The inclusion of SA theory helped to design a new supplementary feeding program that solved the problem (Robertson *et al.* 2006). Another example with practical concern is the investigation of sex ratio shifts related to environmental pollutants. Exposure to mercury or DDT and other organochlorines skewed nestling sex ratios towards the production of females in several species (Fry and Toone 1981; Erikstad *et al.* 2011; Bouland *et al.* 2012). The explanation may come from Trivers–Willard theory or non-adaptive causes, but the pattern can lead to reduced effective population sizes. This could be especially alarming for endangered species such as many seabirds, with already small population sizes and exposed to mercury through their fish diet (Bouland *et al.* 2012).

Box 3. RELEVANT FACTORS FOR THE STUDY OF AVIAN SA

Any socio-environmental parameter that predictably affects the fitness of sons and daughters in a different manner is relevant to the study of SA. In birds multiple factors may influence sex ratio evolution, due to their complex life histories and the environmental unpredictability:

Mating system (e.g. degree of polygyny, local inbreeding; LMC)
Life expectancy (and therefore trade-offs between current and future reproduction)
Sexual difference in philopatry (LRC)
Helpers (LRE)
Parental condition and quality (Trivers-Willard)
Type and amount of parental care (Trivers-Willard)
Environmental condition (territory quality, food availability, stress, time of the year)
Sexual size dimorphism (SSD)
Secondary sexual characters (Attractiveness hypothesis; Trivers-Willard)
Family size (trade-offs between sex, number and hatching position of offspring)

Adaptive models of sex allocation in birds: Evidence

Currently, further extensions of the classic theory make possible to construct more realistic models for birds, although there is still much simplification. For instance, J. H. Myers 1978 and Williams and Williams 1979 addressed the parental problem of how to split the resources into separate offspring, also known as the "size, sex and number trade-off" (see also Carranza 2004; Carranza and Polo 2012).

Reviews from the 1980s claimed that SR variation in birds was rare, of minor magnitude and little adaptive significance (Charnov 1982; Clutton-Brock 1986; Bull and Charnov 1988). Since then, the number of SR studies has greatly increased and now many report significant variation and some also support an adaptive explanation. However, real convincing evidence for adaptive biased sex ratios remains scarce. Mainly five classes of models, with various degree of evidence, aim to provide explanations for adaptive SR patterns in birds (Hardy 1997; Cockburn *et al.* 2002; Komdeur and Pen 2002), according to the large number of factors which could influence avian sex ratio (see Box 3).

Fisher's hypothesis

The theory, at first sight, seems to be supported by the fact that originally motivated it: many birds have approximately equal sex ratios (Clutton-Brock 1986; Gowaty 1991; Ewen *et al.* 2004). However, empirical support is problematic: an unbiased numerical SR is not per se evidence for Fisherian sex ratios until it is accompanied by evidence that the sexes are equally costly to produce. Measuring the relative costs or reproductive value of sons and daughters is extremely difficult, since it involves diverse interacting fitness aspects. A specialized variant of the theory, the *Homeostasis hypothesis* (parents respond to a low number of one sex by producing that rarer sex) remains also very questioned, both theoretically (Leigh 1970) and empirically (Bensch *et al.* 1999). A further problem in birds is that CSD leads also to an expectation of numerical sex ratio equality (see page 10). Postma *et al.* 2011 aimed to distinguish between both possibilities using 14 years of sex ratio data of song sparrows (*Melospiza melodia*), and found no evidence for the existence of either genetic or environmentally induced variation.

Consequently, there seems to be a lack of appropriate tests and results of Fisher's theory in birds, which has not been satisfactorily addressed until the moment.

Social environment hypotheses

They include the Local mate competition (LMC), Local resource competition (LRC) and Local resource enhancement (LRE) hypotheses (see page 7 for predictions), in which the relevant factors are the existence of mating competition among relatives, sex-biased philopatry-dispersion, or a sex-bias in helping behaviour.

LMC theory is a very productive area of SA, but its relevance to birds, as they normally occur in stable populations, is very limited.

LRC among birds was explored by Gowaty (Gowaty 1993), which compared passerine species (where females disperse more) with wildfowl species (where males disperse more) and found a tendency in the predicted direction (passerines favoured the production of daughters and waterfowl the production of sons). However, controversy about the data and methods used (P. J Weatherhead and Montgomerie 1995) and negative results when including data from other Orders (Palmer 2000) has shown that this pattern may not be reliable. Nevertheless, new studies are exploring the topic (Hjernquist *et al.* 2009).

LRE hypothesis (helpers-at-the-nest) is supported by more consistent evidence. So far, patterns of SR adjustment in the predicted direction (groups that lack helpers should bias their offspring towards the helping sex) have been found in several species (reviewed by West and Sheldon 2002; Griffin *et al.* 2005; West, Shuker, *et al.* 2005). A few other species do not show the pattern (Bednarz and Hayden 1991; Legge *et al.* 2001; Koenig *et al.* 2001; Doutrelant *et al.* 2004), but Griffin *et al.* 2005 showed that the degree of SR adjustment can be explained by the variation in the relative benefit that the helpers provide.

Based on these theories, the Seychelles warbler (*Acrocephalus sechellensis*) case is probably the most important and clear example of SR adjustment not only for birds but for all vertebrates. Apart from the striking sex ratios shown, it illustrates how multiple factors can be operating simultaneously in sex allocation (LRE, LRC and also environmental influences; Komdeur 1996; Komdeur, Daan, *et al.* 1997), and provides explicit evidence of the adaptive nature of SR variation (Komdeur 1998).

Trivers-Willard hypotheses

Even in a narrow sense (investment in the sexes is dependent on maternal condition in polygynous societies), the theory provides a good model for birds: (1) inter-familiar variance in parental resources and (2) nonlinearities in the marginal returns in response to investment, are both expected situations in many avian species. Besides, some early studies in birds removed polygyny as a pre-requisite for Trivers-Willards effects (Burley 1981, 1986). Since then, the influence of maternal condition has been profusely investigated and experimentally supported (R. Kilner 1998; Nager, Monaghan, Griffiths, *et al.* 1999; Whittingham and Dunn 2000; Velando 2002; Alonso-Álvarez and Velando 2003; Pike 2005). In addition, similar arguments have been applied to a variety of factors (wide-sense theory): laying date/seasonality, laying-hatching order, egg size, clutch size, territory quality, food availability, number of helpers, parental age, quality and experience and pair bond duration, among others (for literature reviews, see Hasselquist and Kempenaers 2002; Alonso-Álvarez 2006; West 2009).

An extension of the theory that has attracted much interest is the *Attractiveness hypothesis* (Burley 1981; for the mathematical model, Pen and Weissing 2000): females should adjust the SR in response to mate attractiveness or quality, overproducing sons when mated to attractive or high quality males. These fathers would lead to higher quality offspring by passing good genes or through higher quality paternal care. Since in birds the variance in reproductive success is generally higher for males (Møller and Ninni 1998), sons would benefit more than daughters from an increased quality father. Empirical studies have found support for the hypothesis (Ellegren *et al.* 1996; Von Engelhardt *et al.* 2004; Pryke, Rollins, *et al.* 2011) while others not (Rutstein *et al.* 2005). However, there seems to be a widely repeatable and significant trend towards the production of sons when mating more attractive males (West and Sheldon 2002; Delhey *et al.* 2007; West 2009).

Despite the strength of the Trivers-Willard trends in many species, extracting common patterns for birds remains controversial. Many studies have found inconsistencies in the direction or directly no adjustments even if the *a priori* conditions made them expectable (P. J. Weatherhead 1985; Sheldon 1998; Hartley *et al.* 1999; Zann and Runciman 2003; Budden and Beissinger 2004). Even two meta-analyses arrived at different conclusions (West and Sheldon 2002; Ewen *et al.* 2004). Some authors have argued that variation in the extent of SR adjustment across species and populations, however, may be explained by variation in the strength of selection (K. E. Arnold *et al.* 2003) and by low detectability at the clutch level (Rosivall 2008).

Definitive support for the Trivers-Willard theory should come from the demonstration of the fitness consequences of SR adjustment (e.g., showing that maternal condition differentially affects the reproductive value of sons and daughters) as a few experimental studies have done (Nager, Monaghan, Griffiths, *et al.* 1999; Badyaev *et al.* 2002; Saino, Romano, *et al.* 2010). Besides, studies of particular species, which explore different factors throughout different species and populations, are needed in an attempt to explain broad taxonomic variation in the extent of SR adjustment.

Costs of reproduction hypotheses

These alternative arguments compile some of the Trivers-Willard and LRC premises, but differ in the perspective: the emphasis is placed on the reproductive value and fitness of the mother, rather than that of her offspring (J. H. Myers 1978; Wiebe and Bortolotti 1992). Females in poor condition may be averse to invest in the sex with greater demands to minimize the failure risk and/or to increase the subsequent reproduction prospects. A few species produce broods biased towards males or females depending on the maternal rank: primary females, which are assisted by males in the nest provisioning, produce more sons while secondary females produce more daughters (Nishiumi 1998; Westerdahl *et al.* 2000). However, the similarity between the SR trends predicted by these hypotheses and Trivers-Willard make it difficult to distinguish between them.

Brood reduction hypothesis

Instead of focusing on interactions between parents and their brood, these hypotheses deal with the interactions that occur within broods. Certain sex-combinations may be avoided because they lead to siblicide, or favoured if they achieve brood reduction efficiently. Mostly studied in raptors (e.g. Bortolotti 1986; Dzus *et al.* 1996), they seem supported by the occurrence of biased sex sequences, but so far it is not clear whether the possible disadvantages of certain sex-combinations lead to manipulation of primary SR or just to the existence of sex-specific mortality.

Note of language use

Sentences such as "individuals are selected to maximize their reproductive success" do not mean that animals are consciously maximizing their reproductive success, neither that they are consciously aware of the links between behaviours and reproductive success and the consequences of natural selection. Such phrases are used to avoid the constant repetition of long sentences detailing how natural selection works –e.g., individuals who have a greater reproductive success provide a greater genetic contribution to the next generation, and hence natural selection will favor genes that lead to individuals behaving in a way that maximizes their reproductive success (from West 2009).

Inter-specific analyses of sex ratio and vulnerability

Relationships between SR and SSD

Fisher's theory predicts that when individuals of one sex are more costly to produce, the sex ratio at the end of parental care should be biased towards the "cheaper" sex, so that the overall investment in the sexes is equal. This means that patterns of sex allocation depend, at least in part, on the relative cost of producing sons and daughters. In many organisms the cost of producing males and females is likely to be very similar. However, there are species that exhibit marked differences in body size between males and females (sexual size dimorphism, SSD).

Although the idea has been sometimes questioned (Teather and P. J. Weatherhead 1994), it is assumed that in SSD species the larger offspring sex has greater total energy requirements (Slagsvold *et al.* 1986; Richner 1991; Magrath *et al.* 2007), which may affect parental condition (Cameron-MacMillan *et al.* 2007). Furthermore, comparative studies reveal a positive correlation between the degree of SSD and the amount of food/energy demanded by the sexes (Anderson, Budde, *et al.* 1993; Krijgsveld *et al.* 1998). The larger sex would need a greater parental investment (it is more "costly") and the more dimorphic is the species, the greater sex difference in investment may be expected.

If size differences between sons and daughters in SSD species reflect sex-specific costs to the parents (Krijgsveld *et al.* 1998; Magrath *et al.* 2007), we would expect a population sex ratio skewed towards the smaller sex. So far, evidence for influence of avian SSD on sex ratios is blurred: some species have shown the predicted patterns (more daughters in male-biased SSD species or more sons in female-biased SSD species; Howe 1977; Wiebe and Bortolotti 1992; Griffiths 1992; Torres and Drummond 1997), but not others (Newton and Marquiss 1979). Reviews do not illuminate the picture: Clutton-Brock concluded that there was little evidence of variation (Clutton-Brock *et al.* 1985; Clutton-Brock 1991), Slagsvold *et al.* revealed juvenile sex ratios biased towards the smaller sex (Slagsvold *et al.* 1986), while Dijkstra *et al.* determined that the smaller sex was under-represented at fledging (Dijkstra, Daan, and Pen 1998).

From another perspective, increased size may confer selective benefits, but it is likely to come with costs reflected in higher mortality or other vulnerabilities (e.g. reduced physiological status). Such differences between sexes may be worsened by poor conditions, food shortage or environmental stress, and often the males (usually the larger sex) are more adversely affected (Clutton-Brock *et al.* 1985; Roskaft and Slagsvold 1985; Griffiths 1992). Two explanations have been proposed for the generally observed male-bias vulnerability: (1) greater nutritional requirements of the larger sex (**size-dependent vulnerability**) or (2) sex-associated factors (**sex-dependent vulnerability**). The latter includes heterogamety (see page 12) and the *male-phenotype hypothesis*: independent of male size, there exist negative effects of being male. For example, increased testosterone can adversely impact other aspects of development such as immune-competence (Zuk 1990; Fargallo *et al.* 2002; Müller, Dijkstra, *et al.* 2003).

Clutton-Brock's comparative analysis (Clutton-Brock *et al.* 1985) showed that differences in vulnerability (assessed as juvenile mortality) were proportional to the degree of SSD. However, a following number of studies found opposite patterns (e.g. Nager, Monaghan, Griffiths, *et al.* 1999; Nager, Monaghan, Houston, *et al.* 2000; Hörnfeldt *et al.* 2000) or alternatively found no sex difference in pre-fledging growth and mortality in SSD species (Westerdahl *et al.* 2000; Råberg *et al.* 2005). In addition, larger size may be advantageous in situations with strong sibling competition, resulting in sub-lethal effects or increased mortality of the smaller and not the larger sex (Anderson, Reeve, *et al.* 1993; Råberg *et al.* 2005).

Analysis and species models

The previous results create a puzzling picture of the relationships between SSD, sex-specific offspring vulnerability and SR adjustment in birds. A potential solution to this problem is to use the variation between different taxa as a source of data, that is, to use an inter-specific comparative analysis. This kind of approach offers widely interesting and applicable results, because it overcomes the particularities of single species while avoiding the non-independence problem of species characters (Mayhew and Pen 2002).

Comparative studies are thus required in order to examine variation in the extent (or precision) of SR adjustment (Sheldon 1998). For instance, it might be possible to test if Fisher's hypothesis apply, examining whether the offspring sex ratio is correlated with the parental investment ratio (determined by the degree of sexual size dimorphism) in the two sexes (**Chapter 1**). A Fisherian result could be distinguished from a purely heterogametic one (see also page 10), since in species with marked sexually differential production costs, Fisher's equal investment hypothesis predicts a numerical bias, while heterogamety predicts numerical equality (Hardy 1997). In addition, sex-biased mortality and sensitivity performance can be tested in relation to SSD and under varying environments, among a wide range of species. This could be a manner to determine the general validity of the size-dependent explanation of vulnerability patterns, their direction and their intensity (**Chapters 1 and 2**). Comparative analyses also allow the inclusion of many other characters and of various degrees of this character variation, over which natural selection has worked throughout longer timespans than any experimental or observational study can cover (Mayhew and Pen 2002).Therefore, other multiple life-history factors possibly influencing SA and more specifically, influencing the relationships between SSD and SR (see Box 3, page 14) can be included in the analyses (**Chapter 1**).

Finally, comparative analyses can be further complemented by a species-level approach (each species as an independent data point), which provides illustration of the trends and more accessible biological interpretations, and eludes the restrictive assumptions made by comparative methods (T. Price 1997; Martins 2000, see page 21).

The use of avian models, due to some characteristics, benefits the study of these questions. First, females are the heterogametic sex (see page 11). Second, among birds there are monomorphic species, species that exhibit male-biased SSD (male larger than female) and

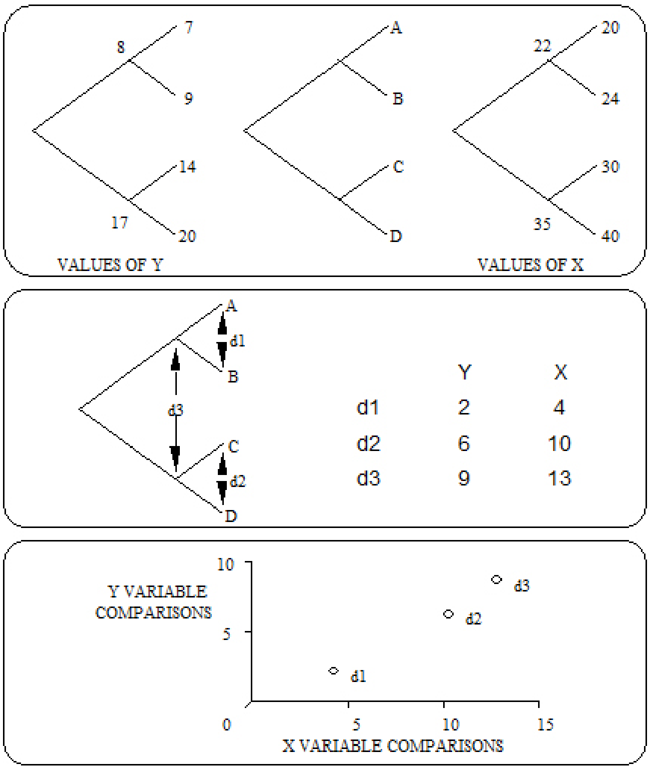


Figure 3: Simplified model of independent comparisons (from Harvey and Pagel 1991, User Guide of CAIC). Values of two traits, X and Y, for each of four species, A to D. Higher nodes are calculated as the average value of lower nodes. Species A and B diverged at the higher node, so differences between them (d1) must have evolved since then (same with d2). These two sets of differences are independent. Furthermore, the differences between the higher nodes (d3) make up a third independent comparison. If the Y-variable comparisons are plotted against the X-variable comparisons, we can see whether evolutionary change in the two traits has been correlated.

some groups (raptors, owls, and some seabirds) that present female-biased or 'reversed' size dimorphism (female larger than male) (Székely *et al.* 2007). In both directions, a number of species and families show extremely pronounced SSDs. This wide range of SSD in birds makes them excellent taxa for comparative analyses across species. Finally, birds have a short period of determinate growth, so most of them achieve adult size shortly after fledging. This makes the developmental period a crucial moment in terms of energetic requirements, especially in case of dimorphic growth. Hence, the effect of sex differences in nutritional requirements, physical condition or survival can be easily observed before (altricial) offspring are independent from their parents.

General methodology

Valid comparative tests across species face one major obstacle, the non-independence of related taxa. Closely related species are likely to be similar because they inherit characters from a common ancestor and therefore, not all the variance in species characters is the result of independent evolutionary events (Felsenstein 1985; Harvey and Pagel 1991). Statistical methods that treat species as fully independent points (by taking raw species characters) are not completely valid, since they may overestimate the amount of evolutionary independent variation, and artificially inflate the significance of any test (the problem known as "phylogenetic overcounting", Mayhew and Pen 2002).

From the techniques that deal with this problem, I chose the method of independent contrasts (based on Felsenstein 1985). The method calculates scores called "contrasts": differences between two sister taxa at each branching point ("node") of the phylogenetic tree, and uses them for analyses (Figure 3). They represent the variation acquired since these taxa shared an ancestor, and are phylogenetically independent (in the absence of confounding third variables). The method requires some assumptions: a Brownian and vaguely parsimonious evolutionary model, and equal rates of evolutionary change per unit branch length in all branches of the phylogeny. Contrasts may be analysed parametrically by linear regression, which must be forced through the origin, since the change in the dependent variable in response to zero change in the independent variable must be also zero (Garland *et al.* 1992). Thus, the expected value of the slope equals the true relation between the compared characters in the absence of phylogenetic effects (Pagel 1993).

A comparative analysis requires two types of data collection: taxon character estimates and phylogenetic information.

Species characters derive usually from second-hand data of studies previously published (collection restricted to the available literature). It is necessary to set criteria for the inclusion of studies in order to reduce the measurement data error, and also to summarize taxon properties with a single statistic. Sex ratios, for example, are sensitive to local conditions, which may make populations rather than species the appropriate level for comparison. However, this would be correct only if the rest of relevant variables are also estimated within each population, which is often not the case (Harvey and Nee 1997). Therefore, an average value should be calculated from the distinct populations (Mayhew and Pen 2002). To ensure the inclusion of correct results in this thesis, specific criteria were used (1) to assess the methods of each study (sample size, measurement differences, sex determining methods. . .); (2) to estimate the degree of SSD and fledging mass; (3) to collect life-history factors; and (4) to calculate mean SR values when necessary.

The phylogenetic information for bird species is being constantly improved: currently, several projects aim to define the bird family tree with branch length estimates (e.g. Tree of Life web project: `http://tolweb.org/Neornithes/15834`, Hackett *et al.* 2008). With the available information at the moment of elaboration, and in common with other studies which had applied this method to the study of avian diversity (Promislow *et al.* 1992; Owens and Bennett 1994), a molecular phylogeny based on DNA–DNA hybridization experiments (Sibley and Ahlquist 1990) was used (Figure 4a). This phylogeny was the first comprehensive attempt to reconstruct a cladogram across a whole class of organisms based on molecular data, and subsequent molecular studies confirmed many of its conclusions (Mooers and Cotgreave 1994). It assumes a phylogenetic topology above the family level and multiple branching among genera within families and among species within genera, with all branch lengths set to equal lengths (Owens and Hartley 1998). However, it has been subject of controversy (Sarich *et al.* 1989) because of biases or inaccuracies. Therefore, I also used a second phylogeny based on the classification of Cracraft (Cracraft 1981), which uses traditional morphological characters, such as skeletal traits (Figure 4b). The use of different cladograms allowed evaluating the extent to which the results were sensitive to different reconstructions of avian evolutionary history. However, the extensive analyses carried out for Chapters 1 and 2 showed that molecular and morphological models of phylogeny led to qualitatively similar results.

From a number of similar programs derived from Felsenstein (Felsenstein 1985), CAIC (Comparative Analyses by Independent Contrasts, Purvis and Rambaut 1995b) is the most popular and used in this thesis work. If the phylogeny is fully known and all the variables are continuous the package applies Felsenstein's method exactly. But if parts of the phylogeny are uncertain and contain polytomies (nodes with more than two daughter branches) that express ignorance of the true branching structure (as in the avian case), CAIC deals with them as soft polytomies (Pagel 1992). CAIC was used here to analyse both continuously distributed variables (such as incubation period) and discrete categorical data (such as presence of helpers), since subsequent implementations also allow the contrast analysis of categorical variables (Burt 1989) –for more information on the algorithms and analyses used by CAIC, see Purvis and Rambaut 1995a–.

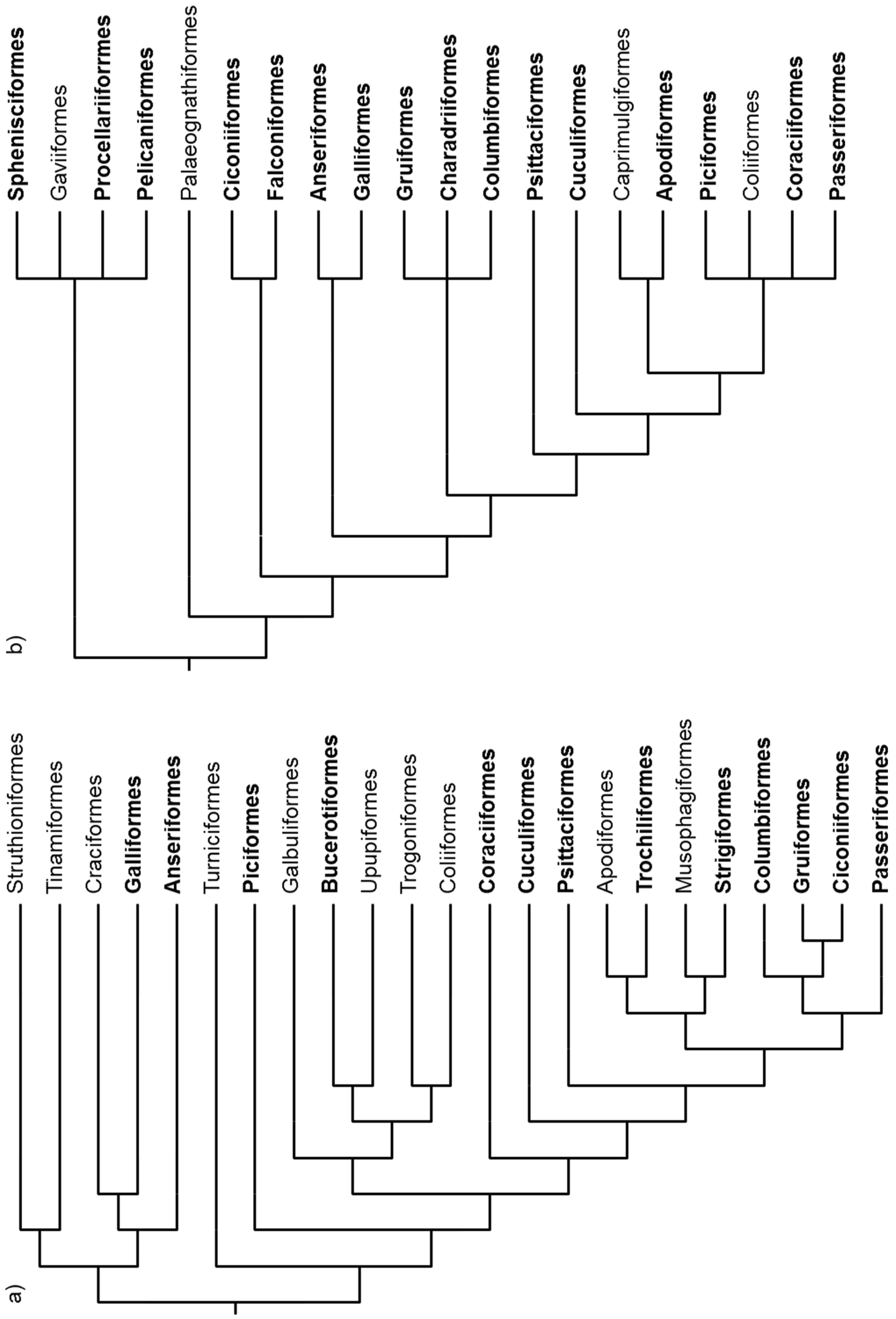


Figure 4: Cladograms used in comparative analyses: a) molecular (Sibley and Ahlquist 1990), b) morphological (Cracraft 1981). In bold Orders included in the study.

Intra-specific analyses: The case of the common tern

Species model

This part of the thesis focuses on the common tern (*Sterna hirundo*), a small-sized migratory seabird. It is morphologically characterised by its pointed wings, forked tail, black cap, slender orange-red bill with black tip and orange-red legs (Figure 5b, page 29). With a mean body mass of 110–140 g and body length of 32–39 cm (Becker and Ludwigs 2004), it is a slightly dimorphic species. In adults, sex differences are significant for bill (6%), head-bill and tarsus length (larger males), and adult males are around 1–3% heavier (K. L. Fletcher and Hamer 2003; Becker and Ludwigs 2004). Besides, males are 3% heavier than females at fledging time (Becker and Wink 2002). No sexual dimorphism in plumage or other physical traits seem to exist.

In order to study the common tern under the light of SA theory, we need previous knowledge of its life-history as well as all of possible sexual differences in behavioural and ecological characteristics (see Box 3, page 14).

Common terns show the typical features of seabirds: high adult survival, delayed maturity, high philopatry, low annual reproductive rate and extended chick-rearing periods. Living up to 30 years, their rate of adult survival is around 90% and age-specific mortality rates seem to be not sex-specific (Nisbet and Cam 2002; Ezard *et al.* 2006; Becker and Zhang 2011). Most individuals breed at age 3 years, after at least one year prospecting at the colony site (Ludwigs and Becker 2002; Dittmann and Becker 2003; Dittmann, Zinsmeister, *et al.* 2005). Date of first arrival and recruitment age are positively linked (Becker, Dittmann, *et al.* 2008). Females seem to reach maturity slightly earlier: their recruitment age is significantly advanced (0.4 years earlier than males, Ludwigs and Becker 2002) and they first breed at a younger age (12% of females start at 2 years old, but only 2% of males, Ludwigs and Becker 2005). The explanation may be that males are more territorial and due to courtship feeding they require more foraging skills than females, and as such they often recruit when 1 year older (Ludwigs and Becker 2002). In spite of the relatively high natal philopatry (Nisbet and Cam 2002; Becker and Ludwigs 2004), young adults present certain sex-differential dispersal: females are more likely than male to prospect other colonies and to emigrate (Dittmann, Zinsmeister, *et al.* 2005; Becker, Ezard, *et al.* 2008). Nonetheless, the sex ratio of breeders is thought to be close to parity (Nisbet and Cam 2002). Average productivity varies from 0 – 2.4 fledglings/pair (mean success 0.8, Becker 1998), and some components of breeding performance decline seasonally (González-Solís, Wendeln, *et al.* 1999; Becker, Wendeln, and González-Solís 2001). Individuals improve their breeding performance with age, although some reproductive senescence seems to occur after approximately age 14 (Rebke *et al.* 2010).

The common tern is a colony breeder, with a monogamous mating system that with high probability is assortative by age and arrival date (Ludwigs and Becker 2005; Ludwig and Becker 2008). They usually establish long-term pair bonds (with no helpers) and demonstrate high mate fidelity, so the occurrence of extra-pair copulations and extra-pair paternity is rare (González-Solís, Sokolov, *et al.* 2001). Yet there is an estimated inter-year divorce rate of 25%, (González-Solís, Becker, and Wendeln 1999; González-Solís, Becker, Jover, *et al.* 1999). Clutches usually contain 2–3 eggs (sometimes 1–4) without sex-specific differences in volume (K. L. Fletcher and Hamer 2004). Replacement clutches after failure are quite common (between 10.6%–22.4% in some years) and do not carry disadvantages for adult's future survival or reproduction (Becker and Zhang 2011). Second clutches (after successful completion of the first) are rare and present only in exceptionally good seasons (Becker and Ludwigs 2004). The semi-precocial chicks hatch asynchronously, and afterwards rely on intense parental care provided by both adults until fledging (fledging age: between 22–29 days). Neither growth rates nor fledging age seem to be sex-dependent (Becker and Wink 2003).

An important characteristic of common terns is the existence of substantial quality differences among individuals. In birds, quality has been described by different parameters including the ability to produce large eggs (Bolton 1991) or clutches (Coulson and Porter 1985), to breed early (Sydeman and Eddy 1995), by the amount of food provided to offspring (Nisbet 1973) or the body condition (Weimerskirch 1992). In the common tern, body mass, for instance, is a measure of condition and is linked to individual quality and reproductive success (Wendeln and Becker 1999; Becker, Wendeln, and González-Solís 2001). Indeed, post-fledging body mass is positively correlated with survival, return and recruitment probability (Ludwigs and Becker 2006; Braasch, Schauroth, *et al.* 2009). In addition, some evidence suggests that body mass may be a highly heritable trait (Ludwigs and Becker 2006). In common terns, individual quality and fitness is further measured by parameters such as age, arrival and laying date, breeding experience, breeding success and lifespan. In general these traits are intercorrelated: older individuals usually arrive and lay large clutches early in the season and show higher fledging success (Ezard *et al.* 2007; Becker, Dittmann, *et al.* 2008). Thus, the clutch size of 3 eggs is commonly used as a combined proxy of indicators for high level of parental quality and care (J. M. Arnold *et al.* 2004; González-Solís, Becker, Wendeln, and Wink 2005).

Regarding environmental factors, common terns are very dependent on the ecological conditions imposed by marine environment, where abundance and availability of food resources is usually unpredictable in time and space (Dänhardt and Becker 2011).

The sex ratio theory applied to common terns

The absence of large SSD in the common tern may imply that there are little reasons to expect SR adjustment or differential costs or mortality between the sexes. However, two indications challenge this idea. First, the evidence of manipulations in other size-monomorphic species with biparental care (Lessells *et al.* 1996; Bradbury and Blakey 1998; Cordero *et al.* 2001; Whittingham, Valkenaar, *et al.* 2002; Andersson *et al.* 2003; Husby *et al.* 2006; Dowling and Mulder 2006; Dijkstra, Riedstra, *et al.* 2010; Zielinska *et al.* 2010). And second, the life-history and behavioral characteristics of the species (from the above description) that comprise potential selective pressures leading to the evolution of sex allocation strategies.

Specifically:

1. Adult common terns show an inter-individual variation in investment of resources, related to parental age, quality and condition. This implies differences in attractiveness to mates important at the moment of pairing, courtship feeding or change of partner.
2. Environmental factors highly influence the reproductive events and the rate of mortality. Moreover, some breeding aspects (e.g. egg mass) decline seasonally.
3. The existence of a certain SSD in adults and fledglings, even if slight, may imply sexual differences in costs or mortality probability.
4. Family (clutch) size is correlated with parental quality. Trade-offs between number, sex and hatching order of the offspring are thus plausible.
5. There exist sex-specific differences in the patterns of philopatry and recruitment, which may affect the amount of local competition among relatives.

Moreover, previous studies on common terns suggest that male and female fitness returns are not equal (Becker and Wink 2003; K. L. Fletcher and Hamer 2004; Ludwigs and Becker 2006; Braasch, Schauroth, *et al.* 2009; González-Solís, Becker, Wendeln, and Wink 2005). These studies suggest that sons are more vulnerable to adverse conditions and/or poor quality parents, and that the offspring is influenced by sex-specific dependent-condition factors: sons in good condition may have particularly good chances of recruiting and pairing compared to sons in poor condition, while the success of daughters might be less dependent on condition.

Together with the pattern of biparental care, the factors exposed above could drive an SR adaptive manipulation in the common tern under the assumptions of Trivers-Willard, attractiveness, seasonal environment and/or LRC hypotheses. As already mentioned, other complexities (clutch sizes larger than one, parental conflict over parental care, trade-offs with future reproduction, etc.) make predictions of adjustment occurrence and its expected directions difficult to establish (Frank 1990). Nonetheless, possible scenarios in the adjustment of family sex ratio in common terns could follow these patterns (adapted from Hasselquist and Kempenaers 2002):

1. **Quality factors: quality differs among individuals**

   High quality males | Low quality males:

   a) Quality inherited by sons:
      - Similar survival of sons and daughters: ♂-biased SR | ♀-biased SR
      - Sons have lower survival: No SR bias | ♀-biased SR

   b) Quality not heritable or heritable to all offspring independent of sex:
      - Same fitness - costs of sons and daughters: No bias
      - Sons have higher fitness - costs: ♂-biased SR | ♀-biased SR

High quality females | Low quality females:

- Same fitness - costs of sons and daughters: No bias
- Sons have higher fitness - costs: ♂-biased SR | ♀-biased SR

2. **Seasonality factors**

   Breeding performance declines with laying date:

   - Similar costs of sons and daughters: No bias
   - Sons are more costly: ♀-biased SR (with increasing laying date)

   Laying date affects age of first reproduction/recruitment probability:

   - Daughters mature faster: ♀-biased SR early in the season
   - Sons mature at an older age: ♂-biased SR early in the season

3. **Environmental factors: local competition differs between sexes**

   Good years | Poor years:

   - Sons are more costly or have lower survival: ♂-biased SR | ♀-biased SR
   - Similar survival and costs of sons and daughters: No bias

4. **Intra-brood factors: chick competitive ability differs between sexes**

   Good rearing conditions | Poor rearing conditions:

   - Sons are more aggressive: No bias | ♀-biased HSR (early positions in clutch)
   - Sons are less competitive: ♀-biased SR (early positions)

Based on these factors it is possible to evaluate a series of specific expectancies (**Chapter 5**). This type of correlative analysis requires an extensive dataset of sexually early-determined chicks, and detailed information about the families: parental condition and quality, laying dates, hatching order, offspring survival until fledging and estimation of environmental conditions. Afterwards it is advisable to test whether the population variance in sex ratios among broods differs from the variance under a binomial distribution. The final step would be to investigate whether these sex ratios are adjusted according to the factors of expected influence (Hasselquist and Kempenaers 2002). The same process is required to investigate the existence and correlation of sex-specific offspring mortality with the factors under study.

However, testing models of SA strategies requires previous knowledge of whether and how the relative fitness of producing sons and daughters is affected by environmental conditions and parental care (Leimar 1996, but see Pen and Weissing 2000). It is therefore important to investigate whether one sex is more costly to produce than the other, for example, because they present sexual differences in growth, metabolic rate, physiological conditions, competitive ability or resistance to diseases/parasites (Burness *et al.* 2000; Saino, Ambrosini, *et al.* 2002; Banbura *et al.* 2008). By exploring, (1) the existence of offspring sexual dimorphism in phenotypic traits apart from size; (2) its relationship with environmental and rearing conditions; and (3) its correlation with parental factors, we could obtain hints of differential parental investment in sons against daughters (Saino, Ambrosini, *et al.* 2002; **Chapter 3**). Moreover, males and females may be similar in size or other phenotypical traits, but one sex may still be more costly to produce. For instance, if sons and daughters develop different strategies during growth that allow them to maximize condition (Becker and Wink 2003) and improve competitive ability. One way of testing this possibility is to explore experimentally the potential sexual differences in the chick's performance of a specific physiological-immunological trade-off with implications in parental provisioning and offspring quality (**Chapter 4**).

General methodology and study site

Fieldwork for the second section of this thesis was carried out in the Banter See colony (Figure 5a) in the harbor area of Wilhelmshaven (Germany). Annually, 90 to 530 pairs of common terns (1992–2008) breed on the 6 identical islands of this site. An integrated population study has been in progress during twenty years to study various aspects of the life history and demography of the species (Becker, Wendeln, and González-Solís 2001). Since 1992, every hatchling has been ringed and marked subcutaneously with a passive transponder before fledging, thus allowing individual identification for lifetime. Besides, all birds marked since 1998 have been molecularly sexed using standard PCR methods, and before 1998 breeders were sexed by behavioural observations. A system of antennas and scales located at resting places automatically records the identity and weight of marked birds. Furthermore, parents are identified by placing an antenna around the nest during incubation (Figure 5b). This approach allows the registration of parameters such as age, arrival date to the colony, body mass, and other individual characters (total fledgling production, number of breeding attempts, etc.). Checks to the colony every second day provide detailed data on every nest: clutch size, laying and hatching dates, laying and hatching order and fate of eggs and chicks (for methods in detail see Becker and Wendeln 1997; Becker, Wendeln, and González-Solís 2001; Becker, Dittmann, *et al.* 2008). Moreover, specific data collection on chicks for this thesis included measurements of morphological traits at different ages, sampling of blood, feathers, parasites, and measures of colour and immunological status (Figure 5c).

Figure 5: a) The Banter See area in Wilhelmshaven, Germany (from http://www.photos-aus-der-luft.de) and the Banter See colony (photo by M. M. Benito); b) Adult common tern (*Sterna hirundo*) over a resting place equipped with an antenna (photo by B. Metzger) and nest surrounded by an antenna (photo by M. M. Benito); c) Methodology: measuring foot web thickness, plasma proteins and foot colour (photos by B. Metzger and R. Nagel).

Objectives

As stated in the Introduction, sex ratio is a complex topic affected by sex determination processes, but also by ecological dynamics and species peculiarities. Its study in birds has provided so far few clear answers. Therefore, the major objective of this thesis was to gain insights into possible factors involved in sex ratio adjustment, using avian species as study models.

The thesis is structured in two sections, and each section addresses specific topics, in general related to sex allocation and sex ratio, but also of broader interest in evolutionary biology.

The **first section** Inter-specific analyses of sex ratio and vulnerability (Chapters 1 and 2) focuses on sexual size dimorphism (SSD) as a key factor in the regulation of offspring sex proportions. The main aim of this section was to assess how the degree of SSD across a wide range of avian species influences sex ratio patterns, and to determine the validity of the size-dependent explanation of vulnerability patterns.
Specific objectives were:

- Provide a complete interspecific review of avian sex ratio literature related to SSD (Chapter 1)
- Assess whether SSD associates to biased sex ratios at different life stages (hatching and fledging) (Chapter 1)
- Assess offspring sensitivity (mortality and physiological negative effects on the body condition) in relation to SSD (Chapters 1 and 2)
- Evaluate the influence of other life-history traits in the relationships between SR and SSD (Chapters 1 and 2)

The **second section** Intra-specific analyses: The case of the common tern (Chapters 3, 4 and 5) focus on a seabird species, the common tern *Sterna hirundo* (Linnaeus, 1758). Its slight sexual size dimorphism gives relevance to other characteristics of the species that could also be involved in shaping sex allocation patterns. The main objective of this section was to investigate, via observational and experimental approaches, the factors that could influence the sex ratio and sex-specific mortality in this species.
Specifically, the objectives of this section were:

- Describe sexual dimorphism in the phenotype and developmental strategies of common tern chicks, which could be related to different reproductive value or cost of each sex (Chapters 3 and 4)

- Obtain hints of a possible differential parental allocation, by exploring whether these sexual differences in phenotype correlate with parental traits (Chapter 3)
- Examine sex ratio and sex-specific mortality at both population and individual level (Chapter 5)
- Assess the influence of environmental factors, breeding time and parental condition and quality on sex ratio patterns at individual level (Chapter 5)

Supervisor’s Report

Hereby, Dr. Jacob González-Solís and Prof. Dr. Peter H. Becker, co-supervisors of the doctoral candidate **María Martínez Benito**, confirm that her PhD thesis entitled **“Avian sex ratio and sex-specific traits in offspring”** presents a series of articles of recognized scientific quality. The dissertation work comprises four articles submitted or published in highly regarded scientific *peer review* journals included in the Science Citation Index, as well as the chapter of a published book.

As supervisors, we have participated in the design, guidance and correction of earlier drafts of the manuscripts written by the candidate. The contribution of the doctoral candidate and the impact factor *(Thomson Institute for Scientific Information)* for each article is detailed below:

Chapter 1. Sex ratio, sex-specific mortality and sexual size dimorphism in birds
M. M. Benito and J. González-Solís (2007)
Journal of Evolutionary Biology 20, 1522–1530. Impact factor (2011): 3.28
DOI: 10.1111/j.1420-9101.2007.01327.x

J. González-Solís and M. M. Benito designed the study; M. M. Benito performed the literature review, collected and analysed data, and drafted the manuscript.

Chapter 2. Sexual size dimorphism and offspring vulnerability in birds
E. Kalmbach and **M. M. Benito** (2007)
In: *Sex, size and gender roles* (eds. D. J. Fairbain, W. U. Blanckenhorn, and T. Székely), Oxford University Press, UK. pp: 133–142. ISBN: 978–0–19–920878–4

E. Kalmbach designed the study, E. Kalmbach and M. M. Benito performed the literature review, collected and analysed the data, and M. M. Benito participated in writing.

Chapter 3. Sex-specific traits in common tern chicks: associations with rearing environment, parental factors and survival
M. M. Benito, J. González-Solís and P. H. Becker
Submitted to the *Journal of Ornithology*. Impact factor (2011): 1.64

J. González-Solís, P. H. Becker and M. M. Benito designed the study; P. H. Becker collected and provided long-term data on traits of adult common terns and on sex and fate of chicks. M. M. Benito collected and analysed chick samples, processed raw long-term data, performed the data analyses and drafted the manuscript.

Chapter 4. Carotenoid supplementation and sex-specific trade-offs between colouration and condition in common tern chicks

M. M. Benito, J. González-Solís and P. H. Becker (2011)

Journal of Comparative Physiology B 181, 539–549. Impact factor (2011): 1.97

doi: 10.1007/s00360-010-0537-z

M. M. Benito, J. González-Solís and P. H. Becker designed the study; M. M. Benito performed the experiment, analysed the samples and data, and drafted the manuscript.

Chapter 5. Sex ratio adjustments in common terns: influence of mate condition and maternal experience

M. M. Benito, H. Schielzeth, J. González-Solís and P. H. Becker (2013)

Journal of Avian Biology 44: 179–188. Impact factor (2011): 2.28

doi: 10.1111/j.1600-048X.2012.00024.x

M. M. Benito, J. González-Solís and P. H. Becker designed the study. P. H. Becker collected and provided long-term data on traits of adult common terns and on chicks, their sex and fate during ontogeny. H. Schielzeth analysed the data and revised the manuscript. M. M. Benito processed raw observational and long-term data, participated in the data analyses, and drafted the manuscript.

We also certify that none of the manuscripts included in this PhD thesis has been used as a part of another PhD thesis.

Barcelona, March 2013

Dr. Jacob González–Solís Bou	Prof. Dr. Peter H. Becker
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Results I.
Inter-specific analyses of sex ratio and vulnerability

1

Sex ratio, sex-specific chick mortality and sexual size dimorphism in birds

Razón de sexos, mortalidad diferencial de pollos y dimorfismo sexual en aves

María M. Benito[1], Jacob González-Solís[1]

La diferencia de tamaño corporal entre pollos macho y hembra puede tener repercusiones importantes en la evolución de la razón de sexos a lo largo de las distintas etapas de la vida. La razón o proporción de sexos se ha estudiado con frecuencia en especies que muestran dimorfismo sexual en tamaño (SSD en sus siglas en inglés), debido a que dichas diferencias podrían suponer para los padres un coste distinto a la hora de criar hijos o hijas. Por esta razón, se considera el dimorfismo en tamaño como un factor con gran potencial para desviar la proporción hacia uno de los sexos de la descendencia. Por ejemplo, el sexo más grande requiere más energía y recursos durante el crecimiento, con el consecuente riesgo de sufrir mayor mortalidad. En última instancia, esta situación podría implicar una sobreproducción del sexo más pequeño por parte de los padres.

Para determinar la relación entre el dimorfismo sexual en tamaño y la proporción de sexos en el momento de la eclosión (razón de sexos primaria) así como cuando los pollos son volantones (razón de sexos secundaria), llevamos a cabo un estudio comparativo basado en 83 especies de aves. El análisis se realizó a nivel de especies independientes y a través de un método de control de las relaciones filogenéticas entre ellas.

En términos globales, encontramos una relación inversa significativa entre el grado de dimorfismo sexual en tamaño de las especies estudiadas y la proporción de machos, eclosionados y volantones, que presentaban sus poblaciones. La mortalidad diferencial entre pollos macho y hembra respecto al grado de dimorfismo también mostró una tendencia inversa, débil pero constante, lo que sugiere que el sexo más grande sufre mayor mortalidad.

Estos resultados sugieren que los ajustes en la proporción de sexos en relación al dimorfismo sexual en tamaño podrían tener lugar principalmente en la fase previa a la eclosión, cuando la manipulación requiere la mínima inversión de energía. No obstante, este sesgo inicial puede ser regulado también durante el crecimiento de los pollos a través de una cierta mortalidad relativa al tamaño. Igualmente, ciertas variables adicionales relacionadas con la estrategia vital (el sistema de apareamiento, pollos altriciales o precociales, la edad de apareamiento o el dimorfismo en plumaje) también parecen influir en estas relaciones, por lo que estas conclusiones deberían ser corroboradas en el futuro.

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Palabras clave
Tamaño corporal; análisis comparativo; razón de sexos primaria; razón de sexos secundaria; asignación por sexo.

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Sex ratio, sex-specific chick mortality and sexual size dimorphism in birds

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Keywords:

body size;
comparative analysis;
fledging sex ratio;
hatching sex ratio;
sex allocation.

Abstract

It has been suggested that sexual size dimorphism (SSD) may influence sex ratios at different life stages. Higher energy requirements during growth associated with larger body size could lead to a greater mortality of the larger sex and ultimately to an overproduction of the smaller sex. To explore the associations between SSD and hatching and fledging sex ratio we performed a species-level analysis and a phylogenetically controlled analysis, based on 83 bird species. Overall, there was a significant inverse relationship between the degree of SSD and the proportion of males at hatching and fledging. Sex-specific mortality related to SSD showed a weak but persistent negative tendency, suggesting a mortality bias towards the larger sex. These results suggest that changes in relation to SSD may take place mainly at the conception stage, but could be adjusted during growth. However, conclusions should be treated cautiously as these relationships weaken when additional variables are considered.

Introduction

Differences in body size between male and female chicks can have important implications for the evolution of sex ratios. Sex ratios have frequently been studied in species showing sexual size dimorphism (SSD) because size differences could result in differential costs of rearing sons and daughters, potentially leading to skewed sex ratios (Stamps, 1990; Krijgsveld *et al.*, 1998). Two decades ago, Clutton-Brock's (1986) original review concluded that there was relatively little evidence of hatching sex ratio variation in birds. However, since Griffiths & Tiwari (1993) identified DNA primers that allowed the reliable diagnosis of bird gender, a plethora of sex ratios studies have been published. Among them, a number of cases reported significant sex ratio variation, not only in relation to SSD but also to several other different factors. Thus, new approaches and case studies call for a re-evaluation of the topic.

The first theory which tried to explain different proportions of males and females in the offspring was formulated by Fisher (1930) and predicts equal sex ratios at the end of parental care if the costs and benefits associated with raising each sex of offspring are equal. In other words, under the assumption that the cost of producing sons and daughters is equal and that future fitness returns are similar for males and females, parents should produce equal numbers of each. In dimorphic species, however, the larger sex is usually assumed to be more costly because of the greater energetic needs or food intake during development (Fiala, 1981; Slagsvold *et al.*, 1986; Teather & Weatherhead, 1988; Stamps, 1990). In this case, according to Fisher's theory, when the costs of rearing males and females differ, sex ratio at independence is expected to be biased towards the less expensive sex, i.e. the smaller sex. Therefore, if parents are able to identify offspring sex and invest according to the rearing costs, equal parental expenditure may result in higher mortality of the larger sex, leading to a biased fledging sex ratio (Emlen, 1997). Alternatively, apart from Fisher's predictions, biased fledging sex ratios may also result through a greater susceptibility to mortality of the larger sex, due to differential sensitivity to adverse

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conditions, starvation, illness, etc. (Roskaft & Slagsvold, 1985; Weatherhead & Teather, 1991).

Investment in sons and daughters can also be achieved through a differential production if parents have some control over the sex composition of the progeny. Despite Mendelian segregation, females have been suggested to overcome constraints of sex chromosome determination and achieve some control of the sex determination at conception (Emlen, 1997; Pike & Petrie, 2003). In such a case, sex-biased investment could take place at an early stage to minimize production costs (Stearns, 1992). We would then expect females to bias primary sex ratio as the cheapest mechanism to balance differential rearing costs between sons and daughters.

Based on a review of the patterns of differential mortality in both birds and mammals, Clutton-Brock *et al.* (1985) and Clutton-Brock (1986) concluded that male-biased mortality during the juvenile stage in species in which males are the larger sex is also common. However, the influence of size dimorphism on hatching and fledging sex ratios remains unclear because it has been reported in some bird species but not in others. Overall, only few studies have found a preponderance of daughters in species where males are larger than females (Howe, 1977; Griffiths, 1992; Torres & Drummond, 1997; Sheldon *et al.*, 1998). Equally, in species where females are larger than males, sex ratios have been found to be male biased in some species but not in others (Newton & Marquiss, 1979; Dijkstra *et al.*, 1990; Wiebe & Bortolotti, 1992; Zijlstra *et al.*, 1992; Appleby *et al.*, 1997; Velando, 2002).

Sex ratios apparently inconsistent with Fisher's theory suggest that multiple other factors could be influencing sex ratio evolution (Cockburn *et al.*, 2002), that can obscure the relationship between SSD and sex ratio. Potentially confounding factors are present both at the interspecific and intraspecific levels. Life-history theory predicts skewed offspring sex ratios in a range of situations in which the costs and benefits of producing sons and daughters differ. These include variability in mating systems (Nishiumi, 1998), dichromatism or other secondary sexual characters (Attractiveness hypothesis: Ellegren *et al.*, 1996; Svensson & Nilsson, 1996), presence of helpers (Local resource enhancement hypothesis or Repayment model: Emlen *et al.*, 1986; Koenig *et al.*, 2001), sexual differences in phylopatry (Local resource competition hypothesis: Clark, 1978) and parental or environmental conditions (Trivers & Willard, 1973). The last hypothesis includes factors, such as the mother's age (Blank & Nolan, 1983) or size (Wiebe & Bortolotti, 1992), the father's or territory quality (Appleby *et al.*, 1997), and the time of year (Lessells *et al.*, 1996).

To overcome the potential peculiarities of each species potentially influencing sex ratios, we need to explore the relationship between sex ratio and SSD across a wide range of bird species. Here, by using data from an extensive survey on avian studies, we provide the first interspecific review since Clutton-Brock's work (Clutton-Brock *et al.*, 1985; Clutton-Brock, 1986) of the relationship between SSD and hatching and fledging sex ratio. We thus investigate whether sexual differences in size could lead to biased sex ratios. For this purpose we tested the correlation between three variables (hatching sex ratio, fledging sex ratio and sex-specific chick mortality 'after birth') and the degree of SSD. Additionally, we explored the influence that other factors may have on these relationships. We analysed all with both a species-level approach (raw species data) and a comparative approach (independent contrasts), having three data sets: whole data set (83 species), studies where birds were molecularly sexed only (44 species), and a subset of seven case studies with hatching and fledging sex ratio data from the same broods.

Methods

Data collection

Data on sex ratios were collated from an extensive literature search of BIOSIS database (September 2004) using the terms 'sex ratio', 'sex allocation' and 'bird'. Sex ratio studies present some difficulties when collating data across many species: limited number of species, small sample sizes, subtle differences in measurement between studies or use of different methods to determine sex (especially for studies before 1993). To avoid these problems as much as possible, and to ensure that we included the correct results, we followed specific criteria. Referring to hatching sex ratio, Fiala (1980) warned that excluding broods that had suffered losses prior to sexing had the potential to bias the sample in favour of the sex with greater survivorship. If broods that had suffered losses (incomplete broods) did not exhibit a sex ratio differing significantly from complete broods, we followed the convention of Fiala (1981) and included sex ratio data of both complete and incomplete broods. From experimental studies overall data were collected if the sex ratio of different groups did not differ significantly; otherwise, only data from control groups was included. When studies presented data of the same population in different environmental conditions, we calculated mean sex ratio values. Finally, we did not include data from studies based on permanent captive populations, experimental conditions that altered the results, fossils or dead birds.

Overall, we collated data from 83 species on sex ratio (50 species on hatching sex ratio, 63 on fledging sex ratio and 30 with both), on adult male and female mass and, when possible, on additional auxiliary variables (see Appendix S1 in Supplementary material). To obtain one unique sex ratio value for each species in each life stage, we calculated the mean value of all sex ratio data available from different years and populations. Final data on sex ratios and references are shown in Appendices S2 and S3. Some additional auxiliary variables that could somehow influence primary and secondary sex ratio were collated (see Appendix 4). These variables were scored following criteria from Bennet & Owens (2002) and Schreiber & Burger (2002) (Appendix 1).

To estimate the degree of SSD we used body mass as the best proxy of size. In some species the body mass of one or both sexes varied greatly throughout the breeding season. In such cases we used body mass at the beginning of the mating period wherever possible. For some species traditionally considered monomorphic, male and female body mass was unavailable. In these cases we used the mean species mass for both sexes, or wherever possible we calculated body mass differences from the percentage of dimorphism. Measuring the magnitude of SSD is a controversial topic in evolutionary ecology, and over time, different ways of measuring it have been proposed and criticized (Ranta *et al.*, 1994). Here, to evaluate the potential dependence of the results on the measure of SSD, we used two indexes: $\log_{10}$(adult male mass/adult female mass) and Storer's index (the relative difference in mass between sexes in percentage):

$$\left(\frac{\text{male mass} - \text{female mass}}{(\text{male mass} + \text{female mass}) \times 0.5}\right) \times 100.$$

Both indexes are widely approved among ecologists because of their simplicity and because they scale the data, getting rid of the effect of size in the measure of SSD. They maintain symmetry around a neutral zero (monomorphy), conforming to the convention of positive values in case males are larger and negative values in case females are larger (Greenwood, 2003).

Comparative methods

Lack of statistical independence among species for the traits of interest was controlled for by using the method of phylogenetically independent contrasts (Harvey & Pagel, 1991; Harvey *et al.*, 1995) as originally suggested by Felsenstein (1985). We used the computer program Comparative analyses by independent contrasts (CAIC) for the analysis (Pagel, 1992; Purvis & Rambaut, 1995a, b). The independent comparisons method requires a phylogeny (Harvey & Pagel, 1991). Here, in common with other studies that have applied this method (Promislow *et al.*, 1992; Owens & Bennett, 1994; Walther & Clayton, 2005), we used a molecular phylogeny based on DNA–DNA hybridization experiments (Sibley & Ahlquist, 1990). We assumed a phylogenetic topology with multiple branching among genera within families and among species within genera, and all branch lengths were assumed equal (Owens & Hartley, 1998). However, to evaluate possible dependence of results on the phylogenetic tree, we also used a second phylogeny, based on the classification of Cracraft, which uses traditional morphological characters (Cracraft, 1981).

Finally, several authors now suggest that, given the restrictiveness of the assumptions that the comparative method makes about the evolutionary model, the species-level analysis might also give valuable information (Price, 1997; Martins, 2000). Thus, we tested relationships on both raw species data and independent contrasts.

Sex ratio values are means ± confidence interval at 95% except otherwise indicated.

Results

Overall sex ratios at different life stages

We obtained overall data from 83 species belonging to 12 orders (Appendix 6). Interspecific sex ratio tended to be male biased (hatching sex ratio $= 0.516 \pm 0.014$, $n = 50$; fledging sex ratio $= 0.509 \pm 0.014$, $n = 63$). Overall, hatching sex ratio differed significantly from parity (one sample *t*-test, $t = 2.157$, $P = 0.036$) whereas fledging sex ratio did not ($t = 1.265$, $P = 0.211$).

Relationships between SSD and sex ratio

At species-level analyses, we found that on average female-biased SSD (Reversed SSD) species produced more males (Appendix 5, Fig. 1a, mean 0.549 ± 0.035) whereas male-biased SSD species produced a mean sex ratio very close to parity (Appendix 5, Fig. 1a, mean 0.503 ± 0.016). The difference in hatching sex ratio between the two types of size dimorphic species was significant (ANOVA, $F_{1,38} = 7.079$, $P = 0.011$). Overall correlation including all the species showed a negative significant association between SSD and hatching sex ratio (Fig. 1a, Table 1a).

Female-biased SSD species presented again a mean fledging sex ratio biased towards males (Appendix 5, Fig. 1b, mean 0.542 ± 0.023), whereas male-biased SSD species maintained a close-to-parity mean sex ratio (Appendix 5, Fig. 1b, mean 0.495 ± 0.018). The difference in fledging sex ratio between the two groups was significant (ANOVA, $F_{1,48} = 8.728$, $P = 0.005$). Furthermore, the overall correlation including all the species again showed that fledging sex ratio was negatively and significantly associated with SSD (Fig. 1b, Table 1a).

We also examined the bivariate correlations between sex ratio and SSD using the evolutionary independent contrasts generated by CAIC (Table 1a). No significant correlations were found between the different sex ratios measures and SSD, regardless of which phylogeny was used.

Relationships using only molecular studies

Given the variability of sexing methods used in the different studies and to avoid possible methodological biases on sex determination, a subsample of 44 species with data collated only from studies using molecular sexing techniques was used to perform the same tests.

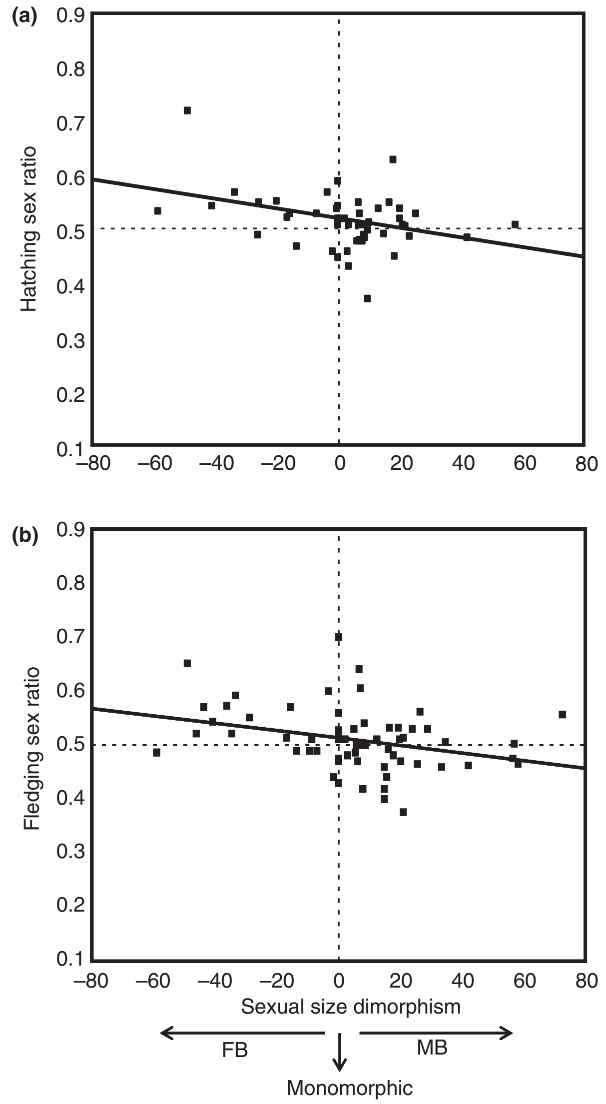

Fig. 1 Relationship between sexual size dimorphism (Storer's index) and (a) hatching sex ratio and (b) fledging sex ratio. Sex ratio expressed as proportion of males. FB, female-biased SSD; MB, male-biased SSD.

Results were not sensitive to the database change and showed the same patterns. For species level data, hatching sex ratio showed a significant negative correlation with the degree of SSD (Table 1b). Similarly, there was a significant negative association between fledging sex ratio and SSD (Table 1b). In contrast with the results obtained using all the studies, phylogenetic analyses restricted to studies using molecular sexing also showed that both hatching and fledging sex ratio were significantly and negatively correlated with SSD (Table 1b). Those results were also consistent regardless of the phylogeny used.

Table 1 Regression models of sexual size dimorphism (Storer's index) on two measures of sex ratio based on species-level data and on independent contrasts (using molecular phylogeny) for (a) all studies, (b) only molecular studies and (c) similar regressions on a measure of differential mortality between sons and daughters from hatching to fledging, for (I) all studies, (II) only molecular studies and (III) data for the same broods.

Dependent variable	Type of analysis	*n*	No. of contrasts	*r*	*P*
(a)					
Hatching sex ratio	Species level	50	–	−0.35	0.012
	Comparative	50	39	−0.26	0.10
Fledging sex ratio	Species level	63	–	−0.30	0.015
	Comparative	63	48	−0.18	0.21
(b)					
Hatching sex ratio	Species level	35	–	−0.47	0.005
	Comparative	35	29	−0.44	0.02
Fledging sex ratio	Species level	26	–	−0.49	0.010
	Comparative	26	24	−0.58	0.002
(c)					
Differential mortality (I)	Species level	30	–	−0.11	0.56
	Comparative	30	26	−0.06	0.78
Differential mortality (II)	Species level	17	–	−0.03	0.89
	Comparative	17	16	−0.35	0.17
Differential mortality (III)	Species level	7	–	−0.92	0.003
	Comparative	7	6	−0.94	0.001

*Sexual size dimorphism is the independent variable in all models. All regressions are forced through the origin. *r*, Pearson correlation coefficient, *P*, probability.

Influence of other factors

We explored the relationship between hatching, fledging sex ratios and mortality and the following life-history traits: plumage dichromatism, mean body mass, coloniality, mating system, presence of helpers, migration behaviour, breeding distribution, nest location, incubation and fledging period, clutch size, hatching sequence, developmental mode, age at first breeding and adult survival rate (for definitions and data see Appendix 1 and 4). Bivariate analyses without phylogenetic control between sex ratios and these potential factors revealed only one significant association: the presence of plumage dichromatism was associated with a higher number of males at fledging (ANOVA, $F_{1,61} = 4.50$, $P = 0.03$) but there was no relationship between plumage dichromatism and hatching sex ratio, nor with any of the different data sets for sex-specific chick mortality. The correlation remained significant when SSD was included in the model (Table 2a).

The use of multiple regression on contrast points produced by CAIC to test for the association between sex ratio and SSD and other life-history variables showed a different pattern. Variables were only included in the multiple regression models if they showed a significant correlation with sex ratios in a previous single regression analysis on contrast points. We used backward

Table 2 Multiple regression models of sex ratio against size dimorphism and life-history factors (listed in Appendix 1) based on species level data for (a) all studies and based on independent contrasts (using molecular phylogeny) for (b) all studies and (c) molecular studies.

Dependent variable	Independent variables	β	P	r^2
(a) Species level (all studies)				
Fledging sex ratio	Sexual size dimorphism	0.092	0.34	0.43
	Plumage dichromatism	0.641	0.00	
Final model: $r = 0.65$, $F_{2,61} = 22.97$, $P = 0.00$				
(b) Comparative (all studies)				
Fledging sex ratio	Sexual size dimorphism	−0.193	0.28	0.26
	Developmental mode	0.392	0.02	
	Age at 1st breeding	0.394	0.03	
Final model: $r = 0.51$, $F_{3,29} = 3.35$, $P = 0.03$				
Differential mortality	Sexual size dimorphism	−0.062	0.72	0.34
	Fledging period	0.573	0.00	
Final model: $r = 0.58$, $F_{2,23} = 5.91$, $P = 0.008$				
(c) Comparative (only molecular studies)				
Fledging sex ratio	Sexual size dimorphism	−0.543	0.00	0.57
	Developmental mode	0.411	0.01	
Final model: $r = 0.75$, $F_{2,20} = 13.25$, $P = 0.000$				
Differential mortality	Sexual size dimorphism	0.413	0.04	0.51
	Mating system	−0.621	0.00	
Final model: $r = 0.71$, $F_{2,14} = 7.17$, $P = 0.007$				

*All regressions made on contrast points are forced through the origin.

elimination to exclude all nonsignificant variables except SSD. Once the effects of the relevant life-history traits were controlled for, we did not find a significant correlation between hatching sex ratio and size dimorphism. Similarly, we found that SSD was not significantly correlated with fledging sex ratio, although two traits had a significant positive effect on the relationship: developmental mode (parental care type, ranging from low amount of care ‘precocial chicks’ to high ‘altricial chicks’) and age at first breeding (Table 2b). Models for the molecular database again showed no significant relationship between SSD and hatching sex ratio, but fledging sex ratio showed a significant negative correlation with SSD and at the same time a positive one with the developmental mode variable (Table 2c).

Sex-specific chick mortality

The relationship between sex-specific chick mortality, calculated as the difference in sex ratio between hatching and fledging for those species in which we had both values, with the degree of SSD showed no correlation for the whole data set at the species level (Fig. 2a, Table 1c) or the subsample of studies with molecular sexing (Fig. 2b, Table 1c). However, as sex ratio values for each species were in most cases calculated as the average of several populations, hatching and fledging sex ratios often came from different populations. To control for this variability among populations, we also performed the analyses on a small subset of seven species for which hatching and fledging sex ratio came from the same broods. This analysis showed a significant negative correlation between sex-specific chick mortality and SSD (Fig. 2c, Table 1c).

Using phylogenetically independent contrasts, there was a clear significant correlation between sex-specific chick mortality and sex ratio data coming from the same broods (Table 1c), even though sample size was drastically reduced. Conversely, analyses for all species (whole database) and for the molecular studies only showed no significant association, although a negative tendency remained. Both molecular and morphological models of phylogeny showed similar results. However, multiple regression models on contrast points showed that, when the effect of size dimorphism was controlled for, differential mortality was positively correlated with fledging period in the model based on all studies (Table 2a) and negatively correlated with mating system, ranging from monogamy to polygamy, for the molecular database (Table 2b).

To explore the sensitivity of results to the index of SSD, we also performed all the analyses with the logarithmic transformation of body masses (log male–log female). This index led to very similar results.

Discussion

The present study partially supports the hypothesis that SSD, which is probably associated with differential rearing costs between sons and daughters, influences sex ratios at fledging and hatching time. Despite the overall effect being rather weak, the resulting trends were in the predicted direction and qualitatively consistent. Thus, all analyses showed a general tendency for

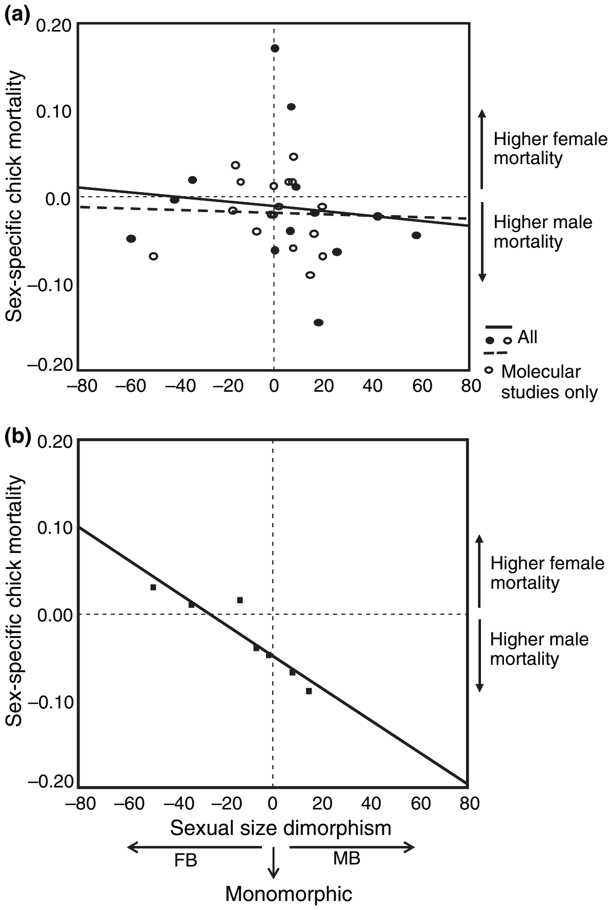

Fig. 2 Relationship between sexual size dimorphism (Storer's index) and sex-specific mortality, calculated as the sex ratio difference (fledging minus hatching sex ratio) for (a) all studies and only studies where sex was determined with molecular techniques, and (b) sample of seven species where hatching and fledging sex ratios were measured in the same broods. FB, female-biased SSD; MB, male-biased SSD.

dimorphic species to skew hatching sex ratio towards the smaller sex. This result was clearer when controlling for phylogeny, especially when using a subsample of studies that used molecular techniques to sex the chicks. This method is the most reliable sexing technique and allows gender to be determined very early during development (Ellegren & Sheldon, 1997). Overall, mean hatching sex ratio presented a shift from parity that may indicate a tendency to overproduce males. However, the effect of SSD on sex ratio seems to be stronger in species with female-biased SSD, as suggested by previous case studies, e.g. in *Falco tinnunculus* (Dijkstra *et al.*, 1990) and *Sula nebouxii* (Velando, 2002).

Skewed fledging sex ratios can result from differential survival of sons and daughters between hatching and independence time. According to Fisher's (1930) theory, sex-specific chick mortality is directly related to the different reproductive costs of males and females, whereas an alternative hypothesis suggests that it could be a function of the lower viability of the larger sex, especially under restricted food availability (Roskaft & Slagsvold, 1985; Weatherhead & Teather, 1991). Although the causes differ between these two hypotheses, both lead to the same prediction of a biased fledging sex ratio towards the smaller sex, and suggest the same mechanism to achieve it, i.e. by greater mortality of the larger sex.

In our study we found that SSD is negatively associated with the proportion of the larger sex at fledging, but overall evidence for greater mortality of the larger sex remains unclear. Hatching and fledging sex ratios were not equal, and this difference should theoretically result from sex-specific mortality over the chick rearing period. However, our results at species level analyses with the whole database showed that sex-specific chick mortality is not related to SSD. Still, these results should be treated with caution because in most cases hatching and fledging sex ratio do not come from the same population. To detect differential mortality between sexes, it is more accurate to measure the sex ratio of the same broods (Komdeur & Pen, 2002). Following this criterion, data from an admittedly small sample of seven species showed a negative and significant relationship between sex-specific chick mortality and SSD. In other words, the larger sex suffers a greater mortality, i.e. more females die in species showing female-biased SSD and more males die in SSD species. This result remained when controlling for phylogeny.

These last results agree with the general belief that differences in mortality between males and females are related to the degree of SSD, with species of larger males suffering a greater male mortality (Teather & Weatherhead, 1989; Griffiths, 1992; Torres & Drummond, 1997; Dijkstra *et al.*, 1998; Krijgsveld *et al.*, 1998). Although not so many studies are apparently available, data on female-biased SSD species also suggest higher mortality of females, the larger sex, at least under food stress (Bortolotti, 1986). Furthermore, the disagreement between our results for all species and the molecular subset and those for the few case studies is only quantitative (and not qualitative) and may result for different reasons. On the one hand, some studies have suggested that a larger size confers an advantage in competitive interactions for food (Anderson *et al.*, 1993; Hipkiss *et al.*, 2002), and even some evidence of higher mortality of the smaller sex has been found (Newton, 1979; Drummond *et al.*, 1991). On the other hand, different survival is focused on the assumption that one of the sexes is more costly than the other because of its larger size. However, it is also possible that sex differences in mortality are the result of fundamental differences between males and females caused by sex-specific production of hormones during early development, which could contribute to the observed mortality patterns (see below). Moreover, possible publication bias towards significant results cannot be excluded as an explanation for the relative abundance of studies finding sex-specific mortality in relation to size dimorphism.

Fledging sex ratio biases, however, could simply result from an initial hatching bias instead of differential mortality between males and females. In fact, sex-specific mortality seems a rather costly mechanism to adjust sex ratios, and therefore control of the sex ratio by the mother should occur as early as possible to minimize wastage of energy, i.e. best at conception. Previous studies found no clear evidence of hatching sex ratio deviations from unity, as expected for heterogametic chromosomal sex determination (Clutton-Brock, 1986). However, new evidence from the last two decades obliges us to reconsider the assumption of primary sex ratios being fixed at parity. Indeed, we found an inverse relationship between hatching sex ratio and SSD, particularly among female-biased SSD species, supporting that hatching biases may be a way to adjust proportion between sexes.

As mentioned above, evidence from some case studies suggests that size dimorphism affects sex ratio at different life stages. However, in these studies sex *per se*, and not necessarily sex differences in size, may be influencing sex ratios, which would also lead to sex-specific chick mortality. For instance, the hypothesis of the male phenotype disadvantage suggests that other characteristics of the male phenotype, such as the embryonic hormone profile (Zuk, 1990), contribute to a higher male mortality. Sex differences in endocrine state during embryogenesis may involve sexual differences in the immune system, potentially causing sex differences in survival probabilities. To disentangle the two hypotheses we need a multispecies approach. Indeed, in our study differences between hatching and fledging sex ratios, although not significant, suggest a slightly greater mortality of male chicks overall. In fact, there is a significant male prevalence at hatching time, especially remarkable in female-biased SSD species, which becomes not significant by the time the chicks fledge (Appendix 5, Fig. 1). When the population sex ratio deviates from parity, an investment unit in the rarer sex has greater fitness returns and this would cause an overproduction of this sex until the sex ratio is equalized (Fisher, 1930). Thus, larger male mortality due to the hormonal disadvantage may lead to an adaptive compensatory overproduction of males to equalize the sex ratio at fledging time.

Apart from SSD and sex *per se*, we can expect some other factors to influence sex ratios at different life stages. Facultative adjustment of offspring at the family level, as first suggested by Trivers & Willard (1973), is based on the assumption that parents can regulate the sex of their offspring in response to environmental conditions. From their original hypothesis a number of extensions have been formulated, accounting for prominent inter- and intraspecific differences between species. We have also analysed the relationships between SSD and sex ratios with several of these potential confounding variables controlled for (see description of variables in Appendix 1). None of these variables were found to strongly affect the relationship between hatching sex ratio and SSD. In contrast, fledging sex ratio increased with the age at first breeding and also with the developmental mode, indicating a higher male proportion in altricial species. Male mortality appears to be lower in species with longer fledging periods and in monogamous compared with polygamous species. Different developmental states (altricial or precocial), developmental rates of chicks (length of fledging period) and mating systems all imply different descriptions of the pattern of parental care. Because offspring of precocial species feed themselves, differential energy requirements of sons and daughters are less demanding on parents and in principle there should be no reason to predict sex ratio biases according to SSD. Regarding the mating system, adjustment of the sex ratio related to the polygyny degree or to the female mating status has been found for several species (Patterson & Emlen, 1980; Ellegren *et al.*, 1996; Nishiumi, 1998). Further, a longer fledging period may increase sex-specific mortality, because females may manipulate sex at conception but males can manipulate offspring sex ratios only by varying their parental care during the chick rearing period. This would be reflected in differential mortality between the sexes during growth, especially for those species with SSD. No other association was found between sex ratio, sexual dimorphism and any other factor. In any case, all these factors make up a complex matrix with possible intercorrelations between them that are not completely understood yet. Clearly, more interspecific and comparative analyses with larger sample sizes are needed, best restricted to studies where sex is molecularly determined and sex ratio at different life stages is monitored in the same broods.

In conclusion, we found consistent, albeit weak negative relationships between SSD and sex ratios at hatching and fledging, plus some evidence of sex-specific chick mortality related to SSD as the mechanism to bias sex ratio between both life stages. Our results suggest that sex ratio adjustments in relation to SSD, or to sex *per se*, may take place mainly at the stage prior to hatching, when manipulation requires the minimum energy investment, although this initial bias could be further adjusted during growth. Nevertheless, other life-history variables seem to influence these relationships and thus this result should be corroborated in the future.

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Supplementary material

The following supplementary material is available for this article:

Appendix S1 Characteristics of the data base.
Appendix S2 Sex ratio data base.
Appendix S3 References for sex ratio data base.
Appendix S4 Life-history traits data base.
Appendix S5 Interspecific mean values of sex ratio for different types of size dimorphic species.
Appendix S6 Phylogenetic distribution and SSD.

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Supplementary material

The following supplementary material is available for this article:

APPENDIX S1 Characteristics of the database
APPENDIX S2 Sex ratio database
APPENDIX S3 References for the sex ratio database
APPENDIX S4 Life-history traits database & Key to Appendix
APPENDIX S5 Figure 1. Interspecific mean values of sex ratio for different types of size dimorphic species
APPENDIX S6 Phylogenetic distribution and SSD

Appendix S1 Characteristics of the database

Life-history trait	**Nr. of species**	**Categories / Units** (see also Key to app. S4)
Sex ratio measures		
Hatching sex ratio	50	♂ / (♀ + ♂)
Fledging sex ratio	63	♂ / (♀ + ♂)
Both	30	♂ / (♀ + ♂)
Sexual dimorphism		
Male - Female body mass	83	grams
Plumage dimorphism	83	0: absence 1: presence
Interspecific variables		
Mean body mass	83	grams
Coloniality	75	1: solitary 2: colonial
Breeding distribution	76	1: polar 2: subpolar 3: temperate 4: subtropical 5: tropical 6-14: combinations
Nest location	73	1: hole 2: ground 3: arboreal
Mating system	77	1: monogamy 2: occasional facultative polygamy 3: lek 4: frequent facultative polygamy 5: polygamy
Helpers	79	0: absence 1: presence
Migration pattern	72	0: resident 1-4: gradient of migration
Incubation period	73	days
Fledging period	74	days
Clutch size	74	eggs per breeding attempt
Hatching sequence	69	1: synchronous hatching 2: asynchronous hatching 3: only one egg
Developmental mode	75	1: precocial 2: semi-precocial 3: semi-altricial 4: altricial
Age at first breeding	50	years
Adult survival rate	34	annual rate among individuals above the modal age at first breeding (%)

Appendix S2 Sex ratio database Hatching and fledging sex ratios calculated as the mean value of available data from different years or populations of each species.

Species		Sex ratio		References
		Hatching	Fledging	
*Acanthiza pusilla**	Brown thornbill	0.54		43
Accipiter cooperii	Cooper's hawk	0.54	0.54	29, 87, 88
Accipiter gentilis	European goshawk		0.52	20, 29
*Accipiter nisus**	European sparrowhawk	0.54	0.49	29, 76, 86, 100
*Acrocephalus arundinaceus**	Great reed warbler	0.52	0.51	77, 107
*Actitis hypoleucos**	Common sandpiper	0.46		3
*Actitis macularia**	Spotted sandpiper	0.57		3
*Aegolius funereus**	Tengmalm's owl	0.72	0.65	50, 53[1]
Agelaius phoeniceus	Red-winged blackbird	0.49	0.46	23, 29, 34, 51, 105, 114
Alectoris rufa	Red-legged partridge		0.49	74
Anas penelope	Eurasian wigeon		0.53	36
Anser caerulescens	Lesser snow geese	0.49	0.50	4, 23, 24, 25
Archilochus colubris	Ruby-throated hummingbird		0.49	73
*Calonectris diomedea**	Cory's shearwater	0.55	0.53	15, 38
Circus aeruginosus	Marsh harrier		0.55	29, 118
Circus pygargus	Montagu's harrier		0.52	7, 67
Corvus corone	Hooded crow	0.51	0.47	95, 105
Corvus frugilegus	Common rook		0.44	105
*Corvus monedula**	Jackdaw	0.48	0.42	6[1]
*Cuculus canorus**	Common cuckoo	0.52		56
*Dacelo novaeguineae**	Laughing kookaburra	0.47	0.49	66[1]
Diomedea exulans	Wandering albatross		0.53	106
Eclectus roratus	Eclectus parrot		0.47	48
*Emberiza citrinella**	Yellowhammer	0.54		79
Falco peregrinus	Peregrine falcon		0.57	19
Falco sparverius	American kestrel		0.51	49, 96
*Falco tinnunculus**	European kestrel	0.52	0.51	11, 28, 29, 60, 64
Forpus passerinus	Green-rumped parrotlet		0.51	17
Geopelia striata	Barred dove		0.43	92
*Haematopus ostralegus**	Eurasian oystercatcher	0.53	0.49	47[1]
Haliaetus leucocephalus	Bald eagle	0.55		12
*Junco hyemalis**	Dark-eyed junco	0.49	0.54	45, 97
Larus argentatus	Herring gull	0.51		18, 89
*Larus audouinii**	Audouin's gull	0.54	0.47	37
*Larus michaellis**	Yellow-legged gull	0.51		2
Larus delawarensis	Ring-billed gull	0.45		71
*Larus fuscus**	Lesser black-backed gull	0.49	0.40	14, 44[1], 75
Larus occidentalis	Western gull	0.53	0.47	54, 90
*Larus ridibundus**	Black-headed gull	0.50		Ležalová, R. 2005 (pers.comm)
*Luscinia svecica**	Bluethroat	0.51		82
Macronectes giganteus	Southern giant petrel		0.51	40
Macronectes halli	Northern giant petrel		0.51	40
*Manorina melanophrys**	Bell miner		0.61	21
*Manorina melanotis**	Black-eared miner	0.37		32
*Melanerpes formicivorus**	Acorn woodpecker	0.48	0.50	59
*Miliaria calandra**	Corn bunting	0.52	0.51	46
*Molothrus ater**	Brown-headed cowbird	0.49		56, 115, 116

Species		**Hatching**	**Fledging**	**References**
*Myiarchus cinerascens**	Ash-throated flycatcher	0.52		33
*Otus scops**	Scops owl	0.53	0.57	10
Parabuteo unicinctus	Harris's hawk		0.57	9
*Parus caeruleus**	Blue tit		0.48	65
*Parus major**	Great tit	0.48		63, 68, 78, 83, 103
*Passer domesticus**	House sparrow	0.51	0.53	26, 108
Passerculus sandwichensis	Savannah sparrow		0.49	109, 110
*Petrochelidon ariel**	Fairy martin		0.52	70
*Phalacrocorax aristotelis**	Shag		0.53	42
Phalacrocorax carbo	Cormorant		0.46	101
Phoeniculus purpureus	Green woodhoopoe		0.46	69
Pica pica	Magpie	0.63	0.48	95
Picoides borealis	Red-cockaded woodpecker	0.59	0.53	41, 58
*Platycercus elegans**	Crimson rosella		0.42	62
*Poecile atricapilla**	Black-capped chickadee		0.53	84
Quiscalus major	Boat-tailed grackle		0.50	8, 94, 105
Quiscalus mexicanus	Great-tailed grackle		0.48	105
Quiscalus quiscula	Common grackle		0.38	105
Sialia mexicana	Western bluebird	0.53	0.64	33, 61
Sitta europaea	Nuthatch	0.51		63, 113
*Sterna dougallii**	Roseate tern	0.45	0.43	98
*Sterna hirundo**	Common tern	0.46	0.44	35, 39[1]
*Strix aluco**	Tawny owls	0.49		5
*Strix uralensis**	Ural owl	0.55		16
*Sturnus vulgaris**	European starling	0.43		13
*Sula nebouxii**	Blue-footed booby	0.57	0.59	99[1], 102
*Tachycineta bicolor**	Tree swallow	0.54	0.56	93, 111
*Taeniopygia guttata**	Zebra finch	0.52	0.70	22, 104, 117
Tetrao tetrix	Black grouse		0.56	52
Tetrao urogallus	Capercaillie		0.56	52, 55
*Troglodytes aedon**	House wren		0.48	1, 112
*Uria aalge**	Guillemot		0.60	80
Xanthocephalus xanthocephalus	Yellow-headed blackbird	0.51	0.47	81, 85, 105
Zenaida macroura	Mourning dove	0.55		30, 91
Zonotrichia leucophrys	White-crowned sparrow		0.51	31, 57, 72

* Species belonging to the molecular sexing sub-sample.

[1] Studies from which special data for differential mortality in the same broods was collated.

References correspond to the different studies used for each species and listed in Appendix S3

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Appendix S4 Life-history traits database

Species	Body mass			Pl	Col	Br	Nst	Mat	Help	Mig	Inc	Fled	Clutc	Hat	Dev	1br	Surv	References
	Male	Female	Mean wt															
*Acanthiza pusilla**	7.3	6.4	6.2	0	1	3		1	0		19.5	17	3.0	1	3		63	ABM, 43, Green 2004
Accipiter cooperii	349.0	529.0	439.0	0														ABM
Accipiter gentilis	712.0	1140.0	926.0	0	1	8	3	1	0	1	36.5	40	3.6	2	4	3	59	CS
*Accipiter nisus**	144.0	264.0	204.0	1	1	8	3	1	0	3	40.5	27	4.9	2	4		60	CS
*Acrocephalus arundinaceus**	33.6	32.9	33.3	0	2	3	2	4	0	4	14.0	13	4.8	2	3	1		CS, 107
*Actitis hypoleucos**	52.5	51.0	51.8	0	1	3	2	1	0	3	21.5	27	4.0	1	1	2	75	CS
*Actitis macularia**	36.9	38.2	37.7	0	1	8	2	5	0	4	21.0	20	4.0	1	1	1		CS, 3
*Aegolius funereus**	101.0	167.0	134.0	0	1	8	3	2	0	0	28.5	32	6.0	2	3	1		CS
Agelaius phoeniceus	63.6	41.5	518.0	1		9												ABM
Alectoris rufa	516.0	439.0	477.5	0	1	3	2	1	0	0	24.0	10	12.7	1	1	1		CS
Anas penelope	735.0	550.0	642.5	1	1	8	2	1	0	3	24.0	43	9.0	1	1	1	53	CS
Anser caerulescens	2744.0	2517.0	2630.0	0	1	2	2	1	0	4	24.0	44	6.0	1	1			CS, NorthernState Univ. Web
Archilochus colubris	3.0	3.3	3.2	1	1	3	3	5	0	3	16.0	18	2.0	1	3	1	37	ABM, H, ADW
*Calonectris diomedea**	708.7	600.8	654.0	0	2	9	1	1	0	4	53.8	91	1.0	3	4	8	93	CS, BMB
Circus aeruginosus	500.0	669.0	584.5	1	1	3	2	1	0	4	34.5	38	4.7	2	4		74	CS
Circus pygargus	261.0	370.0	315.5	1	1	3	2	2	0	4	28.0	38	4.5	2	4	2		CS
Corvus corone	522.0	490.0	506.0	0	1	8	3	1	0	0	18.5	32	4.1	2	3	2	53	CS
Corvus frugilegus	489.0	418.0	453.5	0	2	8	3	1	0	0	17.0	33	4.5	2	3	2	54	CS
*Corvus monedula**	237.0	219.0	228.0	0	2	8	1	1	0	0	17.5	34	4.4	2	3	2	65	CS
*Cuculus canorus**	117.0	106.0	111.5	0	1	3		5	0	4	11.6	19	9.2	2	3	2		CS
*Dacelo novaeguineae**	307.0	352.0	329.5	0	1	9	3	1	1	0	26.5	36	2.5	2	3	1		H
Diomedea exulans	9768.0	7686.0	8727.0	1	2	8	2	1	0	4	79.0	278	1.0	3	2	9	96	CS, BMB
Eclectus roratus	428.0	428.0	428.0	1	2	5	3	2	1	0	26.0	84	2.0	2	3	3		ABM, H, Heinsohn 1997
*Emberiza citrinella**	26.7	26.8	26.8	1	1	8	3	1	0	1	13.0	12	4.0	1	3	1	54	CS
Falco peregrinus	611.0	952.0	781.5	1	1	8	2	1	0	3	30.5	39	3.4	1	4	2	75	ABM, CS
Falco sparverius	109.0	119.0	114.0	1	1	3	3	1	0	3	29.5	30	4.0	2	4	1		CS, BMB
*Falco tinnunculus**	213.0	252.0	232.5	1	1	8	3	1	0	4	28.0	30	4.7	2	4	1	58	CS
Forpus passerinus	29.5	29.5	29.5	1		5	1	1	0		19.0		7.0	2	3			17

Species	Body mass			Pl	Col	Br	Nst	Mat	Help	Mig	Inc	Fled	Clutc	Hat	Dev	1br	Surv	References
	Male	Female	Mean wt															
Geopelia striata	56.0	56.0	56.0	0		5	3		0	0	13.0	12	2.0	1	3			ABM, H
*Haematopus ostralegus**	500.0	536.0	544.0	0	1	8	2	1	0	2	25.5	30	3.0	1	1	4	94	CS, BA
Haliaetus leucocephalus	4130.0	5350.0	4740.0	0	1	8	2	1	0	0	35.0	80	2.0	2	3	5	93	ABM, H
*Junco hyemalis**	20.4	18.8	19.6	1	1	8	1	1	0	3	12.5	11	4.0	1	4	1		ABM, CS, ADW
Larus argentatus	1177.0	944.0	1060.5	0	2	8	2	1	0	1	30.0	48	3.0	2	1	5	91	CS, BMB
*Larus audouinii**	847.0	693.0	770.0	0	2	10	2	1	0	1	28.5	38	3.0	2	2			ABM, CS, BMB, 37
*Larus michaellis**	1275.0	1033.0	1154.0	0	2	3	2	1	0	3	27.5	46	2.7	2	2			ABM, BMB
Larus delawarensis	566.0	471.0	705.0	0	2	3	2	1	0	3	25.5	38	3.0	2	2	3		ABM, H, BMB
*Larus fuscus**	768.0	662.0	715.0	1	1	8	2	2	0	4	25.5	35	3.0	2	1	4		CS, BMB
Larus occidentalis	1136.0	879.0	1011.0	0	2	3	2	1	0	3	25.5	42	3.0	2	1	6		ABM, H, Pierotti 2005
*Larus ridibundus**	294.0	267.0	280.5	0	2	8	2	1	0	2	23.9	35	2.6	2	2	2	76	CS, BMB
*Luscinia svecica**	20.3	18.8	19.6	1	1	8	3	1	0	4	13.5	14	5.0	2	3	1		CS
Macronectes giganteus	5100.0	3600.0	4350.0	0	2	7	2	1	0	3	62.5	118	1.0	3	2			BMB, 40, González-Solís 2000
Macronectes halli	4700.0	3800.0	4250.0	0	2	2	1	1	0	3	59.5	113	1.0	3	2			BMB, 40, Waugh 1999
*Manorina melanophrys**	32.5	30.3	31.4	1	2	3		1	1				1.9					21
*Manorina melanotis**	52.5	47.6	50.1	0	2			1	1									32
*Melanerpes formicivorus**	82.9	78.1	80.5	1	1	10	3	4	1	0	11.5	31	5.1	2	3	2		ABM, H
*Miliaria calandra**	53.6	43.9	48.8	0	1	3	3	4	1	1	13.0	11	4.0	1	3	1	58	CS
*Molothrus ater**	48.9	38.8	43.9	1		9	3		0	3								CS, H2
*Myiarchus cinerascens**	27.2	27.2	27.2	0	1		1	1	0			16	4.0	1	3			ABM, 33
*Otus scops**	79.0	92.5	92.0	0	1	3	1	1	0	1	24.5	25	4.5	2	3			ABM, CS
Parabuteo unicinctus	725.0	1047.0	886.0	0	2	10	3	2	1	0	34.5	40	3.0	2	4			ABM, BMB, ADW
*Parus ater**	9.6	8.9	9.3	0	1	3	3	1	0	0	15.0	19	9.0	2	3	1	33	CS
*Parus caeruleus**	11.0	10.7	10.9	1	1	8	3	1	0	0	14.2	19	11.0	2	3	1	41	CS
*Parus major**	19.1	17.8	18.5	1	1	8	3	1	0	0	13.9	19		2	3	1	52	CS
*Passer domesticus**	30.2	30.2	30.2	1	2	8	1	1	0	0	12.0	14	4.5	2	3	1	55	CS
Passerculus sandwichensis	20.6	19.5	20.1	0														ABM
*Petrochelidon ariel**	11.0	11.0	11.0	0				1	0									70

Species	Body mass			Pl	Col	Br	Nst	Mat	Help	Mig	Inc	Fled	Clutc	Hat	Dev	1br	Surv	References
	Male	Female	Mean wt															
*Phalacrocorax aristotelis**	1940.0	1598.0	1769.0	0	2	8	2	1	0	1	30.5	53	3.0	2	3	4	84	ABM, CS, BMB
Phalacrocorax carbo	2423.0	2085.0	2254.0	0	2	9	2	1	0	1	30.0	53	3.0	2	3	3	88	CS, BMB
Phoeniculus purpureus	86.6	61.9	314.5	0	2	10	3	2	1	0	17.5	29	3.5	2	3			ABM, H
Pica pica	221.6	185.4	203.5	0	1	3	3	1	0	0	21.5	27	6.0	2	4	2	45	CS
Picoides borealis	43.6	43.6	43.6	1	2	3	3	1	1	0	10.5	28	3.5	1	3			ABM, 41
*Platycercus elegans**	124.7	107.3	116.0	0	1	9	3	1	0	0	19.0	35	5.1	2	3			ABM, H, 62
*Poecile atricapilla**	11.3	10.7	11.0	0	1	3	3	1	0	0	12.5	16	6.3	2	3		65	BISO, 84
Quiscalus major	214.0	119.0	166.5	0														ABM
Quiscalus mexicanus	191.0	107.0	149.0	0	2	12	3	5	0	0	14.0	22	3.0	1	3			ABM, ADW
Quiscalus quiscula	119.9	97.1	108.5	0	1	8	3	2	0	4	13.0	14	5.0	2	3			CS, ADW
Sialia mexicana	29.0	27.1	28.1	1	1	3	1	1	1	3	16.0	21	5.0	1	3	1		ABM, ADW, 33
Sitta europaea	24.3	23.5	23.9	1	1	3	1	1	0	0	14.8	24	7.0	1	3	1	43	CS
*Sterna dougallii**	110.0	110.0	110.0	0	2	12	2	1	0	3	23.3	26	2.0	2	1	3		CS, BMB, 98
*Sterna hirundo**	124.0	126.0	133.0	0	2	8	2	1	0	4	24.0	26	2.4	2	2	3		CS, BMB
*Strix aluco**	409.0	533.0	471.0	0	1	3	3	1	0	0	29.0	35	3.0	2	3	2	68	CS
*Strix uralensis**	706.0	863.0	784.5	0	1	8	3	1	0	0	28.0	40	3.0	2	3	3	90	ABM, CS
*Sturnus vulgaris**	82.7	79.9	81.3	1	2	8	3	4	0	4	12.2	19	5.0	2	4		47	CS
*Sula nebouxii**	1283.0	1801.0	1542.0	0	2	5	2	1	0	0	41.0	102	2.0	2	3			ABM,CS, BMB
*Tachycineta bicolor**	20.1	20.1	20.1	1	1	3	1	2	0	3	14.5	20	5.0	2	3	1		ABM, CS, ADW, 111
*Taeniopygia guttata**	12.0	12.0	12.0	1	1			1	0			90			3			ABM, 117
Tetrao tetrix	1290.0	988.0	1139.0	1	1	8	3	3	0	1	26.0	12	7.9	1	1			CS
Tetrao urogallus	4240.0	1985.0	3112.5	1	1	8	2	3	0	0	25.0	18	8.4	1	1	3	50	CS
*Troglodytes aedon**	10.9	10.9	10.9	0	1	12	1	4	0	3	12.0	14	6.0	1	3	1		ABM, ADW, 112
*Uria aalge**	649.0	670.0	660.0	0	2	3	2	1	0	1	33.0	22	1.0	3	4	5	90	CS, BMB
Xanthocephalus xanthocephalus	92.8	51.1	72.0	1	1	3	2	5	0	3	12.0	11	4.0	1	3			H2, ADW
Zenaida macroura	123.0	115.0	119.0	1	1	3	2	1	0	1	15.0	13	2.0	1	3	0		ABM, CS, H
Zonotrichia leucophrys	30.7	27.1	30.0	0	1	8	1	1	0	4	12.3	10	3.0	1	3			CS, H2, ADW

* Species belonging to the molecular sexing sub-sample.

Key to Appendix S4

Body mass:

Mean of adult male and female mass (g).

Mean wt:

Mean adult body mass for the species (g).

Plumage dimorphism **(Pl)**:

0 monochromatism
1 dichromatism

Breeding character (**Col**):

1 territorial
2 colonial

Breeding distribution (**Br**):

1 polar
2 subpolar
3 template
4 subtropical
5 tropical
6 polar and subpolar
7 polar and temperate
8 subpolar and temperate
9 temperate and tropical
10 subtropical and tropical
11 subpolar and subtropical
12 temperate and tropical
13 temperate, subtropical, tropical
14 polar, subpolar and temperate

Nest location (**Nst**):

1 hole (tree holes, cavities, burrows)
2 ground (open nests on ground including raised nests in reeds, lakes)
3 arboreal (open nests in shrubs, trees)

Mating system (**Mat**):

1 monogamy
2 occasional facultative polygamy
3 lek system
4 frequent facultative polygamy
5 polygamy

Helpers (**Help**):

0 absence
1 presence

Migration pattern (**Mig**):

0 resident
1 intracontinental partial migrator
2 transcontinental partial migrator
3 intracontinental complete migrator
4 transcontinental complete migrator

Incubation period (**Inc**):

Mean of incubation period in days (time between laying and hatching).

Fledging period (**Fled**):

Mean of fledging period in days (time between hatching and first flight).

Clutch size (**Clutc**):

Mean of typical number of eggs produced in a single breeding attempt.

Hatching sequence (**Hat**):

1 synchronous hatching
2 asynchronous hatching
3 laying only one egg

Developmental mode (**Dev**): State of development at hatching.

1 precocial
2 semi-precocial
3 semi-altricial
4 altricial

Age at first breeding (**1br**):

Mean of modal age at first breeding (years).

Adult survival rate (**Surv**):

Mean of annual survival rate among individuals above the modal age at first breeding (%).

Key to Appendix S4 (cont.) – References

H2: Cramp, S. and K. Simmons (eds) (1977, 1980, 1983). *Handbook of the birds of Europe, the Middle East and North Africa*. Oxford University Press, UK.

BA: Brown, L.H., E.K. Urban and K. Newman (1982). *Birds of Africa*. Academic Press Limited INC, London.

H: Del Toro, J., A. Elliot and J. Sargatal (1992). *Handbook of the birds of the world*. Lynx cop., Barcelona, Spain.

ABM: Dunning, J.B. (1993). *CRC Handbook of Avian Body masses*. CRC Press, Inc., USA.

CS: Cramp, S. and K. Simmons (1998). *The complete birds of the western Paleartic*, on CD-ROM. Oxford University Press, UK.

BMB: Schreiber, E.A. and J. Burger (2002). Appendix 2 and Chapter 9. In *Biology of Marine Birds*. CRC Press LLC., USA.

ADW: Animal Diversity Web of the University of Michigan, USA. `http://animaldiversity.ummz.umich.edu/`

BISO: Biota Information System of New Mexico BISON. `http://www.bison-m.org/`

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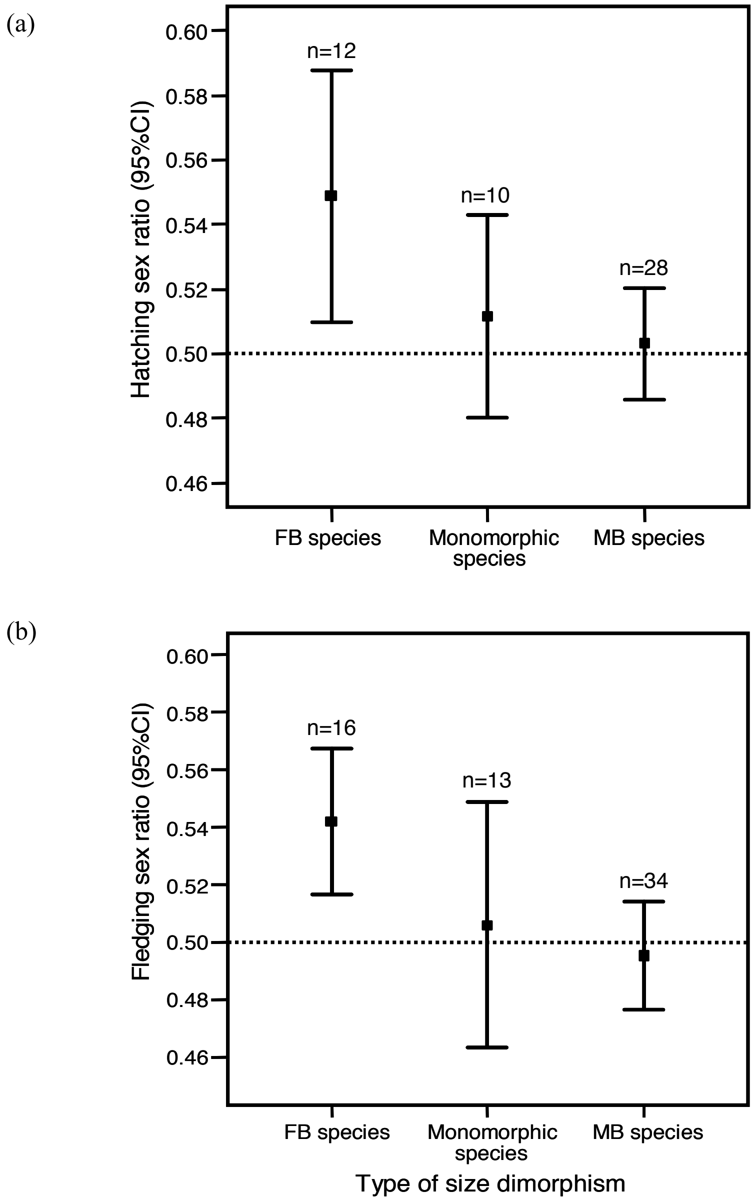
Numbers refer to studies listed in Appendix S3

Appendix S5 Figure 1 Interspecific mean values of sex ratio for different types of size dimorphic species: (a) hatching sex ratio and (b) fledging sex ratio. Sex ratio expressed as proportion of males. We considered dimorphic species those with a sexual size dimorphism degree higher than 3 %. FB, female-biased SSD; MB, male-biased SSD.



Appendix S6 Phylogenetic distribution and SSD

Distribution of the analysed species (within the molecular phylogeny orders, Sibley and Ahlqvist 1990) according to their sexual size dimorphism. We defined monomorphic species as those with less than 3 % body mass difference between sexes (♂= male, ♀= female).

Order	**Family**	No. Species	**Sexual size dimorphism** ♂ > ♀	monomorphism	♀ > ♂
Galliformes		**3**	**3**		
Anseriformes		**2**	**2**		
Piciformes		**2**	**1**	**1**	
Bucerotiformes		**1**	**1**		
Coraciiformes		**1**			**1**
Cuculiformes		**1**	**1**		
Psittaciformes		**3**	**1**	**2**	
Trochiliformes		**1**			**1**
Strigiformes		**4**			**4**
Columbiformes		**2**	**1**	**1**	
Gruiformes		0			
Ciconiiformes		**30**	**13**	**3**	**14**
	Accipitridae	7			7
	Charadriidae	1			1
	Ciconidae	0			
	Falconidae	3			3
	Fregatidae	0			
	Laridae	10	7	2	1
	Phalacrocoracidae	2	2		
	Procellariidae	4	4		
	Scolopacidae	2		1	1
	Spheniscidae	0			
	Sulidae	1			1
Passeriformes		**33**	**24**	**9**	
	Certhiidae	1		1	
	Corvidae	4	4		
	Eopsaltriidae	0			
	Fringillidae	11	10	1	
	Hirundinidae	2		2	
	Maluridae	0			
	Meliphagidae	2	2		
	Muscicapidae	2	2		
	Pardalotidae	1	1		
	Paridae	4	3	1	
	Passeridae	2		2	
	Regulidae	0			
	Sittidae	1	1		
	Sturnidae	1	1		
	Sylviidae	1		1	
	Tyrannidae	1		1	

2

Sexual size dimorphism and offspring vulnerability in birds

Dimorfismo sexual en tamaño y vulnerabilidad de la descendencia en aves

Ellen Kalmbach[1], María M. Benito[2]

El dimorfismo sexual en tamaño suele venir motivado por selección sexual durante la etapa adulta, pero también por selección sobre el crecimiento o el tamaño en etapas anteriores. Aparte de las limitaciones genéticas, las limitaciones fisiológicas durante el desarrollo pueden restringir el tamaño final de manera distinta en cada sexo, provocando que la viabilidad relacionada con el tamaño y los costes en salud aparezcan como efectos secundarios del sexo. Supervivencia, masa corporal e inmuno-compentencia son aspectos claves del desarrollo; si disminuyen inciden en la calidad del individuo o en su mortalidad, y por tanto se convierten en una manifestación de la desventaja que tiene un sexo respecto del otro. Estos (y otros) efectos negativos en la calidad y eficacia biológica de los pollos se denominan en general "vulnerabilidad de la descendencia".

Usando estudios recientes en aves, exploramos los patrones de vulnerabilidad diferencial de la descendencia en relación al dimorfismo sexual en tamaño en ambas direcciones (tanto si el dimorfismo está sesgado hacia el macho o hacia la hembra). En concreto, analizamos la mortalidad y la plasticidad de la masa de pollos volantones en relación al dimorfismo sexual. Si el tamaño es efectivamente la razón principal del incremento de mortalidad en los pollos, entonces los pollos hembra, en especies donde las hembras son más grandes, deberían sufrir desventajas similares a las de los machos en especies donde éstos son más grandes.

Nuestro análisis encontró una correlación interespecífica entre la vulnerabilidad diferencial (mayor mortalidad y masa reducida en pollos volantones criados en condiciones desfavorables) y el grado de dimorfismo sexual en ambas direcciones. Esto indica que estar programando para crecer más conlleva algunos costes de viabilidad. Sin embargo, el resultado de comparar las masas alcanzadas en entornos favorables y desfavorables sugiere que tener que crecer más es una desventaja principalmente cuando está vinculada al fenotipo masculino. Las diferencias de masa en hembras criadas en mejores y peores condiciones fueron independientes del dimorfismo sexual de su especie.

A nivel conductual, un mayor tamaño suele influir positivamente en la capacidad competi-

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tiva. A pesar de las desventajas fisiológicas del sexo más grande, el sexo menor podría ser más vulnerable y presentar mayor mortalidad o retraso en el crecimiento. Las diferencias en la sensibilidad ambiental entre los dos sexos durante la ontogenia, en forma de aumento de mortalidad o de tamaño reducido, podrían seleccionar contra el dimorfismo durante el desarrollo, afectando a los patrones existentes de dimorfismo sexual en una determinada especie. Por tanto, las condiciones ambientales podrían desempeñar un papel importante en la modulación de SSD dentro o entre generaciones. Por último, sugerimos que se efectúen más estudios experimentales con el objetivo de distinguir entre la base fisiológica de la vulnerabilidad asociada al mayor tamaño y los factores de comportamiento que pueden contrarrestar estos inconvenientes.

Palabras clave

Dimorfismo sexual en tamaño; sensibilidad ambiental; descendencia en aves; mortalidad diferencial.

Referencia – Journal Reference

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CHAPTER 13

Sexual size dimorphism and offspring vulnerability in birds

Ellen Kalmbach and Maria M. Benito

13.1 Introduction

Evolutionary theories trying to explain the existence and patterns of sexual size dimorphism (SSD) across taxa often focus on selection on body size at the adult stage, mainly driven by sexual selection acting on males or fecundity selection on females. However, the size dimorphism observed in adults can be determined not only by selection during adulthood (Blanckenhorn 2000), but also by selection on growth or size at earlier stages. It is therefore necessary to include ontogeny as an important period for determining final size dimorphism (e.g., Badyaev 2002; see also Chapters 7, 9, 19, and 20 in this volume).

Besides genetic constraints on how to achieve dimorphic growth while conserving the genes for the complete developmental programme in both sexes, physiological constraints during development can also limit final size. In sexually dimorphic species, size-related viability and health costs can become detectable as sex-biased effects. Increased mortality of the larger sex is the most extreme result, but other sub-lethal fitness effects can also be size- and sex-specific. A main focus in this respect is body mass, or size, of offspring, as this is often related to survival or probability of recruitment and regarded as a prime measure of offspring quality (Hochachka and Smith 1991; Potti *et al.* 2002). More recently, aspects of immunocompetence have been investigated as another measure of sex differences in physiological health and quality (Fargallo *et al.* 2002; Tschirren *et al.* 2003; Laaksonen *et al.* 2004; Bize *et al.* 2005; Chin *et al.* 2005; Müller *et al.* 2005a, 2005b). Although mortality, body mass, and immunocompetence are very diverse aspects of development, sex-biased reductions in offspring quality or survival can all be seen as manifestations of some disadvantage of one sex during the growth period. Collectively, these and any other negative effects on offspring quality and fitness are referred to as offspring vulnerability.

Differential offspring survival in a size-dimorphic species was probably first observed in humans: male fetuses and infants have a higher risk of dying than females (e.g. Süßmilch 1765). Male bias in offspring mortality has also been documented in other mammals (Clutton-Brock *et al.* 1985) and birds (Roskaft and Slagsvold 1985; Teather and Weatherhead 1989; Griffiths 1992; Müller *et al.* 2005a, 2005b). Most of these species have in common that males are the larger sex. To achieve their larger size, males are likely to have higher energy demands during growth, which in turn might make them more vulnerable to a shortage of resources, leading to increased mortality.

Alternative, size-independent explanations have been proposed to explain the observed male-biased offspring vulnerability in many mammals and birds. The one that has received most attention is the male-phenotype hypothesis. Size-independent aspects of physiology, in particular the high levels of testosterone needed for male sexual differentiation, might negatively impact on other aspects of development, such as immunocompetence (Olsen and Kovacs 1996; Fargallo *et al.* 2002). In order to tease apart the importance of male phenotype compared with the size effect on offspring mortality, it is necessary to include species where females are the larger sex.

In this chapter we will use recent studies of birds to explore patterns of sex-specific offspring vulnerability in relation to SSD in both directions; that is, female-biased as well as male-biased SSD. We will combine results on sex-specific offspring performance, and analyze mortality and plasticity of fledging mass in relation to SSD. If indeed size is the main reason for increased mortality of male offspring, then female offspring of species with female-biased SSD should experience similar disadvantages as males in species with male-biased SSD.

13.2 Measuring sex-biased offspring vulnerability in birds

Studies of sex-specific patterns of growth and mortality in birds have benefited hugely from the development of molecular sexing methods in the mid-1990s (Griffiths 1992; Ellegren 1996; Griffiths *et al.* 1998). Bird nestlings can usually not be sexed visually except in extremely size-dimorphic species, but even then only during the second half of the growth period (Cronmiller and Thompson 1980). A few earlier studies used laparotomy, a surgical incision of the abdomen, to inspect the gonads, but this could only be carried out in older chicks, not in hatchlings (Roskaft and Slagsvold 1985). Reports of sex-specific mortality between hatching and fledging could therefore not be based on individual fates. They were mostly inferred by comparing fledging sex ratios in nests with and without mortality (assuming equal hatching sex ratios in both nest categories), or by comparing fledging sex ratios with a sample of dissected clutches (Howe 1977) . As avian hatching sex ratios are frequently skewed in relation to such variables as parental condition or social status, the progressing season or territory quality (e.g. Komdeur *et al.* 1997; Heg *et al.* 2000; Kalmbach *et al.* 2001), comparing hatching and fledging sex ratios between different sub-samples of nests can lead to wrong conclusions about sex-biased mortality.

The most widely reported measure of nestling mortality is the survival probability from hatching to fledging. Using the difference between hatching sex ratio and fledging sex ratio as a measure for sex-specific mortality, a relationship between larger size and increased mortality was found across species with different degrees of SSD (Clutton-Brock *et al.* 1985). Sex differences in nestling mortality correlated with adult size dimorphism: the larger the males were in relation to females, the higher their survival disadvantage as nestlings. However, as only one species with female-biased size dimorphism was included in that review (which showed no sex bias in offspring mortality: Eurasian sparrowhawk, *Accipiter nisus*; Newton 1979), the size–mortality relationship therefore was shown only for species with larger males. Additionally, as the study dates before the advent of molecular sexing, its data suffer from the above-described methodological problems of obtaining true hatching and fledging sex ratios within the same nests. We will remedy this problem by employing strict selection criteria for the studies we include in our comparative analysis of nestling mortality (see Section 13.3.1).

Sex-biased mortality represents the extreme case of sex differences in offspring vulnerability. As mentioned above, growth rate and size at fledging are also regarded as a measure of offspring performance. Because of its likely negative impact on future life stages, reduced size at fledging is seen as a manifestation of non-optimal conditions during ontogeny (Hochachka and Smith 1991; Haywood and Perrins 1992; Potti *et al.* 2002). Assuming that under ideal conditions individuals will grow to the maximum possible size (given their species, genes, and sex), the degree of size reduction under suboptimal conditions gives an indication of how much the growing organism was struggling.

Considering that the larger sex is likely to have a higher energy demand during growth than the smaller one, we would predict that during periods of scarce resources the larger sex would be affected disproportionately. To test this prediction, we will compare fledging mass of males and females under varying circumstances (Section 13.3.2). We use mass rather than some structural measure of size, such as wing or tarsus length, for two reasons. Body mass is probably the easiest of those measures to record in the field, and is the one most frequently reported in publications. Second, our choice of mass reflects the fact that for birds adult SSD is most commonly reported as the dimorphism in mass.

13.3 Comparative analysis of SSD and nestling vulnerability

The modulation of vulnerability differences between the two sexes by environmental conditions is referred to as sex-biased environmental sensitivity. It is generally assumed that poor conditions increase the disadvantage of the weaker sex. In order to investigate environmental sensitivity, comparisons of offspring performance under varying environmental conditions need to be made (Sheldon *et al.* 1998). Most simply, this can be a dichotomy between a ″good″ and a ″poor″ environment. Increasingly, these contrasting situations are created by experimental manipulation of the environment during ontogeny. Such experimental approaches include brood size increase and decrease, manipulation of parental condition and workload, provision of supplementary food, or changes of the parasite load (Richner 1992; Sheldon *et al.* 1998; Nager *et al.* 2000; Bize *et al.* 2005; Råberg *et al.* 2005). However, comparisons might also be made between naturally occurring good and poor conditions, for example between first and last hatchlings in asynchronous broods or between seasons of abundant and low food availability (Wiebe and Bortolotti 1992; Brommer *et al.* 2003; Goymann *et al.* 2005). As restricting data to either experimental or observational studies would greatly reduce the number of available species, we included both types of study in the following comparative analyses.

To correct for the species' phylogenetic relatedness, we employed a comparative approach following the method of phylogenetically independent contrasts (Harvey and Pagel 1991; Garland *et al.* 1992). Contrasts were calculated using the program CAIC (Purvis and Rambaut 1995), and the phylogeny was taken from Sibley and Ahlquist (1990). All statistical results were obtained using this comparative method, and are reported in Table 13.1. However, for illustrative purposes we show species data, including species-level trend lines, in our graphs. These are more accessible because of their biologically interpretable values. Regression lines are only shown for those relationships for which a significant effect was found in the analysis based on phylogenetically independent contrasts.

Table 13.1 Regression results of sex-specific vulnerability against SSD, using phylogenetically independent contrasts. (a) Nestling mortality from hatching to fledging against SSD. The dependent variable was hatching sex ratio, fledging sex ratio, or sex-specific chick mortality (calculated as fledging sex ratio minus hatching sex ratio). (b) Intraspecific fledging mass change under good and poor conditions against SSD. The dependent measure was the mass-change difference (Δflm female–Δflm male; see text), mass-change difference for experimental studies only, male change only, or female change only. SSD is the independent variable in all models. Models are based on phylogenetically independent contrasts. For the analysis presented here we used the molecular phylogeny by Sibley and Ahlquist (1990). The results were qualitatively the same when using a morphological phylogeny. All regressions are forced through the origin. The analyses were run with the program CAIC (Purvis and Rambaut 1995). R^2 is the proportion of variance in the independent variable explained by the predictor variable; *r* is the Pearson correlation coefficient.

Dependent variable	No. of species	No. of contrasts	R^2	*r*	*P*
(a) Nestling mortality					
Hatching sex ratio	45	13	0.02	−0.14	0.622
Fledging sex ratio	45	13	0.09	−0.29	0.303
Sex-specific mortality	45	13	0.29	−0.54	0.047
(b) Fledging mass change					
Female–male difference	21	19	0.32	0.57	0.008
Female–male difference (experimental studies only)	14	13	0.40	0.63	0.015
Male change	21	19	0.25	−0.50	0.025
Female change	21	19	0.00	0.00	0.980

13.3.1 Sex-biased mortality and sex ratios

As highlighted above, for the following sex ratio and mortality analyses, we only used data from studies that report sex ratio at hatching *and* fledging from the same study nests. Sex-ratio data for the cross-species analysis were taken from observational studies or from experimental studies, in cases where the sex ratios between experimental and control treatments did not differ.

Across species, we found a negative correlation between sex-biased mortality and size dimorphism that was consistent for species with male-biased and female-biased SSD (Table 13.1). The larger of the two sexes appears to suffer greater mortality; that is, more females die as nestlings in species with larger females, and more males die in species with larger males. The survival disadvantage increases with increasing size dimorphism. In other words, the larger sex always suffers higher mortality, indicating that to achieve a larger final body size both males and females pay a survival cost. On the species level, overall nestling mortality seemed slightly male-biased (Figure 13.1). This impression is supported by a negative average mortality value in the comparative analysis, suggesting that offspring survival was negatively affected by male-specific traits other than size.

Neither hatching nor fledging sex ratio showed a correlation with SSD (Table 13.1). At the population level, parents neither overproduced the smaller sex (as predicted by Fisher's (1930b) equal-investment sex-ratio theory) nor the larger sex to compensate for its higher mortality up to fledging. Despite the trend of increased mortality of the larger sex, and the unbiased hatching sex ratios, overall fledging ratios were not significantly biased

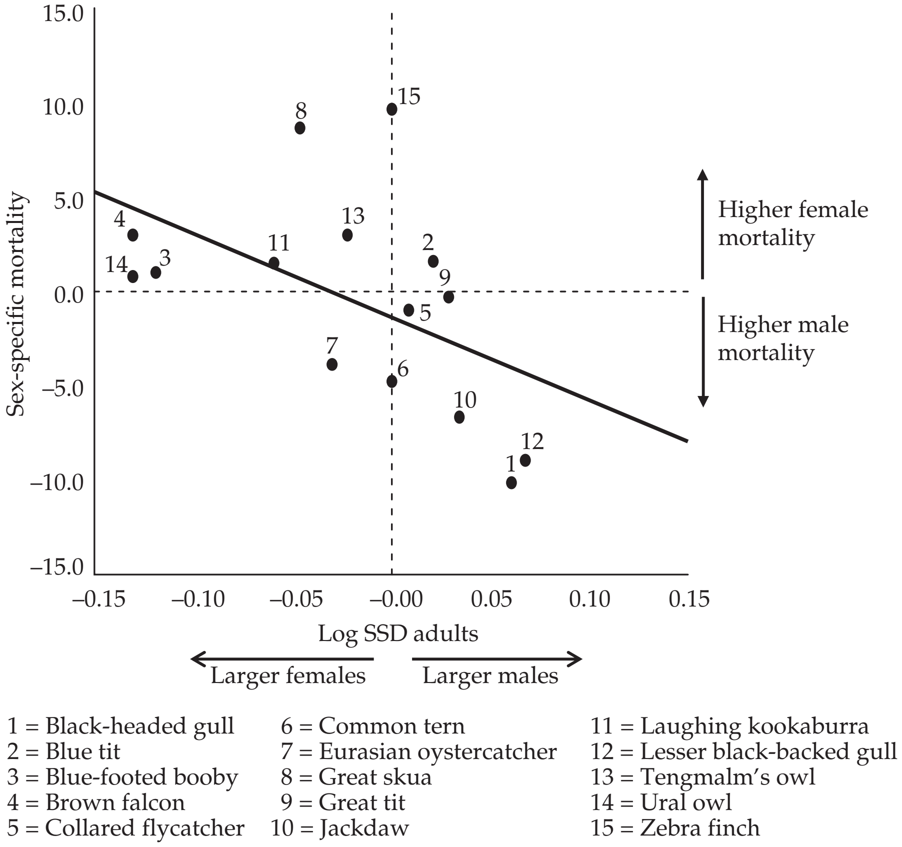

Figure 13.1 Relationship between SSD, calculated as log(male adult weight/female adult weight) and sex-specific chick mortality, calculated as fledging sex ratio minus hatching sex ratio. Species references: 1, Müller *et al.* (2005b); 2, Råberg *et al.* (2005); 3, Torres and Drummond (1999); 4, Mcdonald *et al.* (2005); 5, Sheldon *et al.* (1998); 6, Gonzalez-Solis *et al.* (2005); 7, Heg *et al.* (2000); 8, Kalmbach *et al.* (2005); 9, Oddie (2000); 10, Arnold and Griffiths (2003); 11, Legge *et al.* (2001); 12, Griffiths (1992); 13, Hornfeldt *et al.* (2000); 14, Brommer *et al.* (2003); 15, Bradbury and Blakey (1998).

towards the smaller sex (Table 13.1). This is likely due to the high variation of sex ratios among species and the relatively small number of species we could include based on our methodological criteria.

13.3.2 Fledging mass

In the following cross-species analysis, we used data from studies which reported sex-specific fledging mass under two different conditions that could be classified as either good or poor. In most studies those conditions were created through experimental manipulations, although we also included data from observational studies reporting sex-specific fledging mass (see Table 13.2 for classification of good and poor conditions). For each sex we set the average fledging mass under good conditions as the reference value, and expressed the difference between that and fledging mass under poor conditions as a percentage of the reference mass. We will call this difference Δ*flm* (delta fledging mass). As we are mainly interested in the difference between males and females with respect to their reaction to environmental conditions, we compared Δ*flm* of males and females within each species. We subtracted Δ*flm* of males from Δ*flm* of females to obtain one value per species. When positive, this value indicates that males lose relatively more mass compared to females, whereas when this value is negative males lose relatively less mass. For example, the value of −10.6 for great skua (*Stercorarius skua*) means that males lost 10.6% less of their reference body mass than females during poor rearing conditions (Kalmbach *et al.* 2005).

Across species, and across both directions of size dimorphism, birds of the larger sex suffered a greater mass reduction under poor conditions (Table 13.1; Figure 13.2). For monomorphic species the fledging mass differences are clustered around 0. This suggests that in the absence of size dimorphism neither sex has a consistently higher vulnerability. The overall pattern could indicate that having to grow to a larger size under sub-optimal conditions is similarly difficult for males and females. However, when plotting Δ*flm* for males and females separately, we see that the pattern is mainly generated by a correlation between male fledging mass reduction and SSD (Figure 13.3). The more male-biased the SSD, the larger the impact of poor rearing conditions on male fledging mass, while female mass differences between good and poor conditions are independent of whether they are the larger or the smaller sex. This pattern remains when non-experimental studies are excluded from the data-set (Table 13.1).

Our results prompt an interesting consideration. The relative demands of having to grow large (for a given species) might not be as high as is generally assumed. Only in conjunction with the rest of the male phenotype does aiming for being large—that is, following a developmental program which leads to large size for a given species—appear to make the growing organism more vulnerable. Testosterone and its allies are much-cited candidates for mediating male vulnerability. Remarkably, in the species with the largest females and highest female mass loss (African black coucal), the breeding system is polyandrous. Although female behavior is 'masculinized', daughters' testosterone levels are lower than those of sons and even lower than those of nestlings of other species (Goymann *et al.* 2005).

13.4 SSD and environmental sensitivity of immunocompetence

The immune system provides a potential link for life-history trade-offs (Sheldon and Verhulst 1996). It is relatively expensive to develop and maintain, but crucial for a successful life. Reduced immune capacity of nestlings is likely to indicate sub-optimal conditions during development when resources have to be invested in other parts of the growing organism. Recently, a few studies investigated sex-linked differences of immunocompetence in varying environmental conditions.

In two of four studies there was no differential decrease in immunocompetence under poor conditions (great tit and alpine swift, adult SSD 1.07 and 1.02, respectively; Oddie 2000; Bize *et al.* 2005). In food-restricted nests of Eurasian kestrels (adult SSD 0.78), the (smaller) males showed a slightly

Table 13.2 Circumstances representing good and poor conditions in the studies which were included in the cross-species analysis of fledging mass. Log SSD is log(male adult mass/female adult mass); where available taken from the same study population, otherwise from reference literature. Type of study: obs, observational; exp, experimental.

Species	Log SSD	Type of study	Good/poor environment	Reference
Capercaillie, *Tetrao urogallus*	0.33	obs	Good against poor growth year due to temperature difference	Lindén (1981)
Helmeted guineafowl, *Numida meleagris*	−0.02	exp	Summer against winter rearing conditions	Baeza *et al.* (2001)
Lesser snow goose, *Anser caerulescens cearulescens*	0.06	obs	Seasonal environmental decline; earliest against penultimate category	Cooch *et al.* (1996)
African black coucal, *Centropus grillii*	−0.23	obs	Hatching order; "middle" against "late" chicks; earliest chicks were older at fledging	Goyman *et al.* (2005)
Alpine swift, *Apus melba*	0.01	exp	De-parasitized against parasitized broods	Bize *et al.* (2005)
Ural owl, *Strix uralensis*	−0.13	obs	Good and poor food years (vole cycles)	Brommer *et al.* (2003)
Great skua, *Stercorarius skua*	−0.05	exp	Control eggs against small replacement eggs	Kalmbach *et al.* (2005)
Lesser black-backed gull, *Larus fuscus*	0.06	exp	Control against poorer condition parents	Nager *et al.* (2000)
Black-headed gull, *Larus ridibundus*	0.06	exp	First against last hatched chick in all female and all male broods	Müller *et al.* (2005b)
Common tern, *Sterna hirundo*	0	obs	First against third hatched chicks	Becker & Wink (2003)
Eurasian kestrel, *Falco tinnunculus*	−0.07	exp	(a) Unisex broods in poor food years; (b) control against enlarged brood	(a) Laaksonen *et al.* (2004); (b) Dijkstra *et al.* (1990)
American kestrel, *Falco sparverius*	−0.06	obs	Good against poor food years	Wiebe & Bortolotti (1992)
Blue-footed booby, *Sula nebouxii*	−0.12	exp	Feather-clipping of mothers; chicks of control against chicks of clipped mothers	Velando (2002)
Carrion crow, *Corvus corone*	0.05	exp	Food-supplemented against un-supplemented nests in a food-limited population	Richner (1992)
Collared flycatcher, *Ficedula albicollis*	0.01	exp	Reduced against enlarged broods	Sheldon *et al.* (1998)
Great tit, *Parus major*	0.03	exp	Experimental nests of "large" and "small" nestlings; "large" against "small" nestlings	Oddie (2000)
Blue tit, *Parus caeruleus*	0.02	exp	Reduced against enlarged broods	Råberg *et al.* (2005)
Zebra finch, *Taeniopygia guttata*	0	exp	Abundant against restricted food	Kilner (1998)
Red-winged blackbird, *Agelaius phoeniceus*	0.21	exp	Control against enlarged broods	Cronmiller and Thompson (1980)
Boat-tailed grackle, *Quiscalus major*	0.29	obs	First against third hatched chicks	Bancroft (1984)
Great-tailed grackle, *Quiscalus mexicanus*	0.28	exp	Experimentally synchronized last hatchlings; having female nest mate against having male nest mate	Teather and Weatherhead (1989)

stronger decrease of cell-mediated immunity (CMI) than the (larger) females compared with control nests (Fargallo *et al.* 2002). CMI of male nestlings (larger sex) in large broods of European starlings (adult SSD 1.05) also decreased more strongly than CMI of female nestlings compared to values in smaller broods (Chin *et al.* 2005). So far these studies have reported either no sex bias or a

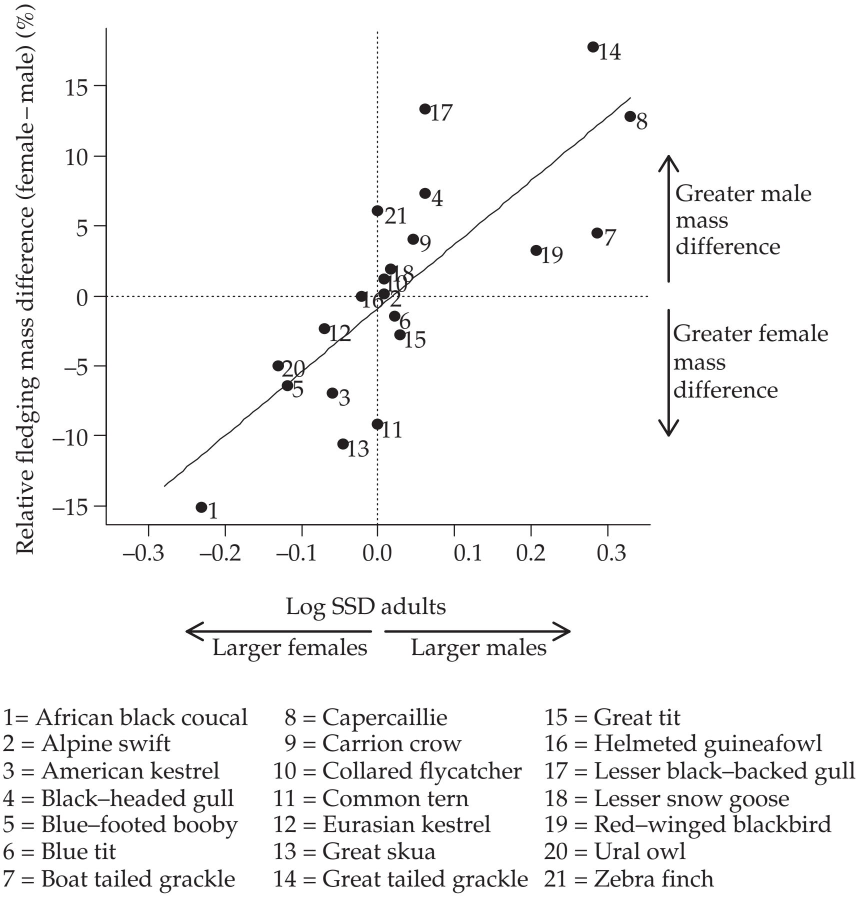

Figure 13.2 Relative change of fledgling mass between good conditions and poor conditions against SSD, calculated as log(male adult weight/female adult weight). Each species value is calculated as female difference (Δ*flm* of females) minus male difference (Δ*flm* of males). Negative values indicate that males lost relatively less mass than females; that females are more vulnerable. Positive values indicate that males lost relatively more mass than females; that males are more vulnerable. The relationship between sex-specific change of fledging mass and SSD is significant using phylogenetic contrasts ($P = 0.008$; see Table 13.1). See Table 13.2 for references.

male bias, but no study has yet found decreased CMI for female nestlings. A second study of Eurasian kestrels, which investigated haematocrit as a measure of physiological condition, found a lower value for (larger) females under increased competition (Laaksonen *et al.* 2004).

The small number of studies and remaining controversy over the interpretation of CMI tests as well as hematocrit values make it clear that at this point no generalization about SSD and immunocompetence of fledglings can be made.

13.5 Intra-brood competition and size-related vulnerability

The dichotomy of good and poor conditions for reasons of comparison is of course a simplification of the much more complex, naturally occurring situation. In reality, rearing conditions vary across a multitude of gradually changing and interacting factors, not just in two extremes (although the latter happens, to some extent, in experimental studies). So far we have assumed physiological disadvantages of large size, which could be regarded as intrinsic vulnerability of the larger sex. However, nestlings interact with each other and size is often implicated in the outcome of intra-brood competition. Following the terminology of intrinsic vulnerability, we will call growth and viability disadvantages that result from social interactions extrinsic vulnerability.

In contrast to intrinsic disadvantages, larger individuals generally have a competitive advantage at the behavioral, extrinsic level (Anderson

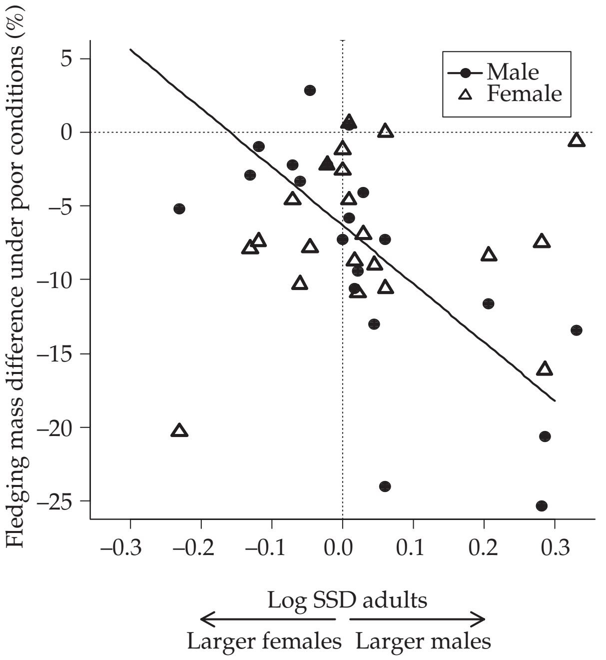

Figure 13.3 Relative difference of fledging mass between good and poor conditions against SSD, calculated as log(male adult weight/female adult weight) for male and female nestlings separately. The regression, based on phylogenetic contrasts, is significant for male nestlings, but not for female nestlings ($P = 0.025$ for males and $P = 0.978$ for females; see Table 13.1).

et al. 1993). Oddie (2000) showed experimentally that increased mortality of the smaller female great tit nestlings was mainly due to their competitive disadvantage. Similarly, Råberg *et al.* (2005) found that female blue tit nestlings (again the smaller sex) suffered more (reduced fledging size). They suggested that brood size, as an indication of the strength of intra-brood competition, could explain part of the variation around the overall pattern. Besides brood size, sex composition, and size and age differences among nest mates determine within-brood dynamics and add another layer of complexity. Depending on the social circumstances, for example in large broods, the competitive disadvantage of the smaller sex can outweigh its physiological advantages.

13.6 Sex-biased vulnerability and the evolution of SSD

Our cross-species comparison highlights two aspects of size-related offspring vulnerability that affect the extent of SSD exhibited in adult birds. First, across species there is a pattern that the larger sex has a viability disadvantage during ontogeny. This will cause a certain amount of viability selection during ontogeny against growing large. How strong this selection is will depend on many other aspects of each species' particular life history. For example it will be modulated by the ability of parents to adaptively skew primary sex ratios in response to environmental conditions, by the strength of sibling competition, by the type of breeding system and reproductive skew between the sexes, or by differential food allocation to offspring (Anderson *et al.* 1993; McDonald *et al.* 2005; Råberg *et al.* 2005).

Second, we found that across species the larger sex shows a stronger modulation of its relative fledging mass according to environmental circumstances. This can lead to a smaller degree of size dimorphism than would be predicted as optimal for adults. In the short term, sex-biased size reduction during ontogeny will create shifting patterns of SSD between cohorts or even within a season, tracking changes in environmental conditions (Cooch *et al.* 1996). If environmental degradation is a continuing process, size dimorphism in a population could decrease over time even though fecundity and sexual selection favor an increase.

Our results show a size-dependent modulation of male fledging mass in response to environmental conditions, but a size-independent mass reduction in female nestlings. This pattern suggests greater plasticity of male growth. On average, quantitative genetic studies indicate a slightly higher heritability of size in female birds (e.g. Jensen *et al.* 2003), which could reflect greater size plasticity in male fledglings. It remains a challenge to uncover the mechanisms permitting sex-biased evolution of growth patterns despite the shared gene pool between the sexes (Merilä *et al.* 1998; see also Chapters 16, 17, and 19).

13.7 Future studies

An important aspect of sex-specific environmental sensitivity is the timing of the occurrence of poor conditions relative to critical stages of offspring development. When cell numbers of specific organs are limited during a small time window of development, metabolism and growth during all subsequent stages can be affected. Such a mechanism is thought to be involved in increased health risks of persons who showed poor growth during gestation (Bateson *et al.* 2004). It is likely that the metabolic machinery to build differently sized individuals of the same species differs from early development onwards. Sex differences in energy allocation to specific organs might already cause higher vulnerability of the eventually larger sex before size dimorphism and differential energy requirements become apparent (Kalmbach *et al.* 2005). Similarly, poor conditions during a developmental phase when the sexes are still equal in size can cause sex- or size-specific effects later (Gorman and Nager 2003). Physiological studies are required to determine sex differences in physiology and energy allocation at very early stages. To tease apart intrinsic and extrinsic size-related vulnerability, growth experiments with hand rearing, having chicks raised as singletons by parents (Kalmbach *et al.* 2005), or creating same-sex and same-size broods (Oddie 2000; Müller *et al.* 2005b), will be useful.

The measures we used for the present analysis are rather broad, including the necessary dichotomous classification into good and poor conditions for the analysis of fledging mass. This was mainly determined by the availability of comparable variables for a larger number of species. Although mortality is no doubt an aspect of fitness, and fledging mass also appears to be fitness-related (Haywood and Perrins 1992; Potti *et al.* 2002), other aspects of an organism's state might be crucial for its subsequent performance. Studies of immunocompetence address this issue.

The between-sex effect of expected size (predicted by the average size of males and females of the species) on mortality and fledging mass indicates that size-related viability selection also occurs within each sex. To address this, it would be necessary to have prior individual-level knowledge of expected size, beyond the classification by sex, and to investigate how individuals of different predicted sizes of a given sex react to varying conditions. This approach was taken by Weatherhead and Dufour (2005), who analysed 30 years' of data for red-winged blackbirds. They found no survival differences between (predicted) large and (predicted) small males. As a predictor of size they used the mid-parent value, but the chicks were reared by their natural parents. A phenotypic correlation between large size and good parental abilities might thus mask size-related offspring vulnerability. The sizes of parents are themselves modulated by plasticity and are not a direct measure of genetic size. Using multi-generation animal models of wild populations or captive selection lines could reduce this problem (e.g. Kruuk *et al.* 2001; Teuschl *et al.* 2007).

13.8 Summary

We found cross-species correlations between sex-biased vulnerability (mortality and reduced fledging mass under poor conditions) and the extent of SSD in both directions (males or females larger). This indicates that being programmed to grow large carries viability costs. However, our comparison between fledging mass reached in good and poor environments suggests that having to grow large is mainly disadvantageous when coupled with the male phenotype. Female fledging mass differences between good and poor conditions were independent of SSD. On a behavioral level, larger size generally influences competitive ability positively. Despite physiological disadvantages of the larger sex, in unmanipulated broods the smaller sex might de facto be more vulnerable; that is, exhibit higher mortality or stunted growth (Anderson *et al.* 1993; Oddie 2000; Råberg *et al.* 2005).

Differences in environmental sensitivity between the two sexes during ontogeny, in the form of either increased mortality or reduced size, may select against dimorphism during development, affecting existing patterns of SSD in a given species. As such, environmental conditions are likely to play a major role in modulating SSD within or between generations. Given that there is a correlation of vulnerability with size predicted by sex, a similar size-related vulnerability would be expected within sexes. However, to determine the predicted size of an individual is much more difficult. We suggest that more experimental studies should be carried out with the aim of distinguishing between the physiological basis for vulnerability of being large and behavioral factors that can counteract such disadvantages.

13.9 Suggested readings

Kalmbach, E., Furness, R.W., and Griffiths, R. (2005) Sex-biased environmental sensitivity: natural and experimental evidence from a bird species with larger females. *Behavioral Ecology* **16**, 442–449.

Le Galliard, J.F., Ferriere, R., and Clobert, J. (2005) Juvenile growth and survival under dietary restriction: are males and females equal? *Oikos* **111**, 368–376.

Råberg, L., Stjernman, M., and Nillsson, J.-Å. (2005) Sex and environmental sensitivity in blue tit nestlings. *Oecologia* **145**, 496–503.

Sheldon, B.C., Merilä, J., Lindgren, G., and Ellergren, H. (1998) Gender and environmental sensitivity in nestling collared flycatchers. *Ecology* **79**, 1939–1948.

Results II.
Intra-specific analyses: The case of the common tern

3

Sex-specific traits in common tern chicks: associations with rearing environment, parental factors and survival

Atributos diferenciales para cada sexo en pollos de charrán común: asociaciones con características de la puesta, factores parentales y supervivencia

María M. Benito[1], Jacob González-Solís[1], Peter H. Becker[2]

La variación fenotípica observable entre la descendencia tiene consecuencias futuras importantes para la eficacia biológica, la supervivencia y el éxito reproductivo del pollo como individuo. Sin embargo, el valor reproductivo y la rentabilidad que esa variación fenotípica tiene para los padres han sido poco estudiados.

Analizamos doce características fenotípicas (morfológicas, serológicas e inmunológicas) de los pollos de charrán común *(Sterna hirundo)* en relación a su sexo, edad y fecha de eclosión, y exploramos la relación para cada sexo entre esos atributos y las condiciones del ambiente de cría (número de hermanos y días de competencia entre ellos) y la supervivencia. Además, estudiamos las diferencias sexuales en el fenotipo de los pollos en relación a factores parentales como la edad, la masa corporal, la fecha de llegada a la colonia y el tamaño de puesta, ya que la existencia de dimorfismo sexual en la descendencia podría reflejar una inversión parental diferenciada para cada sexo.

Tanto la longitud de la estructura morfológica pico-cabeza como la respuesta inmune mediada por linfocitos T variaron en relación al sexo de los pollos: los machos mostraron valores mayores que las hembras para ambas características. La respuesta inmune también se vio afectada por factores ambientales (año), por las condiciones de cría (competencia entre hermanos a partir de los 14 días de edad) y por factores parentales (tamaño de puesta). Ninguna de las doce variables estuvo asociada con la mortalidad, pero la mortalidad de los pollos hembra se relacionó con periodos más largos de competencia en el nido. A pesar de que algunos rasgos parentales influyeron en el fenotipo de los hijos, el dimorfismo sexual no varió en relación a la edad, la masa o la fecha de llegada de los padres. Este resultado sugiere que no existe una gran diferencia en los costes que supone un hijo en comparación con una hija, y por tanto, que no hay un ajuste del esfuerzo o inversión parental asociado al sexo del pollo.

Sin embargo, el hecho de que la respuesta inmune fuera mayor para los pollos macho

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pertenecientes a puestas más grandes y también para aquellos criados junto a hermanos indica una diferencia en las estrategias de desarrollo de machos y hembras que podría estar relacionada con su valor reproductivo. Por tanto, podría esperarse para esta especie una asignación por sexo por parte de los padres que afecte diferencialmente a hijos e hijas (en forma, quizá, de ajuste de la razón de sexos).

Palabras clave
Variación fenotípica; dimorfismo sexual; *Sterna hirundo*; características diferenciales para cada sexo; competencia entre hermanos; factores parentales; valor reproductivo.

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Sex-specific traits in Common Tern chicks: associations with rearing environment, parental factors and survival

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Abstract Offspring phenotypic variation has important consequences for nestling individual fitness, survival and reproductive success, but the sex-specific reproductive value and fitness returns for parents of this variation has been little explored. We analysed twelve phenotypic traits of nestling Common Terns (*Sterna hirundo*) in relation to their sex, age and hatching date, and explored if they were sex-specifically associated with rearing environment and survival. In addition, we studied sex-specific differences in offspring phenotype related to parental factors, since the existence of sexual dimorphism may reflect differential parental investment. Bill-head length and mediated immune-response varied in relation to offspring sex, with males showing larger values than females. Immune response was also affected by environmental (year), rearing (competition over 14 days old) and parental factors (clutch size). None of the morphological, serological and immunological variables was associated with chick mortality, but mortality of female nestlings was related to the longest periods of within-brood competition. Although some parental traits influenced offspring phenotype, sexual dimorphism among nestlings did not vary in relation to parental age, mass or arrival date, which may imply no great differential costs of raising sons or daughters, and therefore, no adjustment of parental effort in relation to nestling sex. However, evidence that immune response was higher for sons in larger clutches and raised with siblings, indicates a difference in the developmental strategies of sons and daughters which might be related to their reproductive value. These results provide thus evidence of certain pathways for parental sex-allocation that may differentially affect sons and daughters.

Keywords Phenotypic variation • *Sterna hirundo* • Sex-specific traits • Sibling competition • Parental factors • Reproductive value

Introduction

In sexually size dimorphic species male and female nestlings also often differ in physiological parameters, in susceptibility to diseases, environmental conditions or sibling competition (Oddie 2000, Råberg et al. 2005). Nevertheless, nestlings of monomorphic or slightly size dimorphic species can also show different rates of growth, developmental patterns and environmental sensitivity, which may lead them to display sexual dimorphism in phenotypical traits other than size (Cordero et al. 2001, Mead et al. 1987). Sex-specific growth may imply differences in the costs and benefits of achieving a particular trait value, which can have important consequences for the chick's individual fitness, survival and reproductive success (Mainwaring et al. 2011, Råberg et al. 2005). Our understanding of how nestling sex-specific traits develop and their future consequences is limited, and it is therefore important to investigate sexual differences, their sources of variation and how they relate to survival.

Among the factors hypothesized to influence sex-specific phenotypic variation in nestlings are these related to environmental and rearing conditions and to parental quality. For instance, rearing environment conditions are influenced by the existence, type and length of sibling competition, and brood size, hatching order

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and sex ratio of the brood, which can determine nestling growth rates (Royle et al. 1999). In broods of two or more siblings, sons and daughters may not compete equally well and thus may not receive equal amounts of parental care or resources (Bonisoli-Alquati et al. 2011). Similarly, brood sex ratio is important because it may not be the same to share the resources with siblings of the same or different sex (Becker and Wink 2003, Teather 1989). Moreover, the rearing environment of nestlings may influence sex-specific trade-offs during growth. Under strong sibling competition, nestlings of one sex may prioritize for example the development of traits that give them an advantage during competition, such as body mass, over traits that enable them to fledge simultaneously with their siblings, such as feather growth (Mainwaring et al. 2009).

Parental factors are also expected to contribute to phenotypic dimorphism between sons and daughters, since nestlings are dependent on the amount and quality of parental care they receive, especially in altricial species. Higher quality parents would likely invest more resources in their offspring than other parents, and their chicks may respond to the higher availability or better resources differentially according to their sex (Hegyi et al. 2011, Metcalfe and Monaghan 2001). Alternatively parents may adjust the level of resources allocated to their sons and daughters if, as the theory of sex-allocation posits, this sex-specific investment results in different reproductive values and thus in different relative fitness benefits for the parents (Charnov 1982). Therefore, nestling sex-specific phenotypic variation could be reflecting the difference in parental investment towards each sex (Lessells 1998, Lessells 2002). Another way in which sexual differences may arise occurs through differential parental investment, when sexually selected traits are genetically heritable. For instance, in species with heritable variation of sexual male traits, females mated to high quality males obtain more benefits from rearing sons, since only sons will inherit sexual ornaments of the high quality father and enjoy greater breeding success, thereby yielding greater fitness returns than daughters (Ellegren et al. 1996). However, the attractiveness hypothesis does not only account for ornamental traits; a good mate condition or high social position are also an incentive to invest differentially (Addison et al. 2008). Experimental evidence supports the link between parental attractiveness and differential investment, usually by mothers (Ligon and Hill 2010, Limbourg et al. 2004, Pryke and Griffith 2010).

In each of these cases, resources allocated by parents will influence the physiological condition of nestlings, as indicated by morphology and blood parameters such as haematocrit, plasma proteins or plasma carotenoids (Banbura et al. 2007, Simmons and Lill 2006). Likewise, development and maintenance of immune function is energetically and nutritionally costly (review in Lochmiller and Deerenberg 2000) and it may be impaired when food is scarce (Fargallo et al. 2002). Thus, these individual morphological, physiological and immunological parameters, which relate to general state and are affected by parental effort, can be analysed and correlated to offspring sex and quality (Banbura et al. 2008, Burness et al. 2000, Saino et al. 2002). Sexual phenotypic dimorphism could then be considered as an indirect expression of differential parental investment (Banbura et al. 2008, Laaksonen et al. 2004) or reflect sex-differential developmental strategies during growth. Therefore, differences in expression of fitness-related traits between offspring sexes might be reflecting differences in the reproductive value of sons and daughters (Saino et al. 2002).

The purpose of this study was to explore whether phenotypic characteristics of the nestlings are sex-specific, and evaluate the importance of post-hatching rearing conditions and parental effects in the offspring phenotypes of both sexes. The species studied here, the Common Tern (*Sterna hirundo*), is a socially monogamous, biparental and colonial seabird. The species is an interesting model because its slight size dimorphism may highlight the occurrence of sexual dimorphism in other traits. We focussed only on first-hatched nestlings (a-chicks), since the inclusion of nestlings of the same hatching position seems to be the best method of controlling the nest hierarchy and dominance effects on many topics.

The first aim of the present study was to detect sexual dimorphism in nestlings, by comparing twelve phenotypical traits of morphology, physiology and immunity in relation to sex, while also considering the effect of the year, hatching date and age of the chick. The second aim was to explore the effects of the rearing conditions on nestling sexual dimorphism, by analysing the association of sex-specific traits with sibling competition, brood size (at hatching and fledging) and the sexual composition of the brood. The third aim was to analyse offspring sexual dimorphism in these traits in relation to parental age and quality, as indicated by body mass mass and arrival date (Ezard et al. 2007, Becker et al. 2008a). Thus we tried to answer the questions of whether these parental factors are related to phenotypic traits, and whether they are differentially related for sons and daughters. Finally, as a fourth aim, we tested relationships between sex, phenotype and survival of chicks, to investigate if sons and daughters differ in sensitivity to environmental and rearing conditions and parental factors.

Methods

Study area and data collection

The study was conducted during the breeding seasons of 2004 and 2005 at the Banter See Common Tern colony in Wilhelmshaven (Germany; 53º27'N, 08º07'E). At this site, an integrated population study has been in progress since 1992 to study various aspects of the life history and demography of this species (Becker et al. 2001). The breeding site consists of six artificial islands (sub-colonies) of equal size and rectangular shape (each 4.9 x10.7 m), arranged in a line with a distance of 0.9 m in between. The concrete walls that surround the islands are equipped with 44 elevated places (0.3 x 0.3 x 0.3 m) for the terns to land and rest on (Becker and Wendeln 1997). These resting platforms are equipped with antennae to remotely and automatically record all transponder-marked birds, and sixteen antennae are combined with electronic balances (for details see Becker and Wendeln 1997, Becker et al. 2001). This approach allows the measurement of variables such as arrival date and body mass of transponder-marked adults (Ezard et al. 2007). All birds marked since 1998 have been molecularly sexed, and before 1998 breeders were sexed by behavioural observations.

The colony was visited regularly during the breeding season. To identify parents of each nest, a portable antenna was placed around the nest for 1–2 days during the incubation period. Each nest was checked every 2–3 days, and laying and hatching date, laying and hatching order and the fate of eggs and chicks were determined. Chicks were ringed and marked with a transponder before fledging (Becker and Wendeln 1997), molecularly sexed using standard PCR methods (Becker and Wink 2003) and recorded until death or fledging.

For this study, 80 chicks (43 males, 37 females) were sampled from two sub-colonies. Only one chick per brood and only a-chicks (first hatched chicks) were studied (44 chicks in 2004, 36 chicks in 2005). When nestlings were 19 or 20 days old (n = 29 and 65, respectively, we measured their body mass using a digital balance to the nearest ±1 g, the wing (ruler, ±1 mm), and tarsus and head-bill length using a Vernier calliper (±0.1 mm). We also collected 100-150 µl of

Table 1. Means (± SE) of phenotypical, parental and rearing variables for chicks of Common Tern at 19/20 days old, by sex (total n = 80). Dates are days from the 1st January (log-transformed for analyses, as the nr. of parasites and the colour parameters luminosity and chroma). Data for isotopic signatures correspond to year 2004, and colour parameters to year 2005.

	Male			Female		
Variable	Mean	SE	n	Mean	SE	n
Morphological						
Body mass (g)	106.6	1.9	43	104.5	1.8	37
Tarsus length (mm)	20.2	0.1	43	19.8	0.1	37
Wing length (mm)	133.4	2.6	43	134.7	2.1	37
Bill-head length (mm)	60.8	0.4	43	59.0	0.3	37
Body condition index	-23.0	0.16	43	-23.4	0.13	37
Physiological						
Haematocrit (%)	37.1	0.6	42	37.0	0.7	37
Protein concentration (g/dl)	1.3426	0.0002	42	1.3425	0.0002	37
Carotenoid concentration (µg/ml)	15.73	0.76	40	14.78	0.95	36
δ^{13}C	-15.28	0.24	22	-16.05	0.46	22
δ^{15}N	20.10	0.12	22	19.87	0.19	22
Foot luminosity (%)	44.61	6.16	21	39.10	8.42	14
Foot saturation (%)	20.06	1.51	21	22.79	2.29	14
Foot hue (°)	58.23	2.01	21	52.88	1.58	14
Immunological						
Immunocompetence index	0.41	0.02	43	0.33	0.02	37
Parasite load (number)	6.2	0.9	38	8.7	1.8	33
Rearing environment						
Sibling competition (days)	7.9	1.2	43	7.9	1.3	37
Hatching date	162.6	1.2	43	164.2	2.0	37
Parental						
Age of the father (years)	9.1	0.7	24	7.2	0.9	13
Age of the mother (years)	7.6	0.9	14	8.4	0.9	17
Arrival date of the father	118.8	1.58	24	127.2	2.87	13
Arrival date of the mother	116.7	2.24	13	119.1	2.89	16
Body mass of the father (g)	130.4	2.8	9	128.4	2.5	5
Body mass of the mother (g)	126.7	2.9	6	132.3	2.3	8

blood from the tarsus vein. Blood samples were stored in heparinised capillaries and centrifuged at 10,000 rpm for 8 min. The haematocrit, measured as percentage of packed red cell volume to total column height (plasma plus packed red cell volume), was determined directly in a micro-haematocrit reader. Two drops of plasma were transferred to a handheld optical refractometer to determine absolute concentration of plasma proteins (±0.1 g/dl), and the rest of plasma was used to analyse carotenoid levels (see below). Thereafter, T-cell-mediated immune response (CMI) of fledglings was measured using the phytohaemagglutinin (PHA) test (Smits et al. 1999). Chicks were injected 0.03 ml of 2 mg/ml PHA (Sigma) in phosphate-buffered saline into a marked site on the right internal foot web whose thickness had been previously measured with a digital micrometer (Mitutoyo, ±0.001 mm).

During the next colony check (two days later), the rest of phenotypical measurements were taken, including the thickness of the foot web again (48h ±17 min later). As the repeatabilities of both initial ($r = 0.98$, $F_{79,160} = 141.3$, $P < 0.001$) and final ($r = 0.97$, $F_{79,160} = 145.5$, $P < 0.001$) measurements, which were taken in triplicate, were high, the mean values were used, and the PHA response was calculated as the change (mean increase) in thickness (mm) of the foot web of chicks. At this time, too, some freshly grown body feathers (breast) were collected for further analysis of stable isotopes ($\delta^{13}C$ and $\delta^{15}N$, see Benito et al. 2011 for method). Besides, foot colour was measured for each chick using a handheld CM-2500d spectrophotometer (Konica Minolta, see Benito et al. 2011 for method). Using the colour space L*S*H, three colour components (Luminosity, Saturation and Hue) were determined and considered as discrete variables. Finally, we collected ectoparasites from the chicks by using the dust-ruffling method described by Clayton and Walther (1997), as this method has been shown to be an accurate predictor of total ectoparasites load (Clayton and Drown 2001). Ectoparasites from individual chicks were kept separated for subsequent count and care was taken to clean all working surfaces between fumigations.

Laboratory analyses

From the centrifuged microcapillaries, we removed the plasma and stored the samples at -60ºC. Carotenoid levels in plasma were determined following a slightly modified protocol from Tella et al. (1998). The concentration of total plasma carotenoids was measured spectrophotometrically. We added 380 μl of pure acetone to 20 μl of plasma (dilution 1:20), vortexed the mixture and precipitated the flocculent proteins by centrifuging the sample at 1,500 *g* for 10 min. We examined the supernatant in a Milton Roy Spectronic 601 spectrophotometer and determined the optical density of the carotenoid peak at 476 nm. Xantophylls, the most common carotenoids in birds, show two absorption maxima in acetone, and we choose the upper peak (at 474–478 nm) following previous researchers (Tella et al. 1998). Carotenoid concentration (mg/ml plasma) was derived from a standard curve of lutein (Sigma-Aldrich; Xanthophyll X-6250).

Definition of other chick and parental variables

Since multivariate measures of size are preferable to univariate ones (Freeman and Jackson 1990), we used principal component analysis, particularly the first axis (PC1), to combine morphological measurements of nestlings. A body condition index was calculated as the quotient between mass of the chick and the 1st factor of a PCA on wing, bill-head and tarsus length.

As “rearing environment variables” we considered other inherent characteristics of the chicks that influence their environment during growth. Brood size at hatching was defined as the number of chicks hatched in one clutch, and brood size at fledging as the number of siblings within a brood alive and ≥18 days old. Sibling competition was the number of days that a chick shared with sibling/s in the nest, and was calculated for each focal chick taking into account the number of siblings and when they died, until age 20 of the sampled chick. Therefore, it ranged from 0 days (sampled chick had a sibling which died the same day of hatching) to 20 days (sampled chick had one or two siblings that survived until this age). A second measurement of sibling competition classified nestlings according to the presence or absence of sibling interactions from age 14 onwards (time when competition for food is strongest, Becker and Wink 2003; and when energy required for growth is at its maximum, Ricklefs and White 1981). For these analyses we used the 61 chicks that were not single chicks (and therefore had siblings to compete with). Finally, we also classified the broods according to their sexual composition (all males, all females, mixed broods).

We use the term ‘parental quality’ in a broad sense to indicate characteristics of individual parents that have been shown to influence breeding performance in

Common Terns. In this study we used age, body mass, date of arrival to the colony and clutch size as proxies for parental quality (e.g. Ezard et al. 2007). Parental age was the age of the adult bird at the moment of the chick sampling. Body mass at arrival (in grams) was recorded as the average mass of the individual bird during the first four days of colony attendance. In Common Terns, body mass has been shown to be a good proxy of body condition independent of size (Wendeln et al. 1997). Arrival date in a given season was defined as the first day of registration at the colony site (log transformed to linearize the relationship with offspring sex) and is regarded as a consistent trait over years (Becker et al. 2008a). Clutch size (number of eggs laid) is also a good indicator of parental quality of Common Terns (Arnold et al. 2004). Adults laying 3-egg clutches arrive and breed significantly earlier, are older and more experienced and raise more fledglings than parents with clutch size ≤ 2 eggs (González-Solís et al. 2005).

Statistical analyses

We first performed analyses to explore individual or environmental variables possibly affecting the sexual dimorphism of the chicks. Because some measurements were lacking from some individuals, sample sizes varied between analyses (see Table 1).

The analysed set of morphological traits included an index of body condition, body mass, tarsus, wing and bill-head length. As physiological variables we considered haematocrit, absolute concentration of plasma proteins, total concentration of carotenoids in plasma, stable isotopes (carbon and nitrogen) of the feathers (only 2004) and feet colour (only 2005). Finally, the results of the PHA test (CMI) and the total number of ectoparasites in the feathers were used as immunological traits.

All univariate analyses of variance, covariance and logistic regressions included year and age of the chick as fixed effects, and hatching date as a covariate. In the case of CMI-response, body mass was introduced as a covariate too, to control its effect on the intensity of immune-responses (Alonso-Alvarez and Tella 2001). Initially, all explanatory variables and two-way interaction terms were introduced in the models, followed by a removal of non-significant interactions and terms.

The relationships between chick phenotype regardless of sex and the parental quality indices (age, arrival date and body mass) were examined first using multiple linear regressions between each chick measurement as the dependent variable and each one of the parental variables, plus year, age and hatching date as independent variables. We tested the significance of variables one by one, starting with the full model and removing variables in the order of significance (except the parental variable of interest) until reaching the most parsimonious model. We used residual plots and the change in R^2 explained by the model after exclusion of the focal variable, in order to test for the statistical significance of the contribution of each element to the

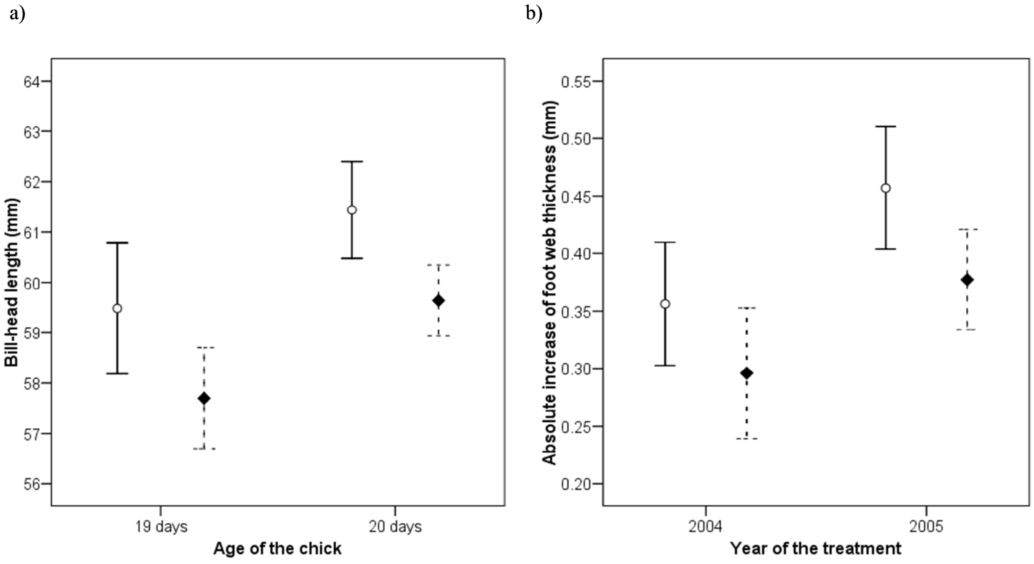

Fig. 1 Mean (95% CI) value for male (open circles) and female (filled rhombus, broken lines) nestlings for **a)** bill-head length in relation to their age **b)** T cell-mediated immune response in relation to their sex and studied year

model. Afterwards, sex and its interaction with the parental variables were subjected to visual inspection of data plots and multiple linear regressions where the phenotypical chick measurement was the dependent variable; sex, the parental index and the significant factors of the previous analyses (year, age or hatching date) were the independent variables, plus the cross-product between the centered parental variable and sex of the chick.

Parental characteristics were not known in all cases (see Table 1). Therefore predictors of maternal and paternal quality were assessed separately (to separate possible effects of male and female parents and to have a maximum sample size). All parents were different between years and only one chick per parent present; hence, there was no need to control for parental identity in the analyses.

However, given that parental information was often not available we used a second approach, considering clutch size (n = 80) instead of direct parental measurements. The parental influence was analysed using ANCOVAs of the effect of sex and clutch size (year and age if needed) on the phenotypical chick variable controlled by hatching date.

Sex-specific chick mortality was tested by means of logistic regressions. We first explored all the bivariate associations between the fate of the chicks and the possible predictors of mortality (sex, the morphological, physiological and immunological variables, and the rearing conditions). Afterwards we assessed one by one the possible interactions between sex and these variables on chick mortality. Variables were only included in the final multivariate logistic regressions if they showed significant effects in the previous two steps. Tests of Hosmer-Lemeshow (not reported in the final results) were used to assess the goodness of fit in all logistic regression models, before considering them acceptable. The significance of explanatory variables was determined by their Wald statistics, distributed as χ^2.

To control for type I statistical errors in simultaneous tests of the same individuals, we adjusted significance level downwards according to sequential Bonferroni correction (Rice 1989). However, most of the tests were non-significant even without applying Bonferroni correction. Given the large number of analyses and the generally non-significant results, most analyses are not reported here or not presented in detail. Results are presented as arithmetic means ± standard error.

Results

Offspring phenotype in relation to sex

The twelve characters measured in chicks were first subjected to multivariate analyses of covariance (excluding foot colour and isotopes, to operate with the greater sample size), with sex, year and age as factors, and hatching date as covariate, to account for the intercorrelations between variables. The only significant effect of sex in GLMs run on nestlings for which all phenotypical measurements were available (n = 68) was found on bill-head length, whereas CMI-response was borderline significant (Multivariate ANCOVA, n = 80: sex on bill-head length: $F_{10,66} = 12.18$, $P = 0.001$; sex on CMI: $F_{10,66} = 3.68$, $P = 0.059$). No significant interactions were observed between the rest of factors and sex ($P > 0.05$ in all cases).

Afterwards, we analysed individually the offspring morphological, physiological and immunological characters in relation to sex.

Dependent	Independent	MS	d.f.	*F*	*P*
Bill-head length	Intercept	44.13	3	9.68	**<0.001**
n = 80	Age of the chick	64.96	1	14.25	**<0.001**
	Sex of the chick	55.01	1	12.07	**0.001**
	Sex*age	0.001	1	2.2E-04	0.988
	Error	4.56	76		
CMI-response	Intercept	0.07	4	5.43	**0.001**
n = 80	Mass of the chick	0.01	1	0.51	0.476
	Year	0.16	1	12.11	**0.001**
	Sex of the chick	0.09	1	6.58	**0.012**
	Sex*year	0.00	1	0.04	0.836
	Error	0.01	75		
CMI-response	Intercept	0.04	14	3.48	**0.001**
n = 61	Mass of the chick	0.002	1	0.20	0.650
	Year	0.09	1	8.07	**0.007**
	Sex of the chick	0.17	1	14.62	**0.000**
	Clutch size	0.07	1	6.15	**0.017**
	Competition (age 14)	0.04	1	3.30	0.076
	Sex*year	0.02	1	1.58	0.210
	Sex*clutch size	0.02	1	1.69	0.190
	Sex*compet(14)	0.09	1	7.75	**0.008**
	Clutch size*compet(14)	0.07	1	5.84	**0.020**
	Sex*clutch size*compet(14)	0.05	1	4.41	**0.041**
	Error	0.01	46		

Table 2. Analyses of variance-covariance of offspring phenotypical variables in relation to sex: sexual dimorphism in bill-head length and CMI-response together with other factors.

Morphology

Mass, wing and tarsus length, and the index of body condition of chicks were not significantly different for males and females (n = 80, F-values associated to $P > 0.05$ in all cases). However, sex and age had a main effect on bill-head length (Table 2, Fig. 1a), with older chicks and males having on average larger values than younger chicks and females.

Physiology

Total plasma proteins and total carotenoid concentration of both male and female chicks were higher in 2005 than in 2004 (n = 79; proteins: $F_{1,71} = 24.87$, $P < 0.001$; carotenoids: $F_{1,71} = 22.62$, $P < 0.001$). Nevertheless, we found no sexual differences, neither in haematocrit level and stable isotopes signatures (n= 79 and 44 respectively, $P > 0.05$ in all cases). Colour variables such as luminosity and saturation showed the same lack of sex influence (n = 36, all $P > 0.05$). However, regardless of hatching date or age, hue of female chicks was significantly lower (6.7º on average), indicating a redder colour than in male foot (n = 36, $F_{1,34} = 5.92$, $P = 0.020$). This result, however, became not significant after Bonferroni correction.

Immunology

To test for sexual dimorphism in the immunological characteristics, we ran ANCOVAs adding the body mass as covariate. Although its effect was not found in our database (mass: n = 80, $F_{1,75} = 0.51$, $P = 0.47$), we included it in all the models.

Sex of the chick had a significant influence on CMI-response, which was also markedly higher in 2005 than in 2004 (Table 2, Fig. 1b). Year, however, did not affect the immune response of sexes differentially (interaction not significant, Table 2), and overall, male chicks showed significantly larger values of CMI than females.

No significant relationship between the number of feather parasites and sex was found, although both year and hatching date affected the parasitological state (hatching date: $F_{1,75} = 34.11$, $P < 0.001$; year: $F_{1,75} = 4.37$, $P = 0.040$), with parasites in higher number in 2005 and increasing with later hatching date. These effects remained significant even after correction of significance values.

Offspring phenotype in relation to sex and rearing environment covariates

The analyses described above were re-run to explore the influence of rearing characteristics on sexual dimorphism of the chicks. We found no significant effect of sex on any morphological, physiological or immunological trait when variables such as brood size (at hatching and fledging), sibling competition and the sexual composition of the brood were included in the models (all $P > 0.05$).

To sum up, sex did not affect nestling phenotype except for bill-head length and T-cell mediated immune response, and in general, no differential effect of age difference or rearing conditions existed on the phenotype of male vs. female nestlings.

Offspring sexual dimorphism in relation to parental factors

After Bonferroni corrections, three parental traits showed a significant relationship with different aspects of chick phenotype. Fathers which arrived later to the colony had chicks with shorter bill-head length (Table 3a, Fig. 2). The relationship remained significant when including sex and the cross-product between sex and the paternal trait to the regression model, but these factors themselves had no significant influence (Table 3b). The carotenoid plasma concentration of chicks was positively influenced both by increasing father age (Table 3a) and maternal body mass (Table 3a), although with no sexual difference (Table 3b). Carotenoid content was higher in 2005 as already known (see above), and additionally, decreased with hatching date in the maternal case (Table 3). However, the relationship between mass of the mother and carotenoids of chicks should be interpreted with caution due to the reduced sample size.

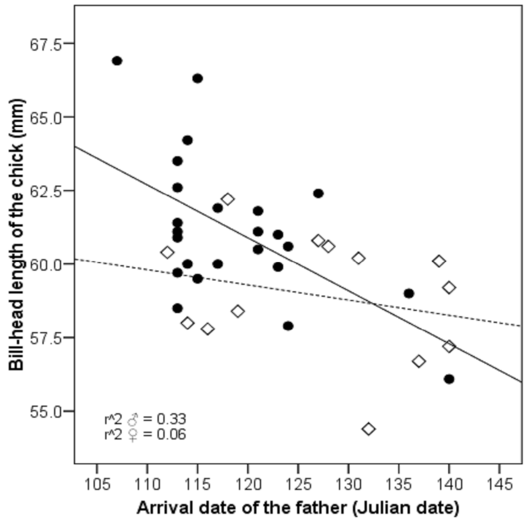

Fig. 2 Sex effect (males: filled circles; females, open rhombus, broken line) of arrival date of the father to the colony: correlation for sons marginally significant

Table 3. Multiple linear regression models of offspring phenotypical variables in relation to parental traits.
a) without sex **b)** with sex (all interactions sex*parental trait were non-significant; details of these models not shown).

	Dependent	Independent	β	*P*	r^2	n
a)	**Bill-head length**	Arrival date father	-0.498	0.001	0.30	37
		Age of the chick	0.218	0.13		
	final model: r = 0.58, $F_{2,34}$ = 8.78, P = 0.001					
	Carotenoids in plasma	Age father	0.411	0.012	0.35	37
		Year	0.308	0.055		
	final model: r = 0.60, $F_{2,31}$ = 8.46, P = 0.001					
		Body mass mother	3.846	0.004	0.86	14
		Year	6.115	0.000		
		Hatching date	-6.788	0.000		
	final model: r = 0.95, $F_{3,9}$ = 25.96, P < 0.001					
b)	**Bill-head length**	Arrival date father	-0.378	0.020	0.39	37
		Age of the chick	0.250	0.082		
		Sex of the chick	0.265	0.088		
	final model: r = 0.63, $F_{3,33}$ = 7.25, P = 0.001					
	Carotenoids in plasma	Age father	0.375	0.027	0.30	37
		Year	0.316	0.051		
		Sex of the chick	-0.13	0.39		
	final model: r = 0.60, $F_{3,30}$ = 5.84, P = 0.003					
		Body mass mother	0.43	0.011	0.84	14
		Year	0.68	0.000		
		Hatching date	-0.77	0.000		
		Sex of the chick	-0.01	0.96		
	final model: r = 0.94, $F_{4,8}$ = 17.31, P = 0.001					

Similarly, we found no significant effect of sex or the interaction between sex and the parental covariates for mainly all variables, indicating that sexual dimorphism of the offspring did not vary in relation to parental age, arrival date or body mass ($P > 0.05$ in all cases). The only exception was nestling CMI-response linked with maternal body mass while controlling by chick mass and year (ANCOVA: n = 14, $F_{5,14} = 3.90$, $P = 0.043$, $r^2 = 0.70$; interaction sex chick*mass mother: $\beta = 0.739$, $P = 0.028$). Male chicks, but not females, showed increased CMI-response with increasing maternal mass (n = 6, $\beta = 0.657$, $P = 0.044$, $r^2 = 0.57$). However, apart from the low sample size, this result was no longer significant after correction.

When using clutch size to evaluate parental effects, T-cell immune response of the chicks was again the only influenced trait: apart from the already described effects of year and sex, chicks from 3-egg clutches tended to have higher CMI responses than chicks from 2-egg clutches (ANCOVA, n = 80; clutch size: $F_{1,71} = 3.71$, $P = 0.058$, sex: $F_{1,71} = 6.70$, $P = 0.012$; Fig. 3a). Moreover, a sex-specific effect of T-cell immune response related to clutch size appeared when the presence of sibling interactions from age 14 onwards (when food competition is strongest) was included (Table 2). Models split by clutch size revealed that, in 3 egg-clutches, males showed significantly higher CMI responses than females, among the chicks that were not alone in the nest (ANCOVA, n = 45; sex: $F_{1,37} = 9.50$, P = 0.004; sex*competition14: $F_{1,37} = 5.18$, P = 0.029; Fig. 3b). Univariate contrasts also indicated that males competing for resources had stronger CMI responses than males alone in the nest ($F_{1,37} = 4.14$, P = 0.049; Fig. 3b).

Age, mass or arrival date of parents did not influence the length of offspring competition or the sexual composition of their clutches (ANOVAs and logistic regressions, $P > 0.05$ in all cases).

Offspring mortality in relation to sex and phenotype

Overall, 19 out of our 80 tested chicks (≈ 24%) died before leaving the colony; of these, 10 were males. Hence, there was no difference in the mortality of male and female chicks ($\chi^2_1 = 0.02$, $P = 0.91$). Bivariate analyses showed year and hatching date as significant predictors of chick mortality (year: 51% of dead chicks in 2004 and 12.5% in 2005; $\chi^2_1 = 5.27$, $P = 0.022$; hatching date: $\chi^2_1 = 8.07$, $P = 0.004$) and we retained them in all initial models of mortality.

None of the characteristics of the chicks appeared to influence their survivorship (bivariate logistic regressions, χ^2-values associated to P > 0.05 in all cases). Moreover, the lack of statistically significant effects of interactions between each variable and sex indicated that males and females, fledged and dead chicks, were phenotypically similar (all F-values associated to *P*-values > 0.05).

Mortality and sex-related sibling competition

Mortality was also analysed in relation with the rearing conditions of the chicks. Brood size at hatching or fledging did not significantly predict overall mortality (n = 80, both χ^2 values associated to $P > 0.5$).

For the following analyses, only the 61 nestlings that were not single-chicks were used. Among them, the brood composition (regarding sex of the sibling/s) was known for 52 chicks: 30 of them belonged to mixed broods and 22 to unisexual broods (11 all-female and 11 all-male broods). Chicks from mixed, all-male or all-female broods did not differ significantly in mortality risk (n = 52, $\chi^2_2 = 4.52$, $P = 0.1$).

However, chick mortality after age 20 (controlled by hatching date, $\chi^2_1 = 4.48$, $P = 0.034$) was affected by the number of days that a chick shared with siblings in the nest before age 20 (omnibus test: n = 61, $\chi^2_4 = 24.14$, $P < 0.001$; competition: $\chi^2_1 = 5.34$, $P = 0.021$). On average, dead chicks shared a 4.8 ± 1.9 days longer period than fledged chicks. Furthermore, the amount of competition affected both sexes differently (interaction sex*competition, $\chi^2_1 = 6.84$, $P = 0.009$). Males of different fate competed with siblings a similar number of days (alive males, 10.4 ± 1.4 days; dying males, 9.5 ± 2.6 days; n = 33, $\chi^2_2 = 2.03$, $P > 0.1$), whereas females which finally died had suffered the longest periods of competition (dying females, 18.4 ± 0.9 days; alive females, 7.2 ± 1.2 days; n = 28, $\chi^2_2 = 5.67$, $P = 0.017$), and also when compared to dying males (n = 17, $\chi^2_2 = 3.76$, $P = 0.050$).

These factors were also intercorrelated. As expected, the longer periods of competition within siblings occurred in 3-chick broods (ANOVA, n = 61, $F_{1,59} = 17.54$, $P < 0.001$), but also in mixed-sex broods (ANOVA: n = 52, $F_{1,50} = 8.49$, $P = 0.005$). The proportion of mixed broods was significantly higher in 3-chick broods (n = 52, $\chi^2_1 = 7.87$, $P = 0.005$). To investigate which of these parameters was more influential on chick mortality, we used stepwise logistic regression with all three variables, sex, their interactions, year and hatching date. Confirming previous results, days of competition, its interaction with sex, and hatching date were the only terms that remained in the model (Table 4).

Discussion

In this study we investigated sexual dimorphism in morphological, physiological and immunological traits in Common Tern nestlings, and the association between dimorphic traits, parental factors and rearing conditions. From all the traits examined we only

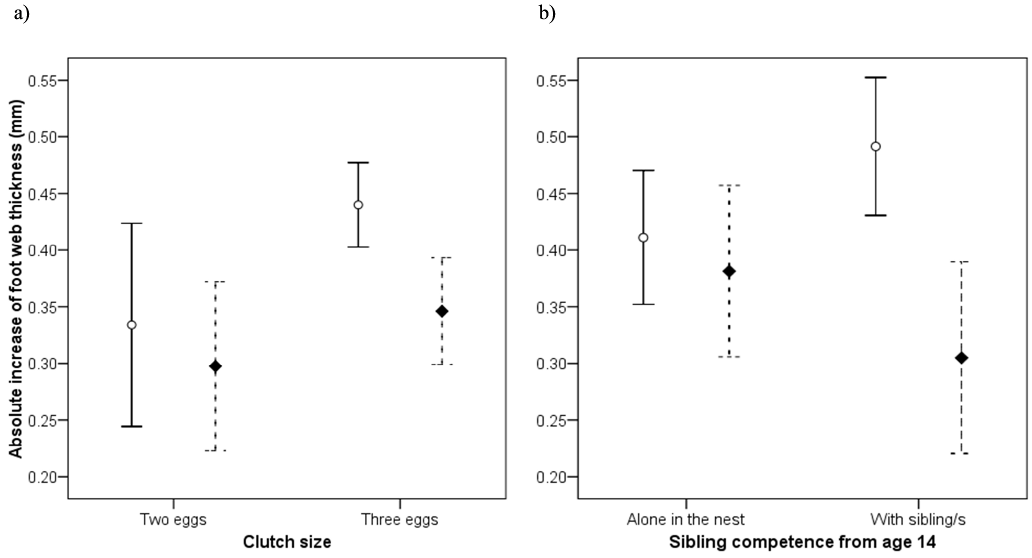

Fig. 3 Mean (95% CI) value of CMI response in male (open circles) and female (filled rhombus, broken lines) nestlings, where sexual dimorphism was observed **a)** with the effect of clutch size **b)** with presence of sibling interactions in 3 egg-clutches

detected sexual dimorphism in two characters: male nestlings showed larger bill-head lengths and T-cell mediated immune-responses than female nestlings. We also found that some traits related to parental quality associate positively with the CMI response of male nestlings. On the other hand we found no differential effect of age, hatching date or rearing variables on the phenotype of sons vs. daughters, and none of the characteristics of the chicks appeared to influence their survivorship. However, although both sons and daughters later fledged or found dead were phenotypically similar, the endurance under sibling competition was longer for female than for male chicks.

Morphologically, a slight sexual dimorphism has already been described: the Common Tern is a monomorphic species in most characteristics but presents a 6% difference in adult bill length (longer in males, Wendeln et al. 1997) and a 3% difference in fledging mass (males fledge heavier than females, Becker and Wink 2003). Here, the larger bill-head length present in male adults and in post-fledglings (Becker and Wink 2003) was already significant at age 19 and 20 days (value also increasing with age), whereas at this age mass did not differ between sexes. Indeed, mass begins to diverge in a sex-dependent way from 18 days old onwards (Becker and Wink 2003), so the lack of dimorphism in our results probably result from a lack of power to detect subtle differences just at the beginning of divergence process. Alternatively, it may be the consequence of the very poor productivity of the studied years (overall colony success: 0.28 and 0.16 fledgings/pair in 2004 and 2005, respectively; cf. Szostek and Becker 2012), which could obscure this process.

T-cell mediated immune-response, likewise, was also found to be sex-specific. Sexual differences in immune defence are a common phenomenon in birds (Moller et al. 1998, Zuk and McKean 1996), even detected at early stages of development (Lobato et al. 2008). Sex-specific patters are, in any case, not completely clear, and lack of differences have been also reported (Lobato et al. 2008, Saino et al. 2002). However, generally males show reduced immune activity compared to females, which has been related to their high concentration of immunosuppressive hormones (Roberts et al. 2004). Moreover, when chicks are experimentally challenged (enlarged broods, food limitation, increased competition) males usually suffer stronger decreases of CMI than females (Dubiec *et al.* 2006, Fargallo *et al.* 2002). Our results, on the contrary, showed consistent stronger responses of males, in larger clutches (3 eggs) and especially in males that had to compete with siblings in final periods of development. Testosterone and corticosterone levels in Common Tern nestlings are low and similar for both sexes (Braasch et al. 2011), and maybe do not play an important role at this age. More plausibly this effect is related to parental quality, as indicated by clutch size (and maternal mass, although evidence was weak given the low sample size). Since development and maintenance of immune function is highly dependent on nutritional resources (Lochmiller and Deerenberg 2000), the higher CMI of sons suggest that high quality parents might be providing better rearing conditions to sons than to daughters, even in years of low productivity as the studied ones. Given the absent (this study) or rather small difference in body mass between sexes of young (Becker and Wink 2003), a likely possibility is that male developmental strategies prioritize the allocation of these extra resources to immune function and competitive ability (Soler et al. 2003), which would also explain the larger response particularly in males with sibling interactions.

On the other hand, female chicks seem more sensitive to competitive scenarios; CMI response was negatively influenced by sibling presence in the last period of development (Fig. 3b). Previous studies (Becker and Wink 2003, Braasch et al. 2011) also found evidences of daughters suffering most from the presence of siblings. However, morphological and physiological condition of daughters was similar to sons, which suggests a different strategy of females

Predictor	β	SE (β)	Wald's χ^2	d.f.	*P*	OR
Constant	19.159	9.405	4.149	1	0.042	-
Hatching date	-0.119	0.056	4.484	1	0.034	0.888
Competition (days)	-6.584	2.930	5.050	1	0.025	0.001
Sex	-0.088	0.068	1.686	1	0.194	0.916
Sex by Competition	0.463	0.177	6.847	1	0.009	1.589

Table 4. Final logistic regression model on mortality of 61 chicks with rearing condition variables and sex.

regarding the trade-off between growth and immune response (Soler et al. 2003) when their rearing conditions include sibling interactions. In order to attain similar size and condition than males, females may reduce investments in immune function in favour of other traits. Evidence of this strategy of female tern nestlings was already found in a previous analysis of the trade-off between carotenoid-based coloration and immune response (Benito et al. 2011). Such a pattern may be optimal if underdevelopment of the immune system at the nestling stage can be compensated later in life (Birkhead et al. 1999). However, this strategy may imply some risks: females that died after age 20 d had also resisted the longest periods of nest competition. Unlike males and surviving females, they experienced the time (pre-fledging ages 14-20) when food demands of offspring increase considerably (Klaassen et al. 1992), and the costs of compensation may make them more vulnerable in the demanding post-fledging period (Metcalfe and Monaghan 2001).

Other parental characteristics were also expected to contribute to phenotypic dimorphism between sons and daughters. We did not find strong evidence that parental factors were related to nestlings phenotypic traits, and the few associations found (carotenoid plasma levels of chicks increased both with age of the father and mass of the mother) were not sex-specific. However, bill-head length was shorter in chicks whose fathers arrived later to the colony, although this affected sons only marginally more than daughters. Arrival date in terns is age-dependent, but nevertheless a consistent individual trait over years (Becker et al. 2008a) and negatively correlated with breeding experience and success (Benito et al. 2013); thus, lower-quality fathers tended to have sons with shorter bills. It is unknown whether bill-head length is a secondary sexual character in terns, but Coulter (1986) found assortative mating by bill size in Common Terns, and high quality individuals are predicted to mate with each other. Altogether, this evidence suggests the possibility of an adaptive reflection of male quality towards sons mediated by the adult female.

One possible implication of the general lack of sexual dimorphism, with only subtle differences among nestlings, may be that no great differential costs of raising males vs. females exist. Therefore, parental investment after birth could be non sex-specific, which is supported by the evidence of parity in sex-specific mortality and fledging sex ratio found in the species (Benito et al. 2013; but see Becker et al. 2008b). Nevertheless, it could also be that differential parental investment in relation to sex does occur, but in order to achieve a similar phenotypic state for sons and daughters; especially if, as some models of differential allocation of parental investment suggest, fathers are selected to favour offspring of one sex and mothers those of the other sex (Lessells 1998). In this case, the study of differential investment might be better approached by studying adult phenotype and sexual characters related to chick characters. Finally, sex-allocation between the offspring in Common Terns may occur previously, in the form of adjusted primary sex ratio related to parental traits (Charnov 1982).

In conclusion, we found dimorphism in bill-head length and immune response as sexual differences in expression of traits. Differences in CMI-response were related to clutch size (parental quality) and post-hatching effects such as year (environmental) and sibling competition (rearing conditions): they may result from divergent strategies of resource allocation during growth of male and female nestlings. If the development of these phenotypic components gives different fitness returns for each sex, then it may be reflecting a difference in reproductive value of sons and daughters. No clear adjustment of relative quality of nestlings of the two sexes occurred in relation to parental age, mass or arrival date; but parental reproductive quality, as measured globally by clutch size, may suggest certain pathways for parental sex-allocation that differentially affect sons and daughters.

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4

Carotenoid supplementation and sex-specific trade-offs between colouration and condition in common tern chicks

Suplementación de carotenos y compromisos diferenciales por sexo entre coloración y condición en pollos de charrán común

María M. Benito[1], Jacob González-Solís[1], Peter H. Becker[2]

Los carotenoides, pigmentos con propiedades antioxidantes e inmuno-regulativas, desempeñan un papel crucial en individuos en desarrollo. Los carotenoides son sintetizados principalmente por plantas y algas, y deben adquirirse a través de la dieta, siendo relativamente escasos. Por tanto, su disponibilidad es un factor limitante que puede conducir a un compromiso entre su utilización para la exhibición de color y las funciones fisiológicas. Sin embargo, las diferencias potenciales entre pollos macho y hembra en este compromiso de utilización han sido poco estudiadas. Siguiendo un diseño experimental, manipulamos la disponibilidad de carotenoides para 39 pollos de charrán común (Sterna hirundo) de 9 días de edad. Durante los siguientes 9 días, suplementamos su dieta natural de pescado con cuatro carotenoides (tres xantófilas: luteína, zeaxantina, cantaxantina y un caroteno: β-caroteno). El objetivo era examinar las respuestas específicas de cada sexo al incremento experimental de carotenos en la circulación de plasma, en la condición física y fisiológica y en la supervivencia. Además, para explorar la base funcional y evolutiva del compromiso, estudiamos la relación entre la concentración de carotenos, la respuesta inmune mediada por linfocitos T y la coloración de los pies.

Después del tratamiento, los pollos del grupo control mostraron concentraciones en el plasma decrecientes para la mayoría de los carotenoides, mientras que en los pollos suplementados aumentaron de forma significativa. La luminosidad y la saturación del color aumentaron en ambos grupos, pero el tono de color cambió significativamente hacia un tono de rojo más intenso en las hembras suplementadas. Los pollos suplementados no mostraron diferencias en la respuesta inmune o en otras características respecto a los pollos control. Sin embargo, las hembras suplementadas mostraron tendencia a desarrollar respuestas inmunes reducidas y firmas isotópicas de nitrógeno crecientes, y los machos suplementados, a desarrollar mayor masa corporal.

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Estos resultados indican que la coloración, en el caso de las hembras, podría desempeñar una función de señalización que compense los costes inmunológicos. En los machos, una disponibilidad adicional de carotenoides podría contribuir a mejorar la condición física. Este estudio sugiere que la respuesta al compromiso de utilización de carotenoides es específica para cada sexo en el charrán común. Por tanto, el suministro de carotenoides por parte de los padres podría ser un componente no reconocido en la asignación por sexo.

Palabras clave
Compromiso fisiológico; pigmentos plasmáticos; asignación de sexo; pollos de charrán común; suplementación.

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Carotenoid supplementation and sex-specific trade-offs between colouration and condition in common tern chicks

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Abstract Carotenoids, as pigments with antioxidant and immunoregulatory properties, play a crucial role in developing chicks. Carotenoids must be acquired through diet and are relatively scarce, suggesting that their availability is a limiting factor leading to a trade-off between colour displays and physiological functions. However, potential differences in this trade-off between male and female chicks have been little studied. We manipulated carotenoid availability in 9 days old common tern *Sterna hirundo* chicks by supplementing their fish diet with four carotenoids during 9 days. Our aim was to examine sex-specific responses to the experimental increase of dietary carotenoids on plasma circulation, physiological and condition variables and successful fledging. Furthermore, to explore the functional and evolutionary basis of the trade-off, we studied the relationships among carotenoid concentration, mediated immune response and foot colouration. After treatment, control chicks showed decreasing plasma levels for most carotenoid types, whereas supplemented chicks had strong increases. Colour luminosity and saturation increased in both treatment groups, while hue only changed significantly towards redder feet in supplemented females. Supplemented chicks presented neither different T-cell-mediated immunity nor other differences compared to control chicks. Nevertheless, supplemented females showed tendencies towards decreased immune responses and increased δ^{15}N signatures, and supplemented males towards greater body mass. Our results indicate colouration may have, in females, a signalling function as to compensate for immunological costs. In males, additional availability of carotenoids may contribute to improve the body condition. This study suggests that trade-off responses to carotenoid availability are sex-specific in tern chicks. Thus, parental carotenoid supply to chicks may be an unrecognised component in sex allocation.

Keywords Physiological trade-off · Plasma pigments · Sex allocation · *Sterna hirundo* chicks · Supplementation

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Introduction

In birds, carotenoids are of major responsibility for feather colouration, but when deposited in the integuments they also result in the expression of many colours in animal fleshy structures (Velando et al. 2006), such as legs and feet (Hudon and Brush 1990). Colours are used in sexual communication, signalling between parents and their offspring and in warning functions, among others. Carotenoids, however, are a large group of biologically active pigments that also perform other physiological functions. They play important roles in different mechanisms directly related to the immune system, and are well known for their activity as antioxidants (reviewed in Møller et al. 2000) and as scavengers of free radicals produced in intense metabolic processes typical of rapid growth or stressful conditions (Surai 2002), although their contribution importance to the former function has recently become controversial (Costantini and Møller 2008).

Carotenoids are synthesised mainly by plants and algae (Goodwin 1984), and should therefore be acquired through the diet. Although still limited, evidence to date posits that they are a scarce resource in nature which may vary geographically and seasonally (Grether et al. 1999, Møller et al. 2000). In the marine environment, some animals (e.g. molluscs, crustaceans, fishes) accumulate carotenoids, being the exclusive source of pigments for seabirds. Moreover, the ability to absorb, transport, and metabolise carotenoids is under genetic and physiological control and may vary substantially among individuals, increasing the variability of the effective availability for the organism (Hill 1991). Thus, under the assumption that carotenoids are scarce and differentially assimilated by individuals, it has been hypothesised that their availability is a limiting factor leading to a trade-off in their allocation to different functions.

For altricial or semi-precocial chicks, the diet is entirely constrained by parental feeding behaviour regarding frequency, quality and type, which might make carotenoids in short supply. These may play a crucial role in developing chicks, because the metabolic processes typical of rapidly growing bird species result in the abundant release of free radicals (Surai and Speake 1998). Few days after hatching, most carotenoids deposited into the egg yolk are metabolised or mobilised into tissues, and therefore food becomes the main source of carotenoid intake (e.g. Blount et al. 2002; Surai et al. 2001). However, the way in which this carotenoid-enriched food is distributed by parents among the young in their brood may follow different strategies to adjust this resource to the reproductive value of the offspring. Several studies on different bird species have shown parental preferences for offspring in relation to their carotenoid-based signals, which are probably providing information about the offspring quality (de Ayala et al. 2007; Saino et al. 2000). These carotenoid-based characters may be perceived by parents as honest signals of the offspring ability to metabolise, utilise and store a scarce resource, diverting carotenoids from immune function to pigmentation, and thus may influence the investment level of parental care (e.g., provisioning rate or quality of the prey delivered) (Kilner 2006). Recently, Griggio et al. (2009) provided direct evidence of differential parental allocation based on carotenoid ornamentation: rock sparrow (*Petronia petronia*) chicks with experimentally enlarged colour patch were fed more frequently. Therefore, a link between diet composition and colour signals may arise, if adults modulate food supply depending on chick colouration. Investigating diet composition during the chick growing period is now possible using the analysis of stable-isotope signatures (δ^{13}C and δ^{15}N) in body feathers. On one hand, the stable-nitrogen isotopic composition of consumers is enriched in nitrogen-15 relative to their prey (Hobson and Clark 1992). Therefore, individuals with higher δ^{15}N values feed on prey of higher trophic levels. Body tissues are enriched in ^{15}N relative to ^{14}N during digestive processes, and thus prey with greater δ^{15}N signatures may be considered of higher nutritional quality (e.g. Forero et al. 2002). On the other hand, the stable-carbon isotopic composition provides information on the sources of nutrients (Hobson and Clark 1993) and in the marine environment carbon-13 is enriched in benthic feeders relative to pelagic feeders.

Despite the increasing number of carotenoid studies carried out in nestlings, the positive effects of carotenoid availability on chicks are still controversial, with some studies showing significant results while others did not. Most common found effects include increased immune response (Cucco et al. 2006, but see Saino et al. 2008); increased colouration (Casagrande et al. 2007), improved growth and/or condition (Cucco et al. 2006, but see Costantini et al. 2007), or others like improved behavioural performances or feather growth (de Ayala et al. 2006, but see Tschirren et al. 2005). In addition, despite having a potentially relevant role in sex allocation, sex-specific requirements and responses of chicks to carotenoid availability are poorly known. Whereas some studies found no sex-related variation in egg carotenoids or contrasting effects of pigments in sons and daughters (Romano et al. 2008; Saino et al. 2003), others found evidence of maternal bias for nutrients towards eggs with the highest reproductive value (Badyaev et al. 2006; Verboven et al. 2005). Moreover, McGraw et al. (2005) showed that supplemented female zebra finches (*Taeniopygia guttata*) incorporated more carotenoids into eggs which produced more sons, which were also more ornamented. Carotenoid-supplemented mothers also produced male hatchlings that were heavier in great tits (*Parus major*) (Berthouly et al. 2008). Thus, carotenoid availability during egg production seems to affect nestling condition in a sex-specific way with potentially long-lasting effects on fitness. However, very little is known about differential effects of carotenoids in pigmentation, self-maintenance and immune defence in developing male and female chicks.

To shed some light on the carotenoid trade-off between signalling and physiological functions in relation to sex, we present a field experiment based on supplementing with carotenoids the diet of common tern (*Sterna hirundo*) chicks, mainly fed juvenile fish captured in marine or limnetic waters (e.g. Frank 1992; Becker and Ludwigs 2004). Growth of common tern chicks is very rapid and, due to the existent though minor sexual size dimorphism in the species, is also a sex-specific process (Becker and Wink 2003) which may be influenced by carotenoid availability.

Regarding colouration, adult plumage is white or grey with crown and nape black, and chick upperparts are buff to grey, patterned with blackish brown. Furthermore, adults and chicks exhibit a great variation in the intensity of orange-red pigmentation in feet and bill (authors, personal observation). Red colouration in bare parts is often based on carotenoids (Olson and Owens 2005) and in gull and tern species reflects individual quality (Kristiansen et al. 2006; Møller et al. 2007) and is related to carotenoid intake (Blount et al. 2002).

In particular, we aimed (1) to describe the patterns of plasma carotenoids in male and female chicks of common tern, (2) to study the effects of carotenoid availability on the immune system, some morphological and physiological proxies of condition, growth rate, and on the integumentary colouration of male and female chicks, (3) to determine sex-specific responses of carotenoid availability on the trade-off between colour and health-related functions, and (4) to explore differences in the food supplied to chicks, as indicated by stable-isotope analyses. If immunocompetence and colour do indeed trade off, unsupplemented chicks may be forced to allocate carotenoids to colouration to the detriment of the immunocompetence or vice versa, while supplemented chicks would be able to allocate carotenoids to each of these functions. Thus, they could easily meet the carotenoid demands of the immune system and use the leftover carotenoids to colour the feet and improve other physiological conditions. Therefore, we predicted that carotenoid supplementation would have positive effects on both immune response and colouration (especially on hue and saturation, respectively representing the type and amount of pigmentation). In consequence, we also expected supplemented chicks to improve growth or condition (measured through haematocrit, plasma proteins, morphology, growth rate and survival). Moreover, if sex-specific growth of common terns is influenced by carotenoid availability, it could be reasonable to expect sex-differential strategies in the use of carotenoids, to increase competitive advantage over siblings or to obtain parental preferences at feeding time.

Materials and methods

Study area and data collection

We conducted the experiment in 2006 at the Banter See common tern colony in the harbour area of Wilhelmshaven (Lower Saxony, Germany; 480 breeding pairs). At this site, an integrated population study has been in progress since 1992 to study various aspects of the life history and demography of this species (Becker et al. 2001). Each clutch (modal clutch size is three eggs) was checked every 2–3 days during the breeding season and laying and hatching date, laying and hatching order, chick mass and the fate of eggs and chicks were determined (Wagener 1998). Each hatchling was individually ringed and marked with a transponder before fledging for lifetime identification (Becker and Wendeln 1997). Chicks were molecularly sexed by plucking a body feather and using standard PCR methods (Becker and Wink 2003) once the experiment was finished.

Experimental design

Chicks were sampled from two sub-colonies (island D and E). Only one chick per brood (not injected with a transponder throughout the experiment) and only a-chicks, which were exactly aged, were studied. Finally 57 chicks were sampled and randomly assigned to the control or supplemented group, of which 39 (21 males and 19 females) survived until the end of the experiment. Chicks of the control group ($n = 21$, 11 males and 10 females) were given an empty pill by means of a gastric catheter. Chicks of the supplemented group ($n = 18$, 10 males and 8 females) were supplemented with a cocktail pill of four carotenoids, provided with the following products: Oro GLO 20 Dry (1.8% lutein, 0.2% zeaxanthin; Kemin Europe NV, Belgium), Carophyll Red (10% canthaxanthin, DSM Nutritional Products, Spain) and β-carotene (12.1% richness, Roig Farma, Spain). The mixture supplied 2 mg of total carotenoids; lutein and zeaxanthin to represent all yellow xanthophylls, canthaxanthin to represent all orange-red xanthophylls and β-carotene. In the absence of information about usual dietary carotenoid intakes of the species, the selected proportion equated that identified in studies of gull eggs (40:25:35%) and represents around twice the amount of carotenoids occurring in an average gull egg (Blount et al. 2002). This amount elevates plasma-carotenoid levels within the physiological range of values already found in a previous pilot study in common tern chicks of this age (3–60 µg/ml plasma, $n = 131$, authors unpublished).

When chicks were 9 days old, we determined their body mass using a digital balance to the nearest ±1 g, scored feet colour (see below) and took 100–150 µl of blood from the tarsus vein. Blood samples were stored in heparinized capillaries and centrifuged at 10,000 rpm for 8 min. The haematocrit, measured as percentage of packed red cell volume to total column height (plasma plus packed red cell volume), was determined directly in a micro-haematocrit reader. Two drops of plasma were transferred to a handheld optical refractometer to determine total plasma proteins (±0.1 g/dl), and the rest of plasma was used to analyse carotenoid levels (see below). Chicks were first supplied with a carotenoids pill later the same day. During a total period of 9 days, chicks were daily given a pill, prepared the day before and kept in the fridge to avoid oxidation. When chicks were 18 days old, 1 day after the last pill intake, we took again the mass and foot colour measurements and blood samples. Besides, we measured head-bill and tarsus length using a Vernier calliper (±0.1 mm) and wing (ruler, ±1 mm), and performed an assay for cell-mediated immunocompetence by a PHA test. This test provides a measure of the proliferative response of circulating T lymphocytes to the injected mitogen phytohaemagglutinin (PHA). After injection, a prominent perivascular accumulation of T lymphocytes is followed by macrophage infiltration, and the acquired immunity is measured as the amount of swelling after a certain time (Smits et al. 1999). Chicks were injected 0.03 ml of 2 mg/ml PHA (Sigma) in PBS into a marked site on the right internal foot web. The thickness of the foot web was measured with a digital micrometer (Mitutoyo, ±0.001 mm) at the injection site prior to and 48 h (±27 min) after challenge (during the next colony check). As the repeatabilities of both initial ($r = 0.94$, $F_{38,78} = 53.68$, $p < 0.001$) and final ($r = 0.97$, $F_{38,78} = 110.4$, $p < 0.001$) measurements, which were taken in triplicate, were high, the mean values were used for statistical analyses and the PHA response was calculated as the mean increase in thickness (mm) of the foot web of chicks. Some freshly grown body feathers (breast) were collected for further analysis of stable isotopes (δ^{13}C and δ^{15}N) at this time too. The feathers were washed in a NaOH solution, rinsed thoroughly, oven dried at 60°C and ground to a fine powder in a freezer mill. Feather powder (0.4 mg) was placed into tin capsules and then oxidised in a Flash EA1112 coupled to a Delta C Finnigan MAT stable-isotope mass spectrometer. The isotopic ratio mass spectrometry facility at the Serveis Cientifico-Tecnics of the Universitat de Barcelona (Spain) applied international standards that were inserted every 12 samples to calibrate the system and compensate for any drift over time.

Plasma-carotenoid analysis

From the centrifuged microcapillaries, we removed the plasma and stored the samples at −80°C. Carotenoid levels in plasma were determined following a slightly modified protocol from McGraw et al. (2003). We added 200 μl of ethanol and 100 μl of *tert*-butyl methyl ether (tBME) to 25 μl of plasma, thawed at room temperature, and vortexed. The mixture was then introduced in an ultrasonic bath for 5 min (at room temperature), and centrifuged in an Eppendorf centrifuge (Biofuge 13) at 13,000 rpm for 4 min. Afterwards, 200 μl of the supernatant were transferred to a new tube and evaporated to dryness under a stream of nitrogen. The remaining residue was dissolved in 200 μl of HPLC mobile phase (methanol–acetonitrile–chloroform, 46:46:8, v/v/v) and vortexed prior to HPLC analysis.

We injected 50 μl of each sample into a Pronto SIL 200-5-C30 HPLC column (250 × 4, 6 mm ID; Bischoff GmbH, Germany) fitted on a D-7000 HPLC system with La Chrom L-7100 pump (VWR, Hitachi, Merck, Ltd.) and L-7450 Diode Array Detector (Hitachi Ltd.). Temperature was maintained at 31°C (Coulometrics column heater, VIC Inc.). Carotenoids were separated using the mentioned mobile phase (with a gradient programme) during 35 min at a flow rate of 1.2 ml/min. This method has been proved to effectively recover all types of carotenoids (McGraw et al. 2008). We confirmed the identity of plasma pigments by comparing their retention times to those for authentic reference carotenoids provided by DHI LAB Products (Denmark), and concentration of each carotenoid type was determined by calculating peak areas (D-7000 HPLC System Manager, VWR, Hitachi, Ltd.). Besides, a few plasma samples were repeated in order to double check the standard HPLC procedure (ICC, $r = 0.77$, $F_{9,10} = 7.83$, $p < 0.002$; $n = 10$).

Colour measurements

Foot colour was measured for each chick on the first (9 days old) and last (18 days old) day of the experiment using a handheld CM-2500d spectrophotometer (Konica Minolta). Measurements were carried out inside the field station at constant temperature. Feet of the chicks were cleaned with water, air-dried and measured twice for the colour. After checking for repeatability (at 9 days old: luminosity $r = 0.83$, saturation $r = 0.86$, hue $r = 0.87$, all $p < 0.01$; at 18 days old: luminosity $r = 0.88$, saturation and hue $r = 0.86$, all $p < 0.02$; $n = 39$), the two measurements were finally averaged. Initially, bill colour was also measured but we felt measurements were not completely reliable because usually the bill of the chick was too small to cover the whole mask of the spectrophotometer (diameter of 8 mm). For that reason, bill colour was not considered for further analyses.

Using the colour space L^*S^*H, three colour components were determined. Luminosity (L) is measured at a scale of 0–100 (lower values indicate darker colour) and is related to the amount of light reflected by the measured surface; Saturation (S) corresponds to the chroma or purity of the colour at a scale of 0–100 (higher values indicate more saturated, brighter colour), and Hue (H) represents redness, expressed as the angle of the colour wheel (lower values indicate redder colour). We considered these three tristimulus parameters as individual variables for colour analysis.

Statistical analyses

All analyses were performed using SPSS 15.0. To test for differences in the pre-experimental situation regarding carotenoids and colour, as well as after treatment for immune response, isotopes and morphological and physiological parameters, we performed two-factor ANOVAs. The variable body mass showed different pre-experimental values between the two groups; to control for these differences in the after-treatment analysis, initial values were introduced as a covariate in the model (ANCOVAs). Changes in plasma concentration (of every carotenoid and of the total amount) and changes in foot colouration of the chicks between the onset and the end of the experiment were tested using repeated measures ANOVA (rmA) on the absolute initial and final values of the variables, with group and sex as fixed effects (also known as mixed-design ANOVA). Correlations were used to test for relationships between colour, carotenoids and immune response. We also give the absolute increments of the colour variables (final minus initial mean values) for an easier interpretation of the results. Repeatability of measures (ICC coefficients) was calculated according to Lessells and Boag (1987). Results are presented as arithmetic means ± standard error [in carotenoids, plus the coefficient of variation (CV)] and all tests are two-tailed.

Results

The study was conducted during 2006, which in terms of reproduction parameters was an average year (clutch size: 2.35 ± 0.04, laying date in Julian days: 146.2 ± 0.8) compared with the mean values of the period 1992–2008 for the colony (clutch size: 2.62 ± 0.01, laying date in Julian days: 142.7 ± 0.3), although the fledging success of 2006 was lower (0.57 ± 0.61 fledglings/pair) than the average of previous 16 years (1.2 fledglings/pair, Becker 1998), probably due to non-optimal weather periods despite otherwise favourable foraging conditions.

Pre-experimental values for plasma carotenoids and colour

When chicks were 9 days old we determined natural levels of six main dietary carotenoids in plasma: lutein, zeaxanthin, canthaxanthin, β-carotene, β-cryptoxanthin and astaxanthin. Tern chicks circulated a total amount of 25.30 ± 1.68 µg carotenoids/ml plasma (CV = 19.8%), (Fig. 1). β-cryptoxanthin was not detected in blood plasma, whereas canthaxanthin presented the highest concentration (9.17 ± 0.75 µg/ml, 36.2%; CV = 20.4%; Fig. 1) followed by zeaxanthin (5.86 ± 0.39 µg/ml, 23.2%; CV = 19.8%), astaxanthin (5.70 ± 0.70 µg/ml, 22.5%; CV = 30.2%), lutein (3.94 ± 0.38 µg/ml, 15.6%; CV = 40.0%) and β-carotene (0.61 ± 0.17 µg/ml, 2.4%; CV = 67.7%). At this age (9 days old, just before the onset of the experiment), chicks from the two randomly assigned groups did not differ in the initial values of plasma carotenoids (ANOVAs, all carotenoids $p > 0.14$, $n = 39$) and male and female chicks showed very similar carotenoid values (ANOVAs, all $p > 0.17$, $n = 39$).

At this stage, we also determined the integumentary colouration. Initial values of foot colour parameters of male and female chicks did not differ significantly

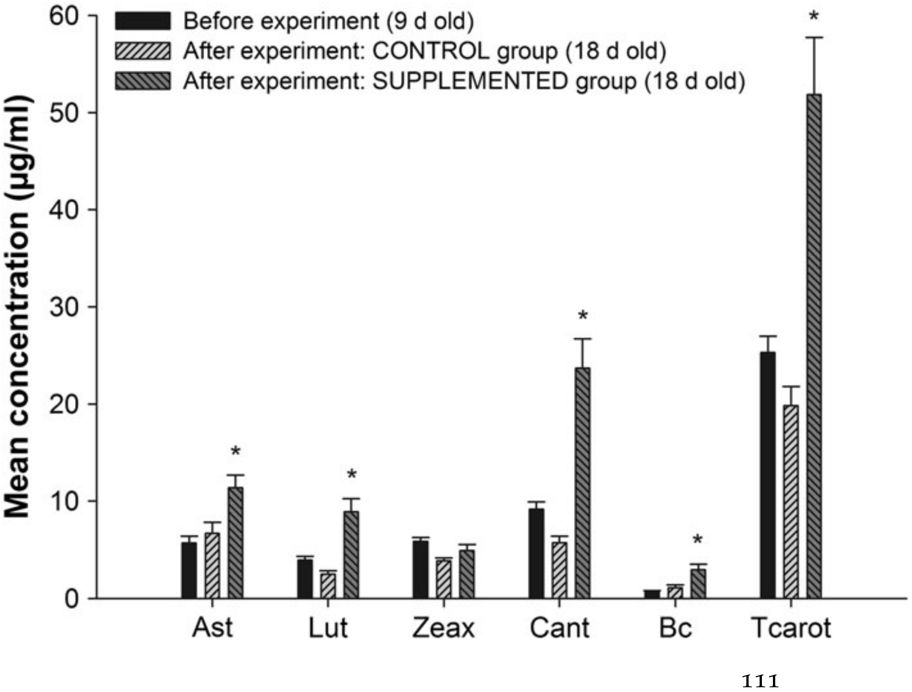
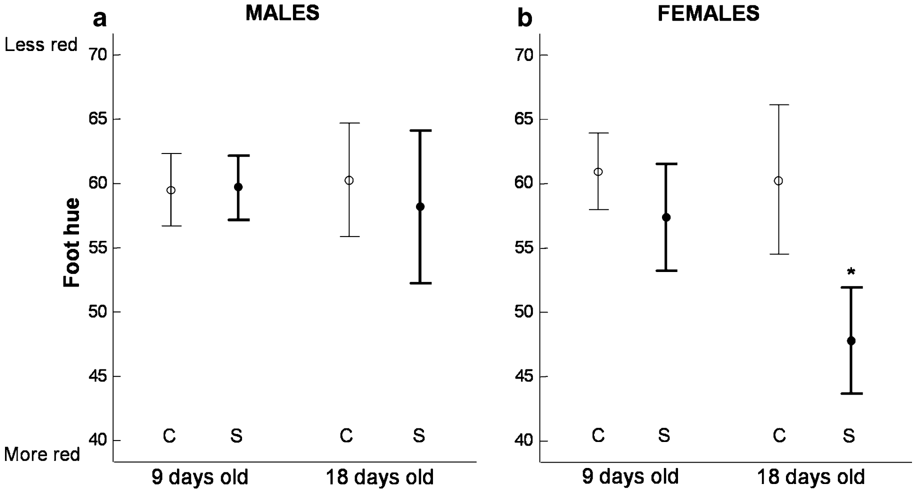


Fig. 1 Concentration (mean ± SE) of plasma carotenoids (*Ast* astaxanthin, *Lut* lutein, *Zeax* zeaxanthin, *Cant* canthaxanthin, *βc* β-carotene, *Tcarot* total carotenoids) before and after the experiment (by treatment group: control $n = 21$, supplemented $n = 18$). *Significant differences between control and supplemented group ($p < 0.01$)

Fig. 2 Change (mean, 95% CI) in hue (°) of the chicks' foot between 9 and 18 days old, for **a** males and **b** females, from the control (*C*) and supplemented (*S*) group, respectively. *Thinner bars* control group ($n = 21$); *thicker bars* supplemented group ($n = 18$). Significant differences are indicated by *asterisks*



(ANOVAs, Hue: $F_{4,35} = 0.11$, $p = 0.7$; Saturation: $F_{4,35} = 0.25$, $p = 0.6$; Luminosity: $F_{4,35} = 0.06$, $p = 0.8$; Fig. 2) and were not significantly different in the two treatment groups, except for luminosity (ANOVAs, Hue: $F_{4,35} = 1.60$, $p = 0.2$; Saturation: $F_{4,35} = 3.98$, $p = 0.08$; Luminosity: $F_{4,35} = 8.09$, $p = 0.007$). Chicks later assigned randomly to the supplemented group showed initial lower luminosity.

Changes in plasma-carotenoid concentration

Carotenoid supplementation strongly affected levels of circulating carotenoids in the plasma. All measured carotenoids except zeaxanthin showed a significant concentration increase in the supplemented group at the end of the experiment (rmA; astaxanthin: $F_{2,16} = 39.99$, $p < 0.001$; lutein: $F_{2,16} = 35.67$, $p < 0.001$; zeaxanthin: $F_{2,16} = 2.17$, $p = 0.13$; canthaxanthin: $F_{2,16} = 36.16$, $p < 0.001$; β-carotene: $F_{2,16} = 16.04, p = 0.001$, Fig. 1). In contrast, chicks from the control group showed a significant decrease in plasma carotenoids between 9 and 18 days' old for both sexes (rmA; lutein: $F_{2,19} = 12.29$, $p = 0.002$; zeaxanthin: $F_{2,19} = 9.88$, $p = 0.005$; canthaxanthin: $F_{2,19} = 11.54$, $p = 0.003$), except for astaxanthin and β-carotene, which showed a non-significant increase (rmA; astaxanthin: $F_{2,19} = 0.06$, $p = 0.8$; β-carotene: $F_{2,19} = 0.18$, $p = 0.7$; Fig. 1).

The change in individual and total carotenoid concentrations between the start and the end of the experiment did not vary differently with sex (rmA, sex and interaction group × sex: all $p > 0.1$, $n = 39$). Similarly, male and female chicks did not differ in plasma-carotenoid concentration at the end of the experiment (ANOVAs, astaxanthin: $F_{4,35} = 0.59$, $p = 0.4$; lutein: $F_{4,35} = 0.04$, $p = 0.8$; zeaxanthin: $F_{4,35} = 0.05$, $p = 0.8$; canthaxanthin: $F_{4,35} = 0.06$, $p = 0.8$; β-carotene: $F_{4,35} = 0.01$, $p = 0.9$).

Changes in foot colouration

Overall, the change over time for the colour variables luminosity and saturation was greater for supplemented chicks (Luminosity (%) change: control group, 5.23 ± 1.79, supplemented group, 11.29 ± 2.35; Saturation (%) change: control group, 3.22 ± 0.88, supplemented group, 5.83 ± 1.02), although treatment-based differences (tested through rmA) were marginally non-significant (rmA; Luminosity: $F_{4,35} = 30.12$, $p < 0.0001$, Luminosity × group: $F_{4,35} = 3.97$, $p = 0.054$; Saturation: $F_{4,35} = 42.50$, $p < 0.0001$, Saturation × group $F_{4,35} = 3.51$, $p = 0.069$).

On the contrary, Hue change (°) throughout the experiment was lower for the supplemented chicks, indicating redder colour (control group, 0.09 ± 1.41, supplemented group, −5.11 ± 1.99), which was supported by a significant difference between groups (rmA; Hue: $F_{4,35} = 5.81$, $p = 0.021$, Hue × group: $F_{4,35} = 6.07$, $p = 0.019$). Furthermore, the change in hue value was significantly related to sex (rmA, Hue × sex: $F_{4,35} = 4.30$, $p = 0.046$), and there was a significant group × sex interaction (rmA, $F_{4,35} = 5.45$, $p = 0.025$). Further rmA analyses split by treatment group showed that, under natural conditions (control group), hue did not change significantly with age or sex (males, 0.77 ± 1.85; females, −0.65 ± 2.23; rmA, age: $F_{2,19} < 0.01$, $p = 0.9$, sex: $F_{2,19} = 0.24$, $p = 0.6$). However, the supplemented group showed a decrease in hue over time that affected sexes differentially (Hue × sex: $F_{2,16} = 4.97$, $p = 0.04$): while males showed similar values females decreased their hue 10° on average (males, −1.54 ± 2.86; females, −9.57 ± 1.8, Figs. 2 and 4).

We did not find any significant correlation between foot colour and circulating carotenoids (each and total) at the end of experiment (Pearson correlations, all $p > 0.1$, $n = 39$), regardless of treatment and sex.

Effect on the immune response and other condition-dependent factors

Carotenoid-supplemented and control chicks (either males or females) did not differ in their cell-mediated response to the PHA injection (ANOVA, group, $F_{4,35} = 0.37, p > 0.5$; sex, $F_{4,35} = 0.77, p > 0.3$). However, responses of males and females in both groups tended to differ (group × sex: $F_{4,35} = 3.18$, $p = 0.083$, Fig. 3a). Within the supplemented group, females reduced their immune response, although the difference was marginally non-significant (ANOVA, $F_{1,17} = 4.18$, $p = 0.058$, Fig. 3a). Relationship between the change in hue and PHA response was further supported by a significant and positive correlation for supplemented females ($r = 0.346$, $p = 0.031$). Thus, chicks with less immune performance were also the chicks with a greater negative change in colour tone, displaying redder colour in the feet. There were no significant correlations (Pearson correlations, all $p > 0.1$, $n = 39$) between the immune reaction and the total or individual amount of circulating carotenoids.

Mortality rate between groups throughout the experiment was equivalent (Pearson's Chi-square test, $\chi^2_1 = 0.48$, $p = 0.4$), but still varied from 26% in supplemented to 35% in control chicks. We tested through ANOVAs other possible effects of the treatment in the chicks, which lead to non-significant results. Morphological measurements included wing length (121.8 ± 2.5 mm; $F_{4,35} = 0.72$, $p = 0.5$), tarsus length (20.5 ± 0.1 mm; $F_{4,35} = 1.12$, $p = 0.3$) and head-bill length (58.7 ± 0.4 mm; $F_{4,35} = 0.89$, $p = 0.4$). There were no group or sex differences in the recorded physiological parameters, which included plasma proteins (1.342021 ± 0.0001 g/dl, CV = 0.05%; $F_{4,35} = 0.38$, $p = 0.7$) and haematocrit (34.1 ± 0.6%, CV = 11.3%; $F_{4,35} = 1.09$, $p = 0.3$). Similarly, the growth rate and survival until fledging (last recorded age) of the chicks were not different between groups or sexes (growth rate, $F_{4,35} = 0.98$, $p = 0.4$; survival, $F_{4,35} = 0.88$, $p = 0.4$). However, the difference in body mass at 18 days (before the immune challenge) showed a group × sex interaction at the border of the significance threshold (ANCOVA, $F_{5,34} = 3.98, p = 0.054$). Although marginally non-significant, supplemented males showed a greater body mass than controls at the end of the experiment (ANCOVA, $F_{3,18} = 3.42$, $p = 0.086$, Fig. 3b). Besides, while isotopic signatures of δ^{13}C values showed no differences between chicks (ANOVA, $F_{4,34} = 1.26$, $p = 0.3$), there was a significant interaction group × sex for the nitrogen isotope (ANOVA, group × sex: $F_{4,34} = 4.21, p = 0.048$): supplemented females showed greater δ^{15}N values, although the difference between sexes turned out to be non-significant within the supplemented group (ANOVA, $F_{3,15} = 3.30$, $p = 0.08$, Fig. 3c).

Discussion

At the end of the supplementation period, carotenoid levels in plasma of supplemented chicks were about twofold compared to control chicks and pre-treatment levels. This result indicates that supplied carotenoids were absorbed by the organism and on average effectively increased circulating levels beyond those obtained from the natural food provisioned by parents. Among the four supplied

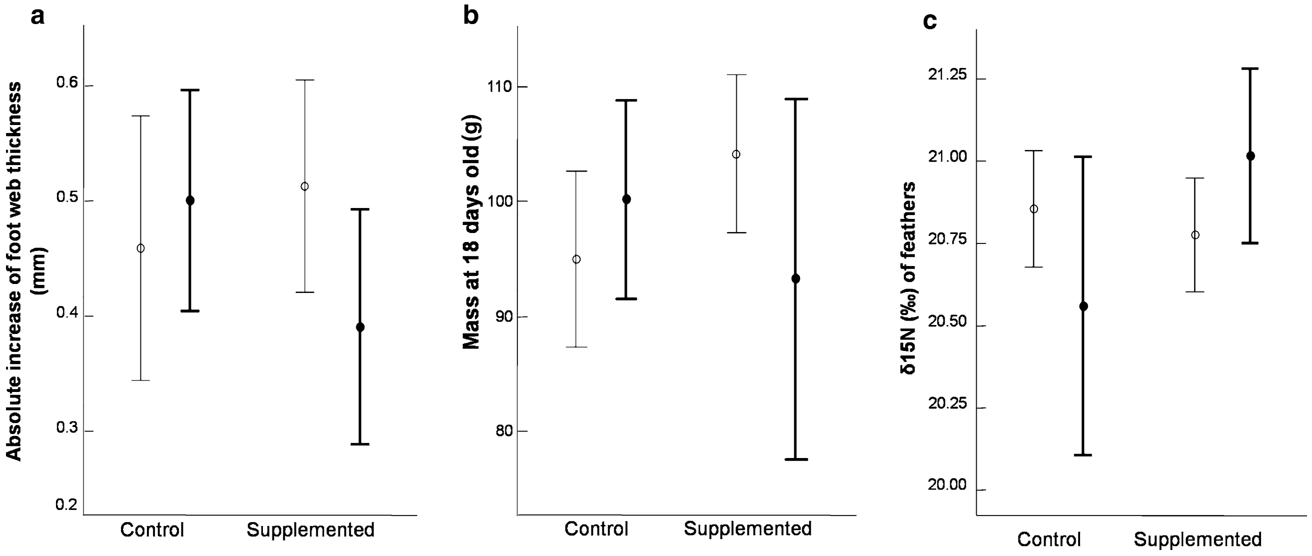


Fig. 3 Effect (mean, 95% CI) of the carotenoid supplementation on **a** the immune response, **b** the body mass and **c** δ^{15}N in feathers of common tern chicks. *Thinner bars* males (total $n = 21$); *thicker bars* females (total $n = 18$)

carotenoids, only zeaxanthin did not significantly increase its level, whereas the unsupplied astaxanthin did. Astaxanthin is acquired from sources like fish and crustaceans, but can also be metabolically derived from precursors such as zeaxanthin (Stradi 1998). Thus, this result may evidence how birds can physiologically favour certain carotenoid types through complex metabolic routes that imply conversion of some forms into others (Goodwin 1986). Alternatively, the lower amount of zeaxanthin supplied to the chicks compared to other carotenoids may also partly explain why we did not detect any increase of this carotenoid.

Contrary to our expectations, carotenoid supplementation had no significant effect on components of feet colouration such as luminosity and saturation, but followed a general pattern in both supplemented and control groups; namely older chicks showed lighter but more saturated coloured skin. Moreover, at the end of the experimental period foot colour was not predicted by the amount of circulating carotenoids in the blood stream, regardless of treatment. In addition, we did not find overall effects of carotenoid supplementation on the condition of the chicks, as indicated by non-significant differences between control and supplemented groups in morphological measures, haematocrit, total plasma proteins, growth rate and survival until fledging. Previous studies have shown that, beyond a specific availability threshold, carotenoid levels in plasma are physiologically regulated and stabilised (Alonso-Álvarez et al. 2004). Therefore, an increase in its availability may not always imply a greater ability of chicks to transform and incorporate these colourants into teguments or to use them to improve the physiological condition. These results might then be reflecting non-linear relationships between carotenoid levels and colour or physiological state. Nevertheless, we did find several sex-specific responses to carotenoid supplementation, and thus our results provide some support to differential strategies between male and female chicks in the use of carotenoids. Hue, the colour tone, changed significantly towards a more reddish colour of feet at the end of the experiment only in supplemented females (Figs. 2b, 4). This may indicate that carotenoid-based colouration of wild tern chicks is under limited expression, consistent with the carotenoid-limitation hypothesis. Nevertheless, most importantly, this result suggests that the assimilation and allocation of dietary carotenoids can differ between sexes (Hill 1992; Romano et al. 2008).

Although we did not detect any general relationship of plasma carotenoids or feet colour with T-cell-mediated response, supplemented females, that showed redder feet than any other group, also showed an unexpected tendency for a lower immune response (Fig. 3a). This is unlikely to result from detrimental effects of an excessive carotenoid supply, since the increase in plasma carotenoids was within the physiological range of common terns (previously measured in a pilot study by the authors), and yet mortality was 9% greater for control than for supplemented chicks during the course of the experiment. Rather than that, lower immune response in females with redder feet may result from a trade-off between immune function and colouration. However, the question which arises is why supplemented females, who theoretically could more easily meet the carotenoid demands, would use carotenoids to improve colouration and at the same time reduce their immunocompetence. A possible explanation is that, contrary to unsupplemented females, supplemented ones would be able to reach a colour threshold beyond which foot colour would improve competitive ability. In this experimental study, the degree of supplementation could have allowed them to almost, but not quite, reach this threshold. Therefore, allocation of carotenoids away from the immune system and instead to foot colour might have pushed them over the threshold and allowed them to benefit from exhibiting a costly signal. Thus, this apparent immunological cost of a redder colouration could possibly be

Fig. 4 Foot colour of 18 days old common tern chicks after 9 days of carotenoid supplementation: control chick (*left* and marked with a *green ring*) and supplemented female (*right* and marked with *yellow ring*) (colour figure in online)

compensated by other benefits for females in terms of sibling competition.

Although the potential function of carotenoid-based colouration in tern nestlings is not fully understood, the improvement of competitive ability of females with redder feet may be supported by their tendency to show greater N signatures. Greater $\delta^{15}N$ signatures result from consuming prey also enriched in ^{15}N compared to ^{14}N, which have been suggested to have greater nutritional quality (e.g. Forero et al. 2002). Moreover, in fishes the enrichment of this stable isotope is considered a continuous measure along the trophic niche within the same species, which is in turn, proportional to age and size of the individual (Cabana and Rasmussen 1994). Therefore, greater $\delta^{15}N$ signatures may suggest that daughters with more colourful feet were fed with prey of higher trophic position or with older and thus bigger specimens of higher energy content. Indeed, previous experiments have demonstrated that common terns are able to discriminate between colours and parents show different behavioural responses to the colouration patterns of the downy plumage of their chicks (Neubauer 1978). If parental preferences in chick feedings are influenced by reddish colour of the feet, daughters may obtain a nutritional advantage compared to their brothers. Alternatively, the increase of carotenoids in plasma or body tissues of females may have interfered with metabolic processes ultimately affecting the relative proportions of nitrogen signatures. However, although this latter hypothesis cannot be completely ruled out we could not find any study supporting this possibility.

In contrast, supplemented males showed no significant differences in response to the PHA challenge but achieved greater body mass than control males at the end of the experiment (although marginally significant, Fig. 3b). Several studies found a positive association between carotenoid levels and body mass (see “Introduction”), although physiological mechanisms underlying it are not fully understood (Surai 2002). Young common terns show a slight size dimorphism by fledging time (male chicks around 3% heavier than females, Becker and Wink 2003). As the difference in growth strategy between sexes already appears in nestling stage, males might have used the surplus of available carotenoids for their higher need in physiological detoxification during growth, thus allowing a better condition. This process is likely mediated by sex-steroid hormones, such as testosterone, the main male sex hormone related to the development of tissues and other traits. Recent research has provided evidence of a link between carotenoids and testosterone levels (Blas et al. 2006), since the hormone regulates the lipoproteins that are the plasma carriers for carotenoids. Since carotenoids can balance the negative effects of testosterone (Blas et al. 2006), the supplementation may have had benefits for the development of male traits, such as their larger size.

Altogether, these results suggest that carotenoid supplementation during growth influences common tern chicks in a sex-specific way, through a differential physiological allocation of carotenoids. When carotenoid availability increased, female chicks seemed to allocate the additionally available pigments preferentially to skin colouration (which might be a signal deployed to get parental attention), apparently paying a small cost in terms of immune system that might be compensated by a better parental care; and male chicks were slightly heavier. Such differences in the response to supplemental carotenoids pose the question of why benefits would be dissimilar for males and females. If sexes prioritise different lines of carotenoids usage in relation to the different ecological, physiological, and parasitological pressures they face (Zuk and McKean 1996), this pattern may be a way to balance the competitiveness between sons and daughters in sibling broods (Becker and Wink 2003) and a sex-specific strategy in the physiological-colouration trade-off to maximise condition. It could also be a pattern imposed on chicks by strategic parents, since in semi-precocial species like the common tern, access until fledging to particularly important resources like carotenoids relies exclusively on parental provisioning. Thus, parents may be expected to eventually allocate a different amount of carotenoids to male and female offspring. However, further investigations will be needed to properly evaluate this hypothesis, and more studies should be carried out on the functions and mechanisms of carotenoids in nestlings, which very likely differ from those of adult birds.

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5

Sex ratio adjustments in common terns: influence of mate condition and maternal experience

Ajustes de la razón de sexos en el charrán común: influencia de la condición de la pareja y la experiencia materna

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La asignación adaptativa por sexo ha sido estudiada principalmente en especies sexualmente dimórficas en tamaño. Como consecuencia, los patrones de asignación por sexo en especies que no muestran un dimorfismo sexual pronunciado son en muchos casos desconocidos. Según la teoría de la asignación por sexo, la inversión parental óptima puede predecirse cuando los costes derivados de la producción de hijos e hijas difieren y/o cuando los beneficios parentales en términos de eficacia biológica varían respecto al sexo de los pollos.

En el caso del charrán común *Sterna hirundo*, algunos estudios previos apuntan a que los hijos (machos) son el sexo más costoso de producir y criar. Nuestro estudio se centró en investigar si, en esa especie, la razón de sexos primaria (en el momento de eclosión), la razón secundaria (en el momento en que los pollos son volantones) y la mortalidad específica de los pollos correlacionaban con distintas variables ambientales y propias de la puesta (fecha de puesta de los huevos, tamaño de puesta, orden de eclosión de los huevos y calidad del año) y con características parentales (condición, fecha de llegada a la colonia, experiencia y éxito reproductor), para lo que utilizamos datos de 7 años consecutivos.

La razón de sexos a nivel de población y la mortalidad específica de los pollos no fueron significativamente diferentes de la paridad (50 %), pero el tamaño de puesta, la masa del padre, la experiencia de la madre y hasta cierto punto la calidad del año se correlacionaron con la razón primaria de sexos. La proporción de hijos tendió a incrementarse en los años más productivos y para los padres con mayor masa, lo que sugiere la posibilidad de que las hembras adultas inviertan más cuando las condiciones ambientales y de pareja son favorables. La proporción de hijas incrementó a mayor tamaño de puesta y mayor experiencia

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materna, lo que podría implicar un declive en el rendimiento reproductor o bien un ajuste de recursos que se lograría al producir más individuos del sexo más barato. No se encontraron patrones claros de mortalidad diferencial, ni globales ni relativos a atributos parentales.

Estos resultados abren una vía para futuros estudios relativos a la asignación adaptativa por sexo en especies casi monomórficas, en las que podrían darse ajustes en la razón de sexos relativos a factores parentales y una asignación diferencial entre la descendencia.

Palabras clave
Razón de sexos; calidad parental; monomorfismo sexual.

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Sex ratio adjustments in common terns: influence of mate condition and maternal experience

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Adaptive sex allocation has frequently been studied in sexually size dimorphic species, but far less is known about patterns of sex allocation in species without pronounced sexual size dimorphism. Parental optimal investment can be predicted under circumstances in which sons and daughters differ in costs and/or fitness returns. In common terns *Sterna hirundo*, previous studies suggest that sons are the more costly sex to produce and rear. We investigated whether hatching and fledging sex ratio and sex-specific chick mortality correlated with the ecological environment (laying date, clutch size, hatching order and year quality) and parental traits (condition, arrival date, experience and breeding success), over seven consecutive years. Population-wide sex ratios and sex-specific mortality did not differ from parity, but clutch size, mass of the father, maternal breeding experience and to some extent year quality correlated with hatching sex ratio. The proportion of sons tended to increase in productive years and when the father was heavier, suggesting the possibility that females invest more in sons when the environmental and the partner conditions are good. The proportion of daughters increased with clutch size and maternal breeding experience, suggesting a decline in breeding performance or a resources balance solved by producing more of the cheaper sex. No clear patterns of sex-specific mortality were found, neither global nor related to parental traits. Our results suggest lines for future studies on adaptive sex allocation in sexually nearly monomorphic species, where adjustment of sex ratio related to parental factors and differential allocation between the offspring may also occur.

Why would parents skew investment towards either sons or daughters? They would have few reasons to do so as long as the costs (resource allocation) and benefits (fitness returns) are similar for both sexes. This would lead to an even sex ratio at the population level (Fisher 1930). However, the relationship between fitness returns and investment for male and female progeny could vary even within a population. Sex-allocation theory predicts that, if diverse ecological conditions produce a sex-specific effect on fitness, parents are selected to differentially allocate resources and thus adjust the sex ratio accordingly to maximize the reproductive value of their progeny (Trivers and Willard 1973, Charnov 1982, Frank 1990). It may happen then, that an even sex ratio in a population reflects the population-wide equilibrium of different individual strategies for adaptive sex allocation (Hasselquist and Kempenaers 2002).

In birds, sexually size-dimorphic species have been intensely studied: size difference can lead to sex-specific effects on offspring fitness and this might facilitate the evolution of sex ratio biases (Myers 1978, Weatherhead and Teather 1991). In the absence of large size dimorphism, there may be little reason to expect significant sexual differences in offspring production or survival (Clutton-Brock et al. 1985). However, some evidence suggests the existence of sex ratio manipulation in size monomorphic species with bi-parental care, either at conception (differential sex production, under maternal control, i.e. Bradbury and Blakey 1998, reviewed by Alonso-Alvarez 2006) or during nestling growth (differential resource allocation, under both parents' control; Cameron-MacMillan et al. 2007). The first process would lead to a biased hatching sex ratio (HSR); the latter to sex-specific mortality patterns and may consequently result in a biased fledging sex ratio (FSR). Higher mortality of one sex may be the result of increased nutrient needs due to larger size (Clutton-Brock 1991) or the result of other size-independent mechanisms, such as vulnerability to adverse conditions of the heterogametic sex (Marr et al. 2006) or the antagonistic action of hormones on male physiology (e.g. testosterone, Zuk 1990).

Factors hypothesized to influence sex allocation decisions include those affecting the parental investment indirectly (environmental and offspring features) and directly (intrinsic parental traits; Hasselquist and Kempenaers 2002), which for slightly dimorphic species have been rarely investigated. Under good environmental conditions, for example, parents are expected to produce more offspring of the more costly sex, because these will mature to be in good condition (Suorsa et al. 2003). There is also evidence of sex ratio adjustment relative to laying date: if age of first reproduction or recruitment probability of one sex are differentially affected by seasonality, they would be produced earlier in the season (Cordero et al. 2001, Andersson et al. 2003, Husby et al. 2006, Dijkstra et al. 2010). Other studies have demonstrated variation in sex ratio with clutch size but especially with hatching order (Lessells et al. 1996, Dijkstra et al. 2010). If sexes differ in their growth and competitive ability, mothers are predicted to bias last hatched eggs towards the sex which is less sensitive or less dependent on resources. On the other hand, parental investment into the sex with highest variance in reproductive success could be theoretically expected under situations such as good maternal condition (Bradbury and Blakey 1998, Whittingham et al. 2002), high experience or high maternal quality (Dowling and Mulder 2006). Similarly, if the fathers' high quality and/or sexually attractive attributes are inherited and have a greater influence on the fitness of sons than on that of daughters, mothers would be induced to skew their reproductive efforts towards male offspring when mated to such males (Charnov 1982, Ellegren et al. 1996, Zielinska et al. 2010).

Despite the studies mentioned so far, other studies of monomorphic species have shown little evidence for sex ratio adjustments (Leech et al. 2001, Westneat et al. 2002, Zann and Runciman 2003, Postma et al. 2011). Such inconsistencies may arise from annual variation in environment conditions, and the topic demands further investigation particularly using long-term data.

In the present study, we examine the aforementioned hypotheses in the common tern *Sterna hirundo*, a small long-living, social monogamous seabird species for which some evidence of sex-ratio biases has been previously reported. By recording offspring sex ratio at hatching and fledging as well as nestling mortality until fledging over seven years, we were able to analyze their covariation with a number of environmental and parental traits.

Several features of the species establish a scenario in which the relative costs/benefits ratio of the sexes may differentially affect their reproductive value. The common tern is a monomorphic species in most characteristics (Wendeln et al. 1997) except for a 6% difference in adult bill length (longer in males) and a 3% difference in chick fledging mass (males fledge significantly heavier than females, Becker and Wink 2003). Egg volume, growth rates and fledging age are similar in both sexes (Becker and Wink 2003, Fletcher and Hamer 2004). However, other indications suggest higher costs of male chicks. Fletcher and Hamer (2004) found that the sex ratio of third eggs (c-eggs), which are usually about 10% smaller and show lower survival (Langham 1972, Becker and Finck 1985), was significantly biased in favour of females, and that they survived better than male third chicks (c-chicks). Sons were also found to suffer higher post-hatching mortality in broods with younger and lighter parents (González-Solís et al. 2005) and, compared to mixed and female broods, only-male broods showed considerably poorer condition in the second chick (b-chick) (Braasch et al. 2011). This evidence suggests that sons are more vulnerable than daughters to adverse conditions and/or poor quality parents and therefore the more costly offspring sex to produce and/or rear.

Sons may also be the sex that provides higher fitness returns under certain conditions. The substantial differences in quality among common tern parents are a crucial factor in offspring fitness and survival; for instance, high parental quality positively influences chick growth rates (Wendeln and Becker 1999). Schauroth and Becker (2008) observed sex-specific differences in post-fledging condition: especially in good years, male chicks increased body mass at a higher rate than females. Males recruit at a significantly older age than females (a difference of 0.4 yr on average, Ludwigs and Becker 2002), as the hurdles towards a first breeding attempt are higher for males; they need to be more skilled to establish a territory and exhibit a strong foraging effort for successful courtship feeding. Since body mass at fledging and post-fledging positively influences return- and recruitment probability and is predictive of future breeding quality (Ludwigs and Becker 2006, Braasch et al. 2009), sons in good condition may have particularly good chances of recruiting and pairing as compared to sons in poor condition, while the success of daughters might be less dependent on their condition.

In summary, these characteristics make the common tern a good model to test some aspects of the sex allocation theory, with the predictions that 1) more daughters, as the faster maturing and presumably less costly sex, would be produced at the end of the season and later within the clutch; 2) sons would be overproduced in good years and females, the dispersive sex, in poorer years; 3) higher quality mothers (either heavier, more experienced or successful breeders) as well as those paired to high quality males would invest more in sons; and 4) offspring mortality would be biased towards males, especially in poor quality years and parents.

Material and methods

Study area and general field procedures

Data were collected from 2002 to 2008 at the Banter See common tern colony in the harbor area of Wilhelmshaven (Lower Saxony, Germany). Colony size in these years varied between 300–530 breeding pairs. An integrated population study has been in progress at this site since 1992, and every hatchling has been individually ringed and marked with a passive transponder before fledging (Becker and Wendeln 1997). This enables lifetime identification of locally born individuals.

The breeding site consists of 6 artificial islands, surrounded by a concrete wall (60 cm high) equipped with 44 resting platforms. Antennas and 16 scales are installed at the platforms, allowing remote and automatic identification and weighing of transponder-marked terns (for details see Becker and Wendeln 1997, Becker et al. 2001). This approach allows the measurement of variables such as arrival date and body mass (for details see Limmer and Becker 2007). Moreover, given the high adult return rate (≈90%, Becker et al. 2001), the electronic system together with manually collected reproductive data provides information for every breeding event over the lifetime of many individuals (Becker et al. 2001). All birds marked since 1998 have been molecularly sexed, and before 1998 breeders were sexed by behavioural observations.

The colony was visited every 2–3 d during the breeding season in order to locate and mark newly initiated clutches and newly laid eggs. To identify parents of each nest, a portable antenna was placed around the nest for 1–2 d during the incubation period. Laying date, hatching order of every chick and the fate of eggs and chicks were determined during these regular visits (for details see Wagener 1998). First-, second- and third-hatched chicks were denoted as a-, b- and c-chicks, respectively. Chicks were ringed on the day of hatching and were then observed every 2–3 d until death or until fledging; fully grown chicks that disappeared from study nests beyond the minimum fledging age of 22 d were also assumed to have fledged.

In this study we focused on first clutches from 3 out of the 6 islands. Of these, 64% corresponded to complete clutches where all eggs hatched and among them, our analysis is focused on broods where the sex of all chicks was known. However, as far as data is available, the hatching sex ratio did not differ significantly between complete and incomplete broods (χ^2-test; $\chi^2_1 = 0.655$, $p = 0.418$), which makes it unlikely that our approach biases the results. Our final dataset encompasses 419 nests with 1032 chicks, distributed among three different clutch sizes (43 chicks in 43 one-egg clutches; 278 chicks in 139 two-egg clutches; 711 in 237 three-egg clutches). All chicks were sexed using standard PCR methods (Becker and Wink 2003). DNA was extracted from blood of quills from body feathers plucked before fledging or from tissue samples collected from chicks found dead on the ground.

Definition of environmental and parental variables

For each nest, the laying date was defined as the date on which the first egg was laid, measured in days from 1 January. Laying date was grouped in periods of five days (pentads), because this allowed us to treat pentads as factorial predictors with little loss of information and suitable for non-linear relationships.

We assessed the fledging success (mean number of fledglings per pair, calculated for the whole colony every year) as an index of 'year quality'. This measurement provides a proxy of environmental conditions in a given year (weather conditions, food availability, etc.). The evaluated years (2002 to 2008) belonged to a poor period with relatively low reproductive success (values in Fig. 1) below the average of this colony (0.97 chicks per pair, Szostek and Becker 2012), except 2003 which was an average year.

As proxies for parental condition and quality, we chose four different traits: body mass, breeding experience, arrival date and average breeding success. Body mass in a given season (in grams) was recorded as the average mass of the individual bird during the first four days of colony attendance. In common terns, body mass has been shown to be a good proxy of body condition independent of size (Wendeln 1997). Breeding experience is the number of previous breeding attempts of each individual, including the current season and the replacement and/or second clutches (range 1–17, log transformed to linearize the relationship with offspring sex); a suitable estimation since renesting birds are high-quality individuals (Becker and Zhang 2011). Arrival date in a given season was defined as the first day of registration at the colony site (counting days from 15 April, log transformed to linearize the relationship with offspring sex). Arrival date in terns is repeatable across years and an important predictor of recruitment age (Becker et al. 2008a). Average breeding success was calculated as the total number of fledglings divided by the total number of breeding years.

Statistical analyses

Goodness-of-fit tests were used to test binary values against a 50:50 expectation (e.g. deviations from equal sex ratios) and contingency tests for differences in proportions between classes (e.g. variation in sex ratio and mortality between years).

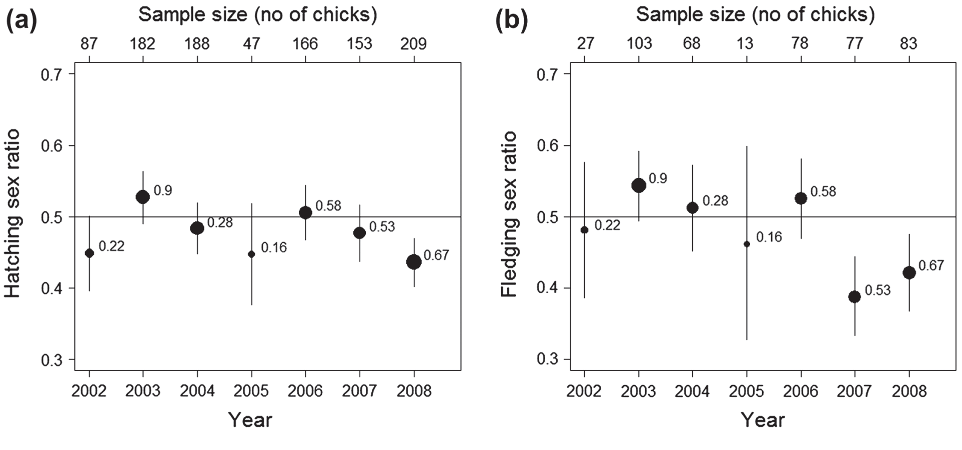

Figure 1. Among year variation in offspring sex ratio at (a) hatching and (b) fledging. Means and standard errors were estimated from a GLMM with binomial error structure and logit link, including year as a fixed factor and mother as a random effect. Estimates (mean ± 1 SE) are backlinked to the probability scale for display. Small numbers show the fledging success of that year (index of 'year quality'). Point sizes are proportional to sample sizes.

Generalized linear mixed models (GLMM) were used to test for the significance of fixed effect predictors (e.g. maternal and paternal traits) while controlling for the non-independence of data points (within mothers, fathers and years). We also report the amount of variance explained by random effects, the significance of which was tested by likelihood ratio tests (LRT). In the analysis of sex specific mortality we also tested for between-mother and between-year variation in sex specific mortality. This was done by fitting a more complex model including the sex × mother or sex × year interaction (random slope model, Schielzeth and Forstmeier 2009). The significance of random slopes was tested by LRT comparing the model with the interaction against a model without (i.e. a random-slope model against a random-intercept model).

The response was binary in all cases (offspring sex or mortality). Therefore, we fitted GLMMs with a binomial error structure and logit link using the lmer function in R (Bates and Maechler 2010). When modelling sex differential mortality, we fitted mortality (fledged = 0, dead = 1) as a response. Main effect predictors therefore test for the effect on overall mortality. To test for sex-differential effects, we included offspring sex and sex in interaction with the relevant predictors. The interaction terms with the sex indicator estimate the sex-specific effect of the respective predictor.

Not all adults in the colony are marked with transponders (about 60%; many are immigrants from other colonies), which means that some parents could not be identified. Therefore we used the 732 chicks (out of 1032) for which we had phenotypic information on at least one of the parents (total number of individually known mothers and fathers, 98 and 119 respectively), although the sample sizes of each model varied depending on the availability of parental predictors. In particular mass information was often not available. Therefore, we fitted all parental models using two datasets, a reduced one which included mass and an extended one excluding the mass (substantially larger sample size). Predictors of maternal and paternal quality were assessed separately (in order to have a maximum sample size and thus a maximal power to find associations). Repeatabilities were calculated from mixed models (R_M, Nakagawa and Schielzeth 2010).

We applied the following strategy to explore GLMMs: we first fitted additive models without interaction terms (except for the interactions with sex in the case of the mortality analysis). In a second step, we verified that effects that appeared significant (or non-significant) in the multiple regression remained significant (or non-significant) when fitted as a single fixed-effect predictor. This procedure allowed us evaluating if the significance of a predictor depended on other predictors in the models, due to collinearity among some of the predictors. Lastly, for certain analyses we explored potential interactions (up to third degree) using a stepwise-backward procedure, removing non-significant terms ($p > 0.05$) and starting with the least significant interaction. Main effects were always retained if they were involved in interactions (as were lower order interactions when involved in higher order interactions).

All statistical analyses were performed using R 2.11.1 (R Development Core Team) using the lmer function (Bates and Maechler 2010) for fitting GLMMs. The level of significance level for all tests was set to $\alpha = 0.05$. All tests (except for tests for the significance of random effects) are two-tailed. Sex ratio (SR) is expressed as the percentage of males in the offspring.

Results

Population-wide effects on sex ratio

Between 2002 and 2008, there were 495 male and 537 female tern chicks that hatched (HSR: 47.96%), and among these, 216 male and 233 female fledged (FSR: 48.11%). Thus, the population-wide sex ratio at hatching and fledging was not significantly biased towards one of the sexes (χ^2-test; hatching: $\chi^2_1 = 1.709$, $p = 0.19$; fledging: $\chi^2_1 = 0.644$, $p = 0.42$). There was no significant variation in sex ratio attributable to mothers (LRT: $\chi^2_1 = 0.000$, $p = 1.00$) or years (LRT: $\chi^2_1 = 0.000$, $p = 1.00$; alternatively tested by a χ^2-test: $\chi^2_6 = 4.334$, $p = 0.63$, Fig. 1). Although both effects were weak, we retained them in all models to control for pseudoreplication within mothers (due to genetic, maternal and early rearing effects) and years (due to shared environmental effects). We also modelled offspring sex ratio as proportion data aggregated on the clutch level. A GLM that included only the intercept did not suggest significant overdispersion (glm function in R, $\omega = 0.94$, bootstrapping CI: 0.84–1.03).

Structural and seasonal variation in sex ratio (predictions 1 and 2)

The patterns of sex ratio variation with respect to laying date and other covariates (clutch size, hatching order and year quality) were very similar for hatching and fledging (Table 1). Year quality tended to have a positive effect (slightly more sons in productive years), while the three other predictors had negative effects. The only significant trend,

Table 1. Fixed effect estimates on the link scale from a GLMM analysis of hatching and fledging SR. Random effects of mother identity and year explained little variance. Year quality was centered to an average year quality of 0.5, clutch size was centered to the model clutch size of 3, hatching order was centered to the first hatched chick and laying date was centered to the modal laying date (day of year 132). Hence the intercept estimates the sex ratio at an average year quality, at the modal laying date, for the first egg of a clutch of three eggs. Negative values indicate female-bias, positive values male-bias. Values in bold depict statistically significant results.

	Estimate	SE	Z	p
Hatching SR				
Intercept	−0.162	0.116	−1.397	0.162
Year quality	0.347	0.274	1.268	0.204
Clutch size	−0.285	0.141	−2.019	**0.043**
Hatching order	−0.003	0.086	−0.043	0.965
Laying date	−0.004	0.006	−0.766	0.443
Fledging SR				
Intercept	−0.087	0.149	−0.581	0.561
Year quality	0.315	0.425	0.742	0.458
Clutch size	−0.314	0.211	−1.485	0.137
Hatching order	−0.128	0.164	−0.783	0.434
Laying date	−0.011	0.008	−1.258	0.208

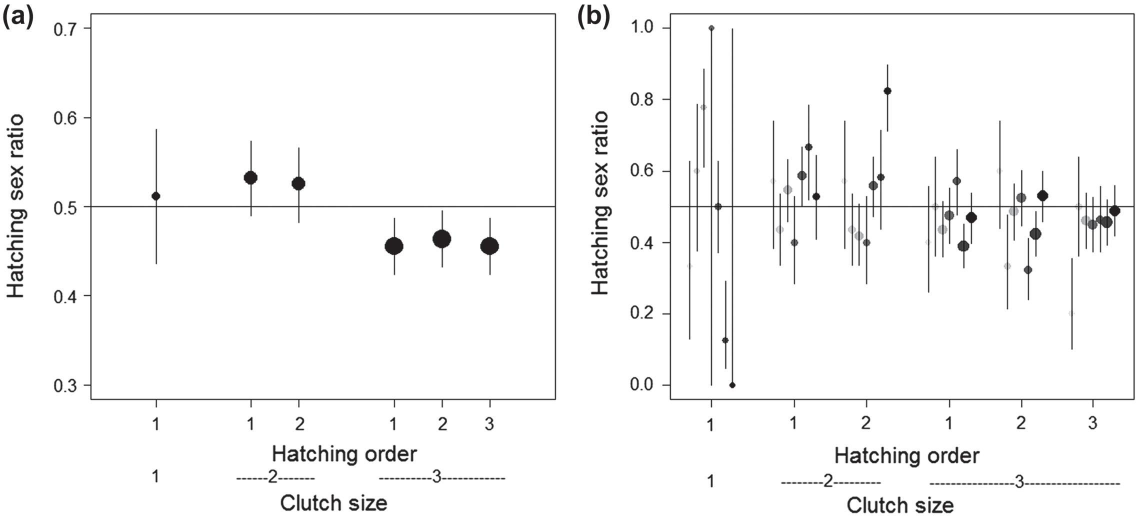

Figure 2. Hatching SR in relation to (a) clutch size and hatching order and (b) clutch size, hatching order and ‘year quality’ index. Estimates (mean ± 1 SE) are backlinked to the probability scale for display. Point sizes are proportional to sample sizes. Point colour indicates year quality index (light colours: low quality, dark colours: high quality).

however, was the negative effect of clutch size on hatching sex ratio (Table 1, Fig. 2a: $SR = 51.16\%$ in 1-egg clutches, 52.87% in 2-egg clutches and 45.85% in 3-egg clutches). The effect was borderline non-significant when tested as the only predictor ($b = -0.204 \pm 0.111$, $z = -1.828$, $p = 0.067$). Considered across the entire brood, 3-egg clutches were significantly female-biased compared to 2-egg clutches ($b = -0.282 \pm 0.142$, $p = 0.047$; Fig. 2a).

We also searched for significant minimal models (stepwise backward procedure) that predict offspring sex ratio including the same covariates and their 11 interactions. For hatching sex ratio we found a significant three-way interaction between clutch size, hatching order and quality of the year ($b = -0.222 \pm 1.001$, $z = -2.219$, $p = 0.026$; Fig. 2b). This seemed to be caused by year-quality dependence of b-chicks in 2-egg clutches (more sons in high quality years), but not in 3-egg clutches (Fig. 2b). For fledging sex ratio we found none of the interactions or main effects to be significant.

Parental effect on offspring hatching sex (prediction 3)

As expected, predictors of maternal condition and quality were highly repeatable among years: mass ($R_M = 0.72 \pm 0.04$, $p < 0.001$), arrival date ($R_M = 0.63 \pm 0.04$, $p < 0.001$) and average breeding success ($R_M = 0.89 \pm 0.01$, $p < 0.001$). While breeding experience and breeding success were positively correlated among each other and negatively with arrival date (Pearson correlation, all $|r| > 0.3$, all $p < 0.002$), body mass was not significantly correlated to any of them (all $-0.13 < r < -0.05$, all $p > 0.19$). Similarly, paternal traits were highly repeatable (mass: $R_M = 0.81 \pm 0.03$, $p < 0.001$, arrival date: $R_M = 0.67 \pm 0.04$, $p < 0.001$, breeding success: $R_M = 0.93 \pm 0.01$, $p < 0.001$) and showed a correlation structure very similar to that in females. Arrival date correlated significantly with laying date in females ($r = 0.59$, $p < 0.001$) and in males ($r = 0.46$, $p < 0.001$). There was no assortative mating for body mass ($r = 0.08$, $p = 0.66$), but breeding experience ($r = 0.70$, $p < 0.001$), arrival date ($r = 0.47$, $p < 0.001$) and average breeding success ($r = 0.76$, $p < 0.001$) were all highly correlated between mates.

One female trait had a significant impact on hatchling sex: females with more breeding experience produced fewer sons (Table 2a, upper). This effect became even more significant when using almost twice as much data (by removing female mass, for which data were often lacking; Table 2a, lower) and remained significant when including only the variable breeding experience ($b = -0.267 \pm 0.134$, $z = -1.990$, $p = 0.046$, Fig. 3a). The additional predictor year quality was significant on the limited dataset (Table 2a, upper), but was only marginally significant in the extended dataset and not significant in the general sex ratio analyses. Hence, this could be a spurious correlation, but we note that the trend was positive in all cases (more sons in productive years).

Also paternal mass seemed to affect offspring sex at hatching: heavier males tended to have more sons (Table 2b, upper). The effect was borderline non-significant in the full model ($p = 0.053$), but was significant when tested alone ($b = 0.007 \pm 0.003$, $z = 1.972$, $p = 0.049$, Fig. 3b). None of the other male quality predictors had a significant effect on the hatching sex ratio (Table 2b), nor did the inspection of interactions for the maternal and paternal models give any further results (details not shown).

Sex-specific chick mortality (prediction 4)

Among the studied years, 583 out of 1032 chicks (56%) died before fledging, and 279 of the dead chicks were males (sex ratio among dead chicks = 47.85%). Although this indicates that more females than males died, overall mortality was not significantly different between the sexes (χ^2-test: $\chi^2_1 = 0.00$, $p = 0.986$). Overall mortality varied significantly between years (χ^2-test: $\chi^2_6 = 32.044$, $p < 0.001$,

Table 2. GLMM models estimating the effect on hatching sex ratio of predictors of (a) maternal and (b) paternal condition and quality. The upper section within each letter refers to models with reduced dataset (including mass) and the lower section to models with extended dataset (excluding mass). Models controlled for mother identity, father identity and year (all of which explained little variance). Sample sizes (n) are given as the number of chicks (number of mothers/fathers). Values in bold depict statistically significant results.

	b	SE	Z	p
(a) Maternal traits				
n = 258 (62)				
Intercept	0.831	0.721	1.153	0.248
Year quality	1.144	0.561	2.04	**0.041**
Mass	0.016	0.018	0.869	0.384
Arrival date	0.029	0.226	0.129	0.897
Average breeding success	0.177	0.295	0.601	0.547
Breeding experience	−0.497	0.230	−2.16	**0.030**
n = 453 (93)				
Intercept	0.714	0.498	1.433	0.151
Year quality	0.711	0.409	1.735	0.082
Arrival date	−0.003	0.144	−0.025	0.98
Average breeding success	0.195	0.230	0.849	0.395
Breeding experience	−0.410	0.176	−2.322	**0.020**
(b) Paternal traits				
n = 277 (81)				
Intercept	−0.128	0.759	−0.17	0.865
Year quality	−0.302	0.560	−0.539	0.589
Mass	0.031	0.016	1.937	0.052
Arrival date	−0.069	0.239	−0.289	0.772
Average breeding success	−0.263	0.293	−0.895	0.370
Breeding experience	0.039	0.244	0.162	0.871
n = 506 (115)				
Intercept	−0.271	0.506	−0.536	0.592
Year quality	0.531	0.400	1.326	0.185
Arrival date	0.089	0.153	0.58	0.562
Average breeding success	−0.100	0.216	−0.464	0.643
Breeding experience	−0.074	0.159	−0.47	0.639

lowest 43% in 2003 and highest 72% in 2005), but sex-differential mortality did not (LRT comparing a random-slope model with a random-intercept model: $\chi^2_2 = 0.332$, $p = 0.846$). Mother identity did not explain variation either in overall mortality (LRT: $\chi^2_1 = 0.00$, $p = 1.00$) or in sex-specific mortality (LRT comparing a random-slope model with a random-intercept model: $\chi^2_2 = 0.113$, $p = 0.944$).

We found strong effects of clutch size, hatching order and laying date on overall mortality (Table 3): more chicks died in 3-egg clutches and specifically in c-hatching position, and chicks born late in the season had a higher probability of dying. However, we did not find any pattern of sex-specific differential mortality in this analysis (Table 3) or in the stepwise model (details not shown). Hence, all factors that influenced chick mortality affected male and female chicks equally.

We also explored if parental traits explain overall and sex-specific mortality, by fitting GLMM with predictors of parental qualities (separately for females and males) and hatching order (the rest of general predictors covary strongly with mortality itself or with parental attributes and were therefore removed). Hatching order showed the same direction of effects (Table 4) as in the analysis without parental qualities (Table 3). Besides, two of the mother indices predicted offspring mortality: experienced females and those which arrived earlier had lower chick mortality (Table 4). However, no offspring sex was more likely to die than the other, and there were little indications of sex-specific effects with respect to parental traits. We found a significant interaction between offspring sex and female body mass (heavier mothers showed more male than female-biased mortality, Table 4a; but not significant when tested alone: $p = 0.13$) and between offspring sex and female arrival date (sons being more adversely affected by late arrival of their mothers, Table 4a). The latter effect was significant in the reduced dataset also when excluding all the other predictors, but clearly non-significant in the full dataset with larger sample size (Table 4b). Hence, both interactions have to be interpreted with some caution.

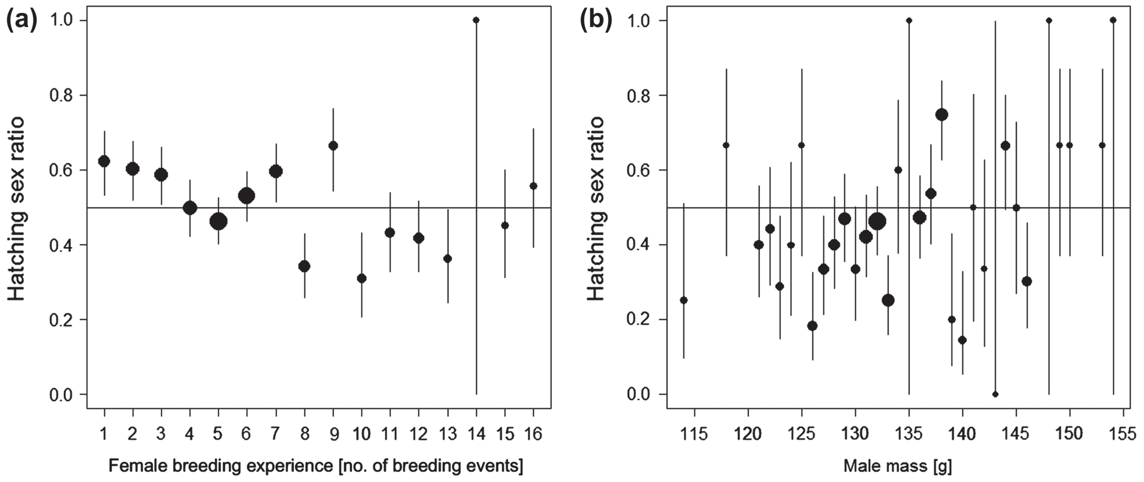

Figure 3. Hatching SR in relation to (a) female breeding experience (n = 98 mothers) and (b) male mass (n = 81 fathers). Means and SE were estimated from a GLMM with binomial error structure and logit link, including female breeding experience/male mass as a fixed factor and mother/father identity and year as random effects. Estimates (mean ± 1 SE) are backlinked to the probability scale for display. Point sizes are proportional to sample sizes.

Table 3. GLMM analysis of sex-specific mortality. Analysis includes mother identity and year as random effects. The table shows fixed effect estimates on the link scale. Clutch size was centered to the modal clutch size of 3, hatching order was centered to the first hatched chick and laying date was centered to the modal laying date (Julian date 132). The sex indicator was centered (females = −0.5, males = 0.5), so that terms that do not involve sex estimate the overall probability of mortality (independent of sex), while terms that do involve sex estimate whether mortality is sex specific (i.e. different probabilities for males and females, positive values indicate higher mortality of males). Significant factors are given in bold.

n = 1032	Estimate	SE	Z	p
Intercept	−1.143	0.221	−5.158	< 0.001
Clutch size	−0.528	0.163	−3.238	**0.001**
Hatching order	1.509	0.111	13.554	**< 0.001**
Laying date	0.022	0.007	2.966	**0.003**
Sex	−0.341	0.275	−1.24	0.214
Sex × Clutch size	−0.047	0.313	−0.151	0.879
Sex × Hatching order	0.303	0.223	1.355	0.175
Sex × Laying date	0.018	0.014	1.258	0.208

Table 4. GLMM estimation of maternal traits effects on offspring mortality (a) with mass (b) without mass (mother identity and year as random effects). The first variable is predictor of mortality, the next three show general effects of maternal quality and the rest show sex-specific effects. The indicator of the sex of chick was centered (females = −0.5, males = 0.5), so that terms that do not involve sex estimate the overall probability of mortality (independent of sex), while terms that do involve sex estimate whether mortality is sex specific (i.e. different probabilities for male and female, positive values indicate higher mortality of males). All predictors that were included in interaction with sex were centered, so that the effect of sex was estimated at typical (average) values of the covariates. Sample sizes (n) are given as the number of chicks (number of mothers). Significant effects are shown in bold.

	b	SE	Z	p
(a) Mortality				
n = 258 (62 mothers)				
Intercept	−0.726	0.293	−2.475	0.013
Hatching order	**1.590**	**0.238**	**6.677**	**< 0.001**
Mass	−0.001	0.022	−0.061	0.951
Arrival date	**0.807**	**0.300**	**2.684**	**0.007**
Breeding experience	−0.521	0.269	−1.934	0.053
Sex of chick	−0.216	0.314	−0.688	0.491
Sex × Mass	**0.107**	**0.045**	**2.379**	**0.017**
Sex × Arrival date	**1.545**	**0.557**	**2.772**	**0.005**
Sex × Breeding experience	0.871	0.51	1.708	0.087
(b) Mortality				
n = 453 (93 mothers)				
Intercept	−0.761	0.253	−3.002	0.002
Hatching order	**1.601**	**0.171**	**9.312**	**< 0.001**
Arrival date	**0.467**	**0.177**	**2.63**	**0.008**
Breeding experience	**−0.662**	**0.196**	**−3.371**	**0.000**
Sex of chick	−0.323	0.227	−1.419	0.155
Sex × Arrival date	0.481	0.338	1.422	0.155
Sex × Breeding experience	0.694	0.371	1.872	0.061

Results for GLMM of paternal predictors only showed a decrease in mortality with paternal breeding experience ($b = -1.088 \pm 0.280$, $z = -3.884$, $p = 0.0001$), but no sex-differential effect of paternal predictors on mortality (interactions of breeding experience, arrival date and paternal body mass with sex, all $p > 0.30$).

Discussion

This long-term study allowed us to test for adjustments of the primary sex ratio in a large sample of common tern broods. We found partial support in relation to our initial sex-allocation hypotheses. The lack of overdispersion in the GLM model might suggest that binomial sampling may be sufficient for producing the between-clutch variation in sex ratios that we observed in our dataset (Postma et al. 2011) and the following patterns should therefore be interpreted with some caution.

Population-wide hatching sex ratio was not significantly different from parity. This confirms the results of previous studies in terns (Fletcher and Hamer 2004, González-Solís et al. 2005) and is in line with the results from other monomorphic species (Pagliani et al. 1999, Leech et al. 2001, Fair and Myers 2002). Nevertheless, population-level parity does not exclude the occurrence of adaptive manipulation of primary sex ratios at individual level. Indeed, we found that the hatching proportion of sons was related to clutch size, to maternal experience, to paternal mass and to some extent to the environmental quality of the year, while expectancies of adjustment related to laying date and hatching position were not supported (prediction 1). Although sex manipulation according to laying date and hatching order seems to be common (Husby et al. 2006, Dijkstra et al. 2010), also in terns (female bias of a-chicks of roseate terns, Szczys et al. 2001; and of c-eggs of common terns, Fletcher and Hamer 2004), no such patterns were found in the extensive dataset examined here. This suggests that parents do not adjust sex ratio according to potential differences in maturing time or competitive ability between sons and daughters, perhaps because they are small (difference in mean age at first breeding is 0.4 yr, Ludwigs and Becker 2002; and the testosterone level of tern male and female nestlings, an important indicator of sibling competition and begging intensity, was recently found to be similar, Braasch et al. 2011).

In addition to a certain year-quality effect, clutch size and two parental characteristics predicted sex ratios (prediction 2 and 3): two-egg clutches tended to be male-biased whereas three-egg clutches showed a female overproduction, females with more experience produced more daughters, and heavier fathers had more sons in their clutches. Interestingly, mother experience and father weight were uncorrelated ($r = -0.11$, $p = 0.25$) showing that the two effects are largely independent.

Two of these results confirm a differential allocation in the predicted direction. Independent of experience, female terns that were paired to heavier males biased their production towards sons (prediction 3). This supports the male attractiveness hypothesis, thus suggesting that a good mate condition is an incentive to allocate towards sons (Addison et al. 2008). One of the assumptions of the mate quality hypothesis is that sons will inherit the paternal desirable trait (Ellegren et al. 1996), and previous research in the colony consider body mass as highly heritable (Ludwigs and Becker 2006), as shown by high parent–offspring correlations for mass at fledging (unpubl.). In addition, we found some support for the environmental hypothesis (prediction 2), although the effect was not clearly significant, possibly due to the general low productivity of the considered period. Females, irrespective of quality or condition, tended to invest more in sons in productive years, which seems a good allocation strategy since sexual differences in body size between sons and daughters are more pronounced when environmental conditions are favourable (Schauroth and Becker 2008). Overall this evidence supports our conjecture that males are the more costly sex, and that a higher reproductive variance of sons may be strongly related to conditions experienced throughout development.

The negative associations found between both clutch size and maternal experience with number of male offspring were a surprising result, apparently inconsistent with the idea of increased allocation towards sons with increasing maternal quality (prediction 3). We could not formulate an a priori prediction for the modification as a whole of the clutch sex ratio, but we interpret this bias as related to parental characteristics. In the common tern, clutch size is a good indicator of differences in parental quality (Arnold et al. 2004, González-Solís et al. 2005). Adults laying 3-egg clutches arrived and bred significantly earlier, were older and more experienced and raised more fledglings than all other parent groups (Benito et al. unpubl.). Therefore, in this study both variables would reflect the same effect: mothers with more experience were also those laying 3 eggs and produced more female offspring, while the opposite occurred for less experienced mothers which laid just two eggs.

One reason could be that mothers suffer a decline in breeding success after some years of breeding experience and then turn to produce more of the less costly sex. If breeding performance decreases after several years, a female-biased sex ratio with increasing maternal age would be a logical prediction. However, in common terns, reproductive senescence occurs only after a period of improved performance which lasts until they are 14 yr old (Rebke et al. 2010). Another possibility may be that experienced mothers, which are also older, high-quality individuals arriving earlier and laying three eggs but nevertheless working near their capacity limit (Bauch et al. 2010), have to balance their resources between the number and the sex of their offspring. A three-egg clutch represents around 50% of female body mass in common terns (Bauch et al. 2010), and several studies have shown that, in gulls and terns, the capacity of parents to rear a brood decreases substantially with the laying of an additional fourth egg, and the relatively poor condition of females after laying reduces its ability to allocate resources to the offspring (Heaney and Monaghan 1995, Monaghan et al. 1998). Thus experienced mothers may be producing more daughters that are less demanding to rear, as a mechanism to maximize the survival probabilities of their three chicks. Although overall mortality was higher in 3-egg clutches, particularly experienced mothers reared more successful chicks, which supports this interpretation.

Finally, we cannot disregard a non-adaptive hypothesis such as female physiological changes occurring with age that may alter the ratio of W- and Z-oocytes (as suggested for X and Y in humans, although with low empirical support, Martin et al. 1995).

Chick mortality during growth showed little indications of being sex-specific. This result was as expected if sex differences in mortality were only the result of large sexual size dimorphism, and neither supports size-independent mechanisms, contrarily to prediction 4. Clutch size, hatching order and laying date are important mortality factors, as already found for terns and larids (Becker and Finck 1985, Bollinger 1994), but sons and daughters seem equally affected. Consequently, fledging sex ratio was similar to that at hatching. These results contrast with those by previous studies in terns (Szczys et al. 2001, Fletcher and Hamer 2004, González-Solís et al. 2005, Becker et al. 2008b), which found predominant female ratios at fledging and a differential loss of male chicks in certain conditions (although they used only one or two years of data, which makes them very sensitive to sporadic relationships). It is not clear yet what is the general pattern regarding sensitivity in size-monomorphic species. Experiments in the collared flycatcher *Ficedula albicollis*, showed both lack of sex-biased mortality in different environments (Sheldon et al. 1998) and poorer performance of sons in bad conditions (Rosivall et al. 2010). Larger female sensitivity has also been found (i.e. in zebra finches *Taenopygia guttata*, Bradbury and Blakey 1998), suggesting that no general rule exists.

The influence of direct parental factors in chick mortality has more rarely been investigated. Under the assumption of higher costs of sons we expected a lower survival rate of male chicks especially when raised by poor-quality parents (prediction 4). However, our results over a large sample size state that high-quality mothers (experienced and arriving earlier) are more successful breeders, but no clear sex-specific effects of parental traits on chick survival appeared. As a whole, the lack of sex-biased mortality patterns indicates that sex ratio manipulation (e.g. through differential parental investment of resources like selective feeding) does not take place during the parental care period, and that variation in parental traits do not influence much the survival of each sex.

In conclusion, our results suggest pathways for sex ratio adjustments related to parental factors that could act also in slightly dimorphic species. The lack of evidence for sex-differential mortality suggests that the adjustment mechanisms may not occur during the offspring growth period. Instead, our study suggests that adjustment of primary sex ratio may be important in shaping allocation patterns towards sons and daughters in common terns. Since sex determination is under maternal control in birds, mothers might adjust primary sex ratios in response to different allocation components such as the offspring number, condition of the partner and their own breeding experience. Previous results from common terns indicate higher costs and reproductive value of sons and our findings are partly in line with such evidence, but more research is needed to fully identify the benefits of sex ratio adjustments.

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General Discussion

Global Results & Discussion

The existence of the two sexes, their origin and their differences is one of the most challenging biological phenomena still lacking a universal explanation (Maynard Smith 1980; Charnov 1982). The scientific study of sex allocation deals with the variation in the quantity and quality of males and females and its ultimate and proximate causes. Mechanisms of sex allocation, although successfully applied to a number of taxa, are still poorly understood in vertebrates. This thesis investigated causes of variation in avian sex allocation, with a focus on the role of sexual size dimorphism and other features of life-history, associated with differences in the reproductive value of sons and daughters.

Sex allocation and sexual size dimorphism

Sex ratio and sexual size dimorphism

This section presents the results of the tests of a population model, which makes testable predictions about SR under the assumption that facultative adjustment will be favoured when the fitness benefits compensate the fitness cost (West, Reece, *et al.* 2002). Good examples of tests of population sex ratio models involve species where it is possible to estimate the strength of selection for such adjustment, for example species with sexual size dimorphism (SSD; West and Sheldon 2002).

In SSD species, producing and rearing the larger sex would force parents to invest more heavily on these offspring, because they are assumed to have greater demands (Slagsvold *et al.* 1986; Nicolaus *et al.* 2009; Kalmbach, Griffiths, *et al.* 2009; Kristensen *et al.* 2013). Fisher's prediction states that the sex ratio will be biased towards the smaller and cheaper sex at the end of parental care period, such that overall investment into both sexes is equal in the population. Total investment of parental resources in the two sexes can be balanced by adjusting sex ratios at birth (HSR) or during the period of parental care (FSR). Under the assumption that offspring of the species that are more sexually size dimorphic will have a greater sex difference in demands (Clutton-Brock *et al.* 1985; Magrath *et al.* 2007), the differences in the reproductive costs of sons and daughters due to SSD are expected to correspond to the investment ratio in male and female offspring, and therefore, to correlate with biases in the sex ratio at different life stages.

In partial support of this prediction, the species-level and comparative analyses carried out across bird species in Chapter 1 showed that population hatching and fledging sex ratios were influenced by the degree of SSD. Being the overall effect weak, the trends were in the predicted direction and qualitatively consistent. Dimorphic species showed a general

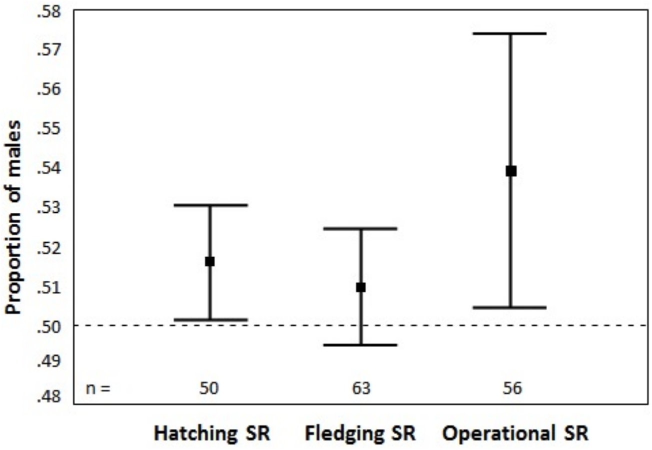


Figure 6: Interspecific mean values of sex ratios (95% CI) at different life stages. Sex ratio expressed as proportion males.

tendency to bias hatching SR towards the smaller (cheaper) sex, in both directions of SSD: higher proportion of daughters in species where males are relatively larger and vice versa (Fig. 1a and Table 1, chapter 1; page 44). At species-level without phylogenetic control, the mean interspecific sex ratio presented a significant male prevalence (Figure 6). However, this was dependent on the type of SSD. Monomorphic and male-biased SSD species showed a SR closer to parity (Fig. 1a, Appendix S5, chapter 1; page 66), whereas deviations were particularly remarkable among species with female-biased SSD. At fledging time, these patterns (Fig. 6; Fig. 1b and Table 1, chapter 1 –page 44–; Fig. 1b, Appendix S5, chapter 1 –page 66–) were similar but softened with respect to those at hatching.

The scarce previous comparative analyses of SR in birds with SSD have shown little evidence for consistent hatching sex ratio biases at the population level (Clutton-Brock 1986; Pen, Weissing, *et al.* 2000). Fledging sex ratio biased towards the smaller sex was indeed found in two reviews (Slagsvold *et al.* 1986; Pen, Weissing, *et al.* 2000). However, another one found the opposite pattern: more of the larger sex at fledging (Dijkstra, Daan, and Pen 1998). The results presented in this thesis include new data for many species (especially female-biased SSD species were lacking) and use relatively new techniques (of sex determination and of analysis). Indeed, the results were clearer when controlling for phylogeny and with data restricted to molecular studies, which use more reliable sexing techniques that allow sex-determination very early in the development.

Previous studies on single species have reported significant biased sex ratios at hatching, especially among species showing high size-dimorphic differences (e.g. Dijkstra, Daan, and Buker 1990; Velando 2002; Kalmbach, Nager, *et al.* 2001; Øigarden and Lifjeld 2013). This is not surprising, since the most extreme and precise sex ratio adjustments are predicted in species where the fitness benefits of facultative SR adjustment are high and the costs are low, as expected if the degree of SSD is associated with differential costs of sons and daughters (Komdeur 2012). Therefore, general adjustment of hatching sex ratio, particularly among female-biased SSD species, may be a way to equalize investment between sexes with different costs. However, no significant trends for HSR (nor FSR) with SSD appeared in the similar comparative analyses of Chapter 2 (Table 13.1, page 75). Although the discrepancy may come from the relatively small number of species used in Chapter 2 (15 against 50), caution is needed before asserting that biased sex ratios in presence of SSD are the result of equal investment into sons and daughters in presence of SSD.

The extent of the influence that the patterns of primary and secondary sex ratio variation have in tertiary sex ratios is largely unknown. As the ratio of males to females ready to mate, the tertiary or operational sex ratio (OSR, Box 1, page 4) greatly influences the intensity of the mechanisms of mating competition in a population, and hence also the intensity of sexual selection (Kvarnemo and Ahnesjoe 2002). Although not presented in any chapter, the OSR of 56 avian species was also analysed (database in Appendices A and B, pages 233 and 235). The mean interspecific value showed a significantly male-biased proportion, higher than in any other life stage (Figure 6).

A general acceptance of Fisher's argument led to consider tertiary sex ratios as approximately 1:1 in wild bird populations. However, Mayr (Mayr 1939) and subsequent reviews (Payevsky 1993; Donald 2007) concluded that skewed adult sex ratios are common in birds, with males outnumbering females by around 33% on average (although there might be also an influence of phylogenetic factors). This might be due, rather than to skewed offspring sex ratios, to higher female mortality rates between independence and the sexual mature stage (Donald 2007). However, adult sex ratio values are often very difficult to collect and may be severely biased by the type of counting method. Whereas OSR refers strictly to the ratio of males and females able to breed in a population at a given time (in breeding grounds or for individuals in breeding age; Kvarnemo and Ahnesjoe 2002), most of the OSR studies used in this analysis were actually measuring adult sex ratios during the breeding *and* post-breeding season, as well as in wintering or migrating populations. Even when OSR is apparently correctly measured, inaccuracies are still possible if sub-adults or inmatures present in breeding grounds belong mainly to one sex. Therefore, although the trend presented here is in agreement with other reviews, we should be aware of the limited validity of these data.

The analysis at the species-level of the relationship between the degree of SSD and adult sex ratios revealed no significant pattern (Figure 7). This may indicate that the costs of size dimorphism are mainly incurred during juvenile growth, and that the suggested female-biased mortality after fledging is the result of other factors rather than size. Heterogamety, sexual differences in foraging behaviour, postnatal dispersion, predation or reproductive effort (Promislow *et al.* 1992; Owens and Bennett 1994; Awkerman *et al.* 2007), and the type of mating system (Breitwisch 1989), are suggested as major determinants of unbalanced adult sex ratios. Male-skewed OSR might create sufficient variance in mating success between males as to drive the evolution of sexual ornaments (Dearborn *et al.* 2001) and of SSD. In any case, these results, although coincident with previous observations (Donald 2007), should be confirmed by a phylogenetically-controlled analysis.

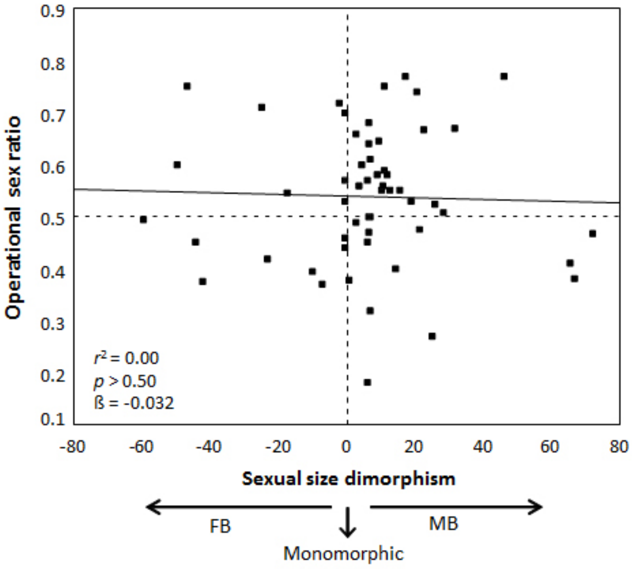


Figure 7: Relationship between SSD (Storer's index) and operational sex ratio. FB, ♀-biased SSD; MB, ♂-biased SSD.

Offspring vulnerability and sexual size dimorphism

Shifts of the FSR with respect to parity could simply result from an initial hatching bias, but also from a differential survival of sons and daughters between hatching and independence time. Many studies report a greater sensitivity of one of the sexes (reviewed in Jones *et al.* 2009), but it is unclear which factors are responsible for a differential performance of sons and daughters that may eventually lead to sex-biased mortality.

Differential vulnerability may be the result of parental active manipulation of juvenile conditions after birth. Sex-biased parental care has been found in several species (R. M. Kilner 2006; Griggio *et al.* 2009; Mainwaring *et al.* 2011; Leech, Rowe, *et al.* 2006). Differential mortality during the period of parental investment does select for biased sex allocation according to Fisher's theory, so primary sex ratios would correlate with mortality rates in case adaptive manipulation is involved. In species where female mortality is greater, for example, primary sex ratio would be biased towards females. Although not specifically tested in this thesis, the male prevalence at hatching in all types of species (but especially in female-biased SSD ones), as well as the comparison of hatching SR with fledging SR patterns (Fig. 1, Appendix S5, chapter 1; page 66) does not support this possibility.

More likely, sex-specific differences in mortality or other measures of vulnerability are reflecting differences in the growth patterns between the sexes. The comparative analysis of Clutton-Brock (compiled in Clutton-Brock 1991) showed higher male mortality in male-biased SSD species, also found by case-studies (in both directions of SSD: Griffiths 1992; Krijgsveld *et al.* 1998; Torres and Drummond 1997; Bortolotti 1986; Kalmbach, Furness, *et al.* 2005). Opposite results (e.g. Nager, Monaghan, Houston, *et al.* 2000; Hörnfeldt *et al.* 2000) or directly no sex mortality difference in species with rather marked SSD (Westerdahl *et al.* 2000; Råberg *et al.* 2005) have also been found. The results presented here (using sex ratio data strictly monitored in the same broods) clearly showed the existence of a sex-specific mortality negatively correlated with size dimorphism (Fig. 2b and Table 1c, chapter 1 –pages 44, 45–; Table 13.1 and Fig. 13.1, chapter 2 –pages 75, 76–). The larger sex suffers higher mortality: sons exhibit a relatively greater mortality in male-biased SSD species and daughters in female-biased species, and the survival disadvantage increases with increasing size dimorphism. Vulnerability, measured as change of fledging mass under different conditions, was also related to the degree of SSD (Table 13.1 and Fig. 13.2, chapter 2; pages 75, 79): the larger sex (either males or females) suffered a greater mass reduction when rearing conditions were poor. The minor mass changes in offspring of monomorphic species suggest that in absence of SSD no sex has more vulnerability than the other.

Altogether, these patterns indicate that both males and females pay a cost, in the form of mortality or negative fitness effects, for achieving a larger final body size. Hypothesis of size-dependent vulnerability suggest two opposite mechanisms: (1) increased vulnerability of the larger sex under poor rearing conditions due to its higher nutritional needs or (2) increased vulnerability of the smaller sex (Oddie 2000; Råberg *et al.* 2005; Rowland *et al.* 2007) due to the advantage of the larger sex in intra-brood competition (Hipkiss *et al.* 2002). Because both Fisher's argument and the first mechanism predict fledging sex ratios skewed towards the smaller sex and suggest the same mechanism to achieve it (greater mortality of the larger sex), it would be difficult to distinguish the real cause of sex-specific mortality. In any case, size seems indeed the main reason for increased mortality, because daughters of female-biased SSD species experience similar disadvantages than sons in male-biased SSD species. Size-dependent effects, specifically by increased sensitivity of the larger sex, are possibly the cause of differential vulnerability.

Other indications, however, suggest more to the story. Size-independent explanations (e.g. "male-phenotype hypothesis") have also been proposed, accounting for the general male bias in offspring vulnerability observed in birds. Overall nestling mortality appeared slightly male-biased (Fig. 2b, chapter 1 –page 46–; Fig. 13.1, chapter 2 –page 76–), as did the average mortality value in the comparative analysis of Chapter 2. Moreover, further analyses on fledging mass change showed that the general pattern described above was dependent on the type of SSD: while female mass differences seem independent of SSD, in males they are strongly correlated to the extent of size dimorphism (Fig. 13.3, chapter 2; page 80). This suggests that, besides size, offspring survival is negatively affected by sex-specific traits.

Therefore, although being the larger sex carries viability and survival costs during growth, it is mainly disadvantageous in conjunction with the male phenotype. Male hormonal profile is a likely candidate for mediating male vulnerability (Olsen and Kovacs 1996). The high levels of testosterone and other hormones needed for male sexual differentiation may negatively impact other aspects of development (Fargallo *et al.* 2002; Møller, Sorci, *et al.* 1998; Müller, Groothuis, *et al.* 2005). Our results are supported by a recent meta-analysis, which concluded that sex-specific patterns of vulnerability can be explained by a combined influence of SSD and other factors, rather than by size or sex factors on their own (Jones *et al.* 2009).

In any case, if the differences in mortality found in this and other studies are not due to parental decisions, they may be acting to moderate the strength of selection for sex ratio biases in the populations, because a higher baseline mortality of the larger sex would reduce the differences in the costs of producing males and females (Komdeur 2012).

Life-history factors in the relationship SR-SSD

Sex ratios have been frequently studied in SSD species because this characteristic is considered a major determinant of SR variation. However, birds show life-histories that add a layer of complexity to sex allocation (Box 3, page 14). Fisher's theory is based on certain assumptions that may not hold in this context, as these factors may differentially affect the fitness returns of sons and daughters. The life-history traits selected for this analysis were presence of plumage sexual dimorphism, coloniality, mating system, presence of helpers, migration behaviour, breeding distribution, nest location, incubation and fledging period, clutch size, hatching sequence, developmental mode, age of first breeding and adult survival rate (definitions and data in Appendices S1 and S4, chapter 1; pages 51, 61–65). They are all considered by different sex allocation models as potential modulators of adaptive sex ratio variation.

The relationships between hatching/fledging sex ratio and size dimorphism, once the effects of the relevant life-history traits were controlled for, did not show significant patterns. The importance of SSD in shaping sex ratios may be diminished by other characteristics of the species that outweigh or oppose to that of size. Dichromatic species, for example, showed a higher proportion of males at fledging time (Figure 8) regardless of the degree of SSD. Plumage dichromatism is a strong sign of sexual selection, even in monogamous species (Webster *et al.* 2007), and a higher variance in attractiveness of the partner could motivate females to invest differentially in their offspring (*Attractiveness hypothesis*). Since at hatching no relationship appears, plumage dimorphism could be expected to influence sex-differential mortality between both life stages. However, this is not the case, suggesting that dichromatism may be also intercorrelated with other factors.

Altricial development of the chicks and increased age of first breeding were also associated with a higher number of males at fledging; in addition, the rate of male mortality decreased with longer fledging periods (Table 2a-b, chapter 1; page 45). These trends were independent of SSD degree or type.

However, other life-history factors showed an effect on sex ratio *and* SSD (restricted database of molecular studies; Table 2c, chapter 1, page 45): more males than females were found at fledging in altricial species, and the mortality of males was lower in monogamous species. Mating system of the species and developmental mode of the offspring imply different descriptions of parental care. Precocial chicks may show different energy requirements between the sexes, but as they feed themselves they are likely much less demanding on parents than altricial chicks, where both sexes are highly dependent on parental provisioning.

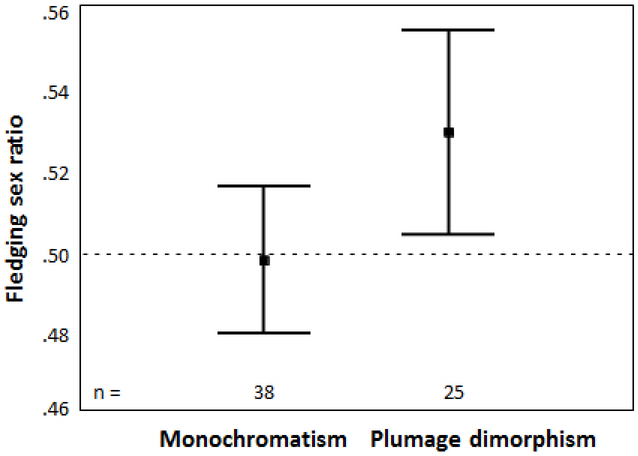


Figure 8: Interspecific mean value of fledging sex ratio (95% CI) according to the presence or absence of sexual dimorphism in plumage.

Apart from that, sons may suffer more in polygynous species where there is a greater reproductive male variance and breeding success in males is more strongly influenced by body size than in females (Nishiumi 1998). The influence of SSD and the life-history factors, however, showed opposing signs in both cases. This proves the complexity of these relationships and the difficulty to predict patterns. In some cases, the effects may all act in the same direction making explicit predictions possible (e.g. Seychelles warbler, Komdeur 1998). In others such as the cases presented here, factors can act in divergent directions, making hard to know what pattern to expect in the absence of quantitative estimation of the importance of each effect. However, these analyses are still useful to make relative (comparative) predictions for how the population sex ratio or the extent of sex ratio adjustment should vary across species rather than what should happen in a particular species (Griffin *et al.* 2005; West 2009).

The evidence, even if mixed, that some life-history variables affect sex ratios and the modulating role of SSD on them, points toward a “conditional sex allocation” scenario rather than an “equal-investment” one. Since several of its assumptions are not fulfilled, the validity of Fisher’s argument for the biased-sex ratios related to SSD at population level is still uncertain.

The strength of the comparative method for sex ratio analyses

Although comparative methods have received increasing attention in the last years and are firmly encouraged (West 2009), recent tendencies in studies across species have come back to their origins. Several studies suggest that species-level analysis, rather than evolutionarily independent contrasts, may be more appropriate for some forms of comparative analysis (Martins 2000; T. Price 1997).

The rigour of the quantitative studies is based on the underlying data, which in comparative studies varies widely in accuracy, quantity, and in methods of collection. One of the frequent

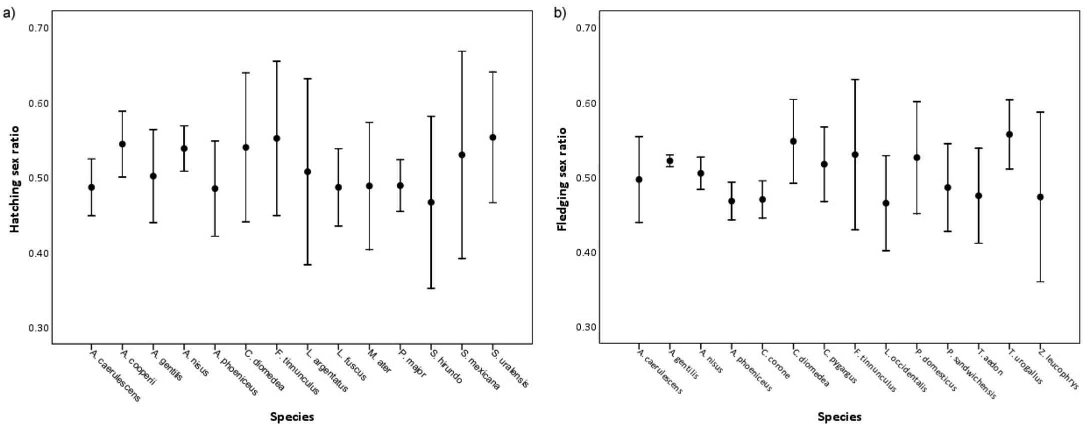


Figure 9: Mean values of a) hatching and b) fledging sex ratio (95% CI) for those species with more than one reported value.

criticisms made about comparative analysis is that intraspecific variation is too great to enable meaningful comparisons between species. However, when the range of studied taxa is wide, variation among species is usually greater than variation within species. So, although there is variation among individuals within a population, and across populations of the same species, this variation is often small compared to the variation across species or higher taxonomic levels. Figure 9 shows that this is the case for sex ratio in birds, specially at fledging; the higher variation at hatching may be due to the higher number of species with only two recorded values. Along with the results of Chapters 1 and 2, this confirms that at least in some cases, species-level and phylogenetic approaches can lead to consistently similar results, making both types of comparative analysis valuable and complementary.

Sex allocation in the common tern

To test theories of sex allocation in a single bird species, it is important to cover three aspects: investigate the variance in sex ratios at different levels within the population, investigate the difference in the costs of producing sons and daughters (maybe affected by environmental and parental factors), and investigate whether the sex ratio is adjusted according to these factors (Hasselquist and Kempenaers 2002).

Sex-specific traits in the offspring

When investigating variation in sex ratios, the factors that have potential sex-specific effects on the offspring fitness should be identified (see Komdeur and Pen 2002). So far the focus has been placed on sexual size differences as generators of sex ratio variation, but the common tern is only slightly size dimorphic (around 3% larger males). According to the results of the first part, common tern populations would show a slight male prevalence at hatching and a slightly male-biased vulnerability. This would turn into a slightly male-biased FSR; in any case, all ratios would be close to parity.

However, apart from size, the quality of the external and social environment, the quality of the parents, the number and asymmetry among the offspring, etc. are likely influential too (Sheldon 1998). It is therefore important to study whether one sex is more costly to produce than the other, for example, because it has a higher metabolic rate or food requirements (Teather and P. J. Weatherhead 1988; Krijgsveld *et al.* 1998), grows faster (Teather 1987), begs more or shows higher aggressiveness (Ayala *et al.* 2007). Any sexual dimorphism described in the common tern chicks could imply a difference between the sexes potentially able to modify the relative reproductive value of each sex. Moreover, any sex-linked differences in sensitivity would indicate the effects of possessing a male or female phenotype without the confounding influence of great differences in body size.

Common tern offspring (in these particular studies, a-chicks) shows two remarkable exceptions to the generalized lack of sexual dimorphism in most morphological, physiological and immunological traits (Table 1, chapter 3; page 91). Shortly before fledging, sons and daughters show different bill-head lengths and CMI (cell-mediated immune-response), which are both larger in males (Chapter 3). Larger bill-head lengths are also found in male adults and in post-fledglings (Becker and Wink 2002). In opposition, body mass, the only sexually dimorphic characteristic previously found in tern fledglings, did not differ between sexes in this study. The reason probably results from a lack of statistical power to detect subtle differences just at the beginning of divergence process, since mass begins to deviate in a sex-dependent way from 18 days old onwards (Becker and Wink 2003).

Dimorphism is also present in the sex-specific development of some growing strategies (Chapters 3 and 4). Sex-specific trade-offs during growth for male chicks seem to involve the allocation of resources preferentially to body condition (Chapter 4) and to immune function (Chapter 3; see also Cucco *et al.* 2006; Soler *et al.* 2003), maybe to compensate detrimental effects of male-phenotype condition (Blas *et al.* 2006). Precisely the high concentration of immune-suppressive hormones in males is often invoked as the cause of their general reduced immune-activity when compared to female birds (Møller, Sorci, *et al.* 1998; Roberts *et al.* 2004; but see Hasselquist, Marsh, *et al.* 1999). Moreover, when chicks are experimentally challenged (enlarged broods, food limitation, increased competition) males usually suffer stronger decreases of CMI than females (Fargallo *et al.* 2002; Chin *et al.* 2005; Dubiec *et al.* 2006). Common tern male chicks, on the contrary, showed consistent stronger responses than females, especially in 3-eggs clutches and when competing with siblings during the final periods of development (Fig. 3 and Table 2, chapter 3; page 94, 97).

Thus, when more resources are available due to good environmental conditions or good parental care –as simulated by the experimental supplementation of a limiting resource in nature (Chapter 4) and as measured through clutch size as indicator of parental quality (Chapter 3)–, sons seemed to prioritize the development of traits such as size and CMI. This would allow them to reach the slight size dimorphism by fledging time and may indicate higher viability and enhanced survival rates, since increased CMI is positively related to individual traits such as body mass (Alonso-Álvarez and Tella 2001), survival probabilities (González *et al.* 1999) and protein nutritional status (Lochmiller *et al.* 1993). Male chicks investigated in the experimental carotenoid study, however, did not show increased immune responses, maybe due to lack of statistical power (lower sample size), to interactions with the carotenoid metabolism or to year influences (Fig. 3a, chapter 4; page 113).

If apparently sons have better prospects, why their phenotype and mortality are similar to these of daughters? The lack of relationships between sex, phenotype and survival of chicks (Chapters 3 and 4) could mean, despite the above results, that sons and daughters do not differ in sensitivity to environmental and rearing conditions. However, another explanation would be that female chicks have their own beneficial growing strategies regarding resource allocation, which allows them to balance the situations of sibling competition and maximize the access to parental care. In order to attain similar size and condition than males, females may reduce investment in immune function in favour of other traits (Chapters 3 and 4). Supporting this interpretation, female chicks allocated more resources to their skin coloration (Fig. 2 and Fig. 4, chapter 4; pages 112, 114). This conspicuous signal may be a potential advantage in obtaining parental attention, although it may entail a small cost in terms of immune system. Such strategy in the trade-off between carotenoid-based coloration and immune response, together with the evidence that females endure longer periods of sibling competition (Table 4, chapter 3; page 98), might be optimal patterns if underdevelopment of the immune system at the nestling stage can be compensated later in life (Birkhead, F. Fletcher, *et al.* 1999).

Apart from the already mentioned slight SSD, previous evidence of the species points towards males as the more vulnerable sex to adverse conditions or poor quality parents and therefore, the more costly offspring sex. Sons suffered higher post-hatching mortality when reared by younger and lighter parents (González-Solís, Becker, Wendeln, and Wink 2005) or in certain hatching positions (K. L. Fletcher and Hamer 2004). In addition, mixed broods seem advantageous for sons (Becker and Wink 2003) while in male-broods the second chick displayed a poorer condition (Braasch, Palme, *et al.* 2011). The results of this thesis regarding trade-offs in males might imply (1) that sons benefit more from improved conditions, (2) that their reproductive value could be more condition-dependent than that of daughters and (3) that under certain conditions, they also have advantages in sibling competition. However, overall evidence does not allow concluding that sons and daughters show, at least based on phenotypic traits, great differences in production/rearing costs.

As semi-altricial offspring, common tern chicks are dependent on the amount and quality of parental care they receive. Therefore, parental factors were expected to contribute to certain phenotypic dimorphism between sons and daughters. Higher quality parents would likely invest more resources in their offspring than other parents, and their chicks may respond to the higher availability or better resources differentially according to their sex (Metcalfe and Monaghan 2001; Hegyi *et al.* 2011). Alternatively parents may adjust the level of resources allocated to their sons and daughters if this results in different reproductive values and thus in different relative fitness benefits for the parents (Frank 1990).

Parental factors such as age, body mass and arrival date were not clearly related to pheno-

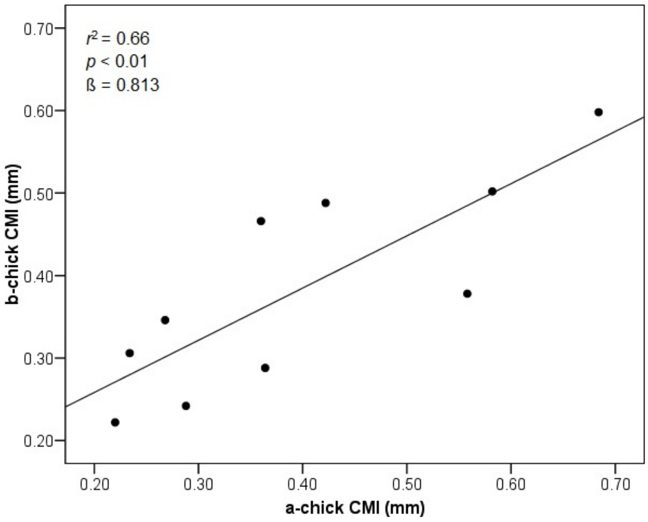


Figure 10: Intra-brood values of absolute increase thickness in foot web (mm) after PHA test, for siblings of common tern ($n = 10$ broods). a-chick: first-hatched chick; b-chick: second hatched chick.

typic traits and mortality of the nestlings, at least sex-specifically (Fig. 2 and Table 3, chapter 3; pages 95, 96). However, immune-responses of male chicks were positively associated with parental quality –in the form of clutch size; see page 25– (Fig. 3, chapter 3; page 97). The relationship was especially remarkable in sons sharing the nest with siblings at final stages of development. These results highlight: (1) a male advantage in sibling competition at its strongest point (from 14 days old onwards, Klaassen *et al.* 1992) and (2) the high quality of the parents successfully raising more than one chick until fledging (see also Apanius and Nisbet 2006). Phenotypic variance in immune response has a significant genetic component (Drobniak *et al.* 2010) as confirmed by the significant brood-specific CMI found in a few nests where both the a– and b– nestling were sampled (Figure 10). Thus, rearing conditions (clutch size, presence of siblings), as a reflection of the quality of parental care or condition, play a role in an enhanced immunological status of the offspring (Hõrak *et al.* 1999).

Overall, these results suggest that parental investment after birth in common terns is not distinctly sex-specific. The life strategy used by seabirds may be not to maximize the offspring reared in a given season, but to have a high number of reproductive attempts to rear some offspring (Forbes and Mock 2000). Another possibility could be that differential parental investment in relation to sex occurs in order to achieve a similar phenotypic state for sons and daughters; especially if, as some models of differential allocation of parental investment suggest, fathers are selected to favour offspring of one sex and mothers those of the other sex (Lessells 1998). Finally, the differential ways in growth trade-offs found for sons and daughters of common tern may not reflect sex-specific chick responses to resources allocation but patterns imposed on chicks by strategic parents, since at least until fledging access to important resources relies exclusively on parental provisioning.

Therefore, an overview of these results underlines:

1. the potential of parents to affect the development of their offspring by differential supply of particularly important resources such as carotenoids
2. the influence of parental reproductive quality on the sexual differences, even if slight, of the offspring.

Although these influences suggest the existence of differential reproductive efforts from the parents and ways in which parental allocation may differentially affect their offspring, so far it is not clear whether this is reflecting a difference in the reproductive value of sons and daughters.

Population and individual patterns of sex ratio

Optimal sex-allocation theory makes predictions about mean sex ratios but also about the statistical variance (see pages 8 and 10). Sex ratio variance would mostly follow the binomial expectation in organisms with CSD, but under some circumstances (for instance, when the Trivers-Willard assumptions apply) overdispersion (greater than binomial variance) may be favoured by selection (e.g. Frank 1990). Therefore, variances in population sex ratios are of interest because they can indicate parental control of sex allocation (Krackow 2002).

The long-term study carried out to test adjustments of sex ratio in a large sample of common tern broods (Chapter 5) revealed that population-wide patterns of sex ratio (at hatching and fledging; Fig. 1, chapter 5, page 125; page 126) were slightly female-biased but not significantly different from parity. Although more daughters than sons died during development, no significant sex-specific mortality was found (Table 3, chapter 5; page 129). Similarly, mother identity did not explain variation in sex ratios or mortality. So far these observations would fit to the theoretical situation of a species nearly monomorphic, with similar production and raising costs of sons and daughters, and where mechanisms of nestling mortality show no impact of heterogamety or male-phenotype effects. Other studies in species with slight SSD have found similar results (Leech, Hartley, *et al.* 2001; Fair and O. B. Myers 2002; Ramsay *et al.* 2003; Pagliani *et al.* 1999), also in common terns (González-Solís, Becker, Wendeln, and Wink 2005; K. L. Fletcher and Hamer 2004). In addition, the lack of overdispersion in the model on data aggregated at the clutch level may indicate that sex allocation in this population is random, as would be if defined by pure binomial sampling. These evidences together suggest that sex ratio manipulation does not occur in this population.

However, from such analyses we can only infer variance dispersion for that particular level of aggregation. For example, if sex ratio adjustments take place within rather than between clutches, this will not necessarily appear as overdispersion. Moreover, mechanisms of variance increase seem to be potentially widespread (Krackow 2002), and their effects may be operating in opposite directions, so that the resulting distribution does not differ from a binomial one. Therefore the absence of deviation in a particular sample cannot be assumed as evidence for the absence of factors affecting sex ratio variance (Edwards 1960) or the inexistence of sex ratio manipulation at individual level (Hasselquist and Kempenaers 2002). For example, the Seychelles warbler, being one of the most dramatic examples of adaptive sex allocation, shows no bias in population sex ratios (Komdeur, Daan, *et al.* 1997; Komdeur 1998). Some authors suggest that models of evolutionary stable strategies make no clear predictions about the sex ratio at population level, even in cases of facultative adjustment (Cockburn *et al.* 2002; Pen and Weissing 2000).

Analyses at the individual level in the common tern partially supported some of the sex-allocation hypotheses (in the Trivers-Willard sense) based on life-history traits of the species. The hatching proportion of sons was related to paternal mass and showed a tendency to be affected by the environmental year quality (Table 1 and 2, Fig. 1 and 3b, chapter 5; pages 125, 126, 128), although not by the seasonality within the year (Figure 11).

The influence of these two factors was indeed expectable. Environmental factors are known to heavily influence avian breeding success and also hatching sex ratios (Suorsa *et al.* 2003; Merking *et al.* 2012; Pryke and Rollins 2012). Small seabirds like terns, with a specialized diet, small foraging range and tight energy budget are particularly sensitive toward local changes in food supply and adverse weather conditions (Furness and Tasker 2000). Additionally, common tern condition, measured as body mass, is a stable and age-independent individual feature with substantial effects on reproduction (Wendeln and Becker 1999; Wendeln, Becker, and Wagener 1997). The condition of the male partner, irrespective of female condition or quality, seems thus to be an incentive to allocate towards sons.

This evidence supports the male-attractiveness hypothesis and to some extent the environmental hypothesis. This may be a good allocation strategy if sons are actually the more costly sex, if its reproductive value is more strongly condition-dependent than that of daughters (Addison *et al.* 2008; Zielinska *et al.* 2010) or if sons benefit more from improved rearing conditions, as suggested by the previous phenotypic results.

So far, these results confirmed a differential sex allocation in the predicted direction. The negative association between clutch size and maternal experience with the number of hatched sons, however, is apparently inconsistent with the expectancy of increased allocation towards males with increasing parental quality. Experienced mothers, mostly laying 3 eggs, overproduced daughters (Fig. 3a and Table 2, chapter 5; page 128). There are several possible explanations for this observation. The first one would be the existence of reproductive senescence, but in common terns, especially in females, it has been found only after a period of improved performance that lasts until 14 years old (Rebke *et al.* 2010). Physiological changes with age that alter the frequency of gametes may also occur, but they are not completely supported yet (Martin *et al.* 1995). This result may also be the consequence of a trade-off between the number of offspring, their sex and their survival probabilities. Experienced mothers are also older, high-quality individuals that arrive early, lay three eggs and rear more successful chicks until fledging. Nevertheless, they are probably working near their capacity limit (Bauch *et al.* 2010) and may need to balance their resources, or may try to avoid future competition, as females more likely prospect other colonies and emigrate (Dittmann,

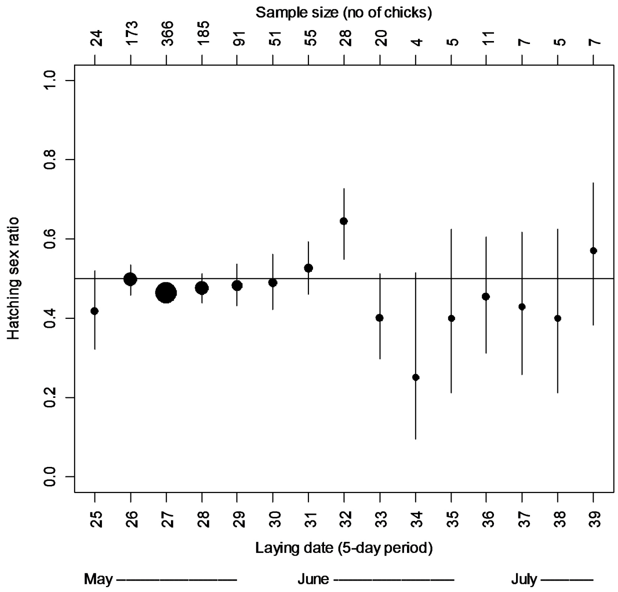


Figure 11: Hatching SR in relation to laying date (grouped in pentads, $1 = 1^{st}$ January – 5^{th} January; years 2002 – 2008). Means and SE were estimated from a GLMM with binomial error structure and logit link, including laying date as a fixed factor and mother and year identity as random effects. Estimates (mean ± 1 SE) are backlinked to the probability scale for display. Point sizes are proportional to sample sizes.

Zinsmeister, *et al.* 2005; Becker, Ezard, *et al.* 2008).

Alternatively, a higher production of daughters might provide a good terminal investment over fewer numbers of chicks altogether, or over sons, because of a higher reliability of fitness returns from daughters over sons. In case that quality is passed mainly from mother (and not father) to offspring, not all the grand-offspring would be of the same value. For a high-quality mother, a daughter would be more valuable than a son because of her relatively higher than average quality, which would be again inherited by her daughter's offspring (Leimar 1996). Therefore, the production of daughters of high quality may involve unrecognized fitness returns.

Overall, the effects of adjustment described in the sex ratio of common terns follow different directions, and thus might cancel out, being the reason of the unbiased population patterns found.

In this context, an important question is how much variance exists in the reproductive success of male and female terns. To date, the answer is unknown. Theoretically, in most seabirds there should be no conflict over parental care because the interests of the sexes are similar, and partners depend onto each other to rear the offspring (Birkhead and Parker 1997). Consequently, extra-pair paternity is rare (González-Solís, Sokolov, *et al.* 2001). Besides, there is no evidence of sex-linked variances in adult survival and operational sex ratio is thought to be practically even (Ezard *et al.* 2006; Becker and Zhang 2011). This may mean that there is little potential for differences between males and females in their reproductive success

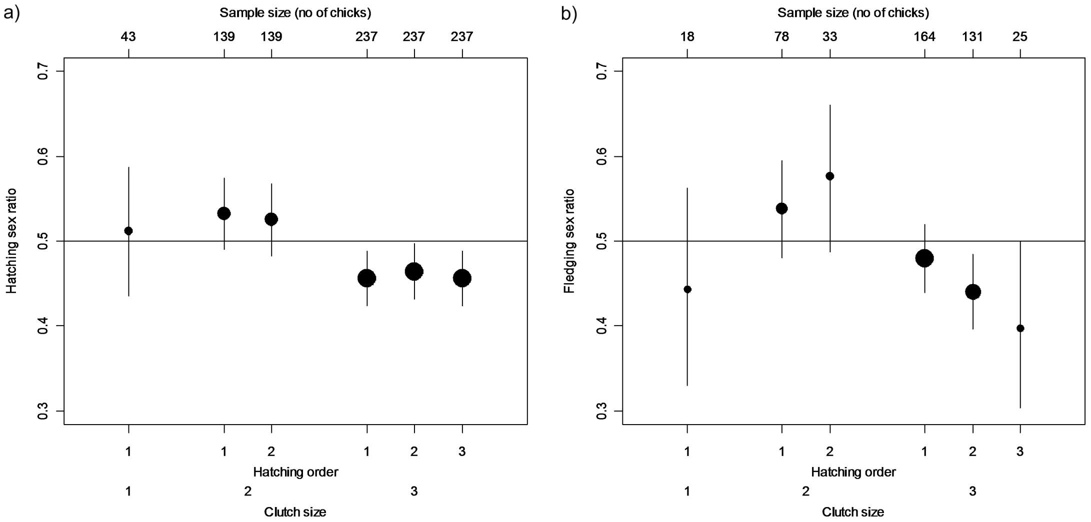


Figure 12: a) Hatching and b) fledging SR in relation to clutch size and hatching order. Means and SE were estimated from a GLMM with binomial error structure and logit link, including egg position as a fixed factor and mother identity as a random effect. Estimates (mean ± 1 SE) are backlinked to the probability scale for display.

variance. However, the high constancy in body condition within individuals and the great differences between individuals may also result in differences in lifetime reproductive success (LRS; see Box 1, page 4) and fitness (Wendeln and Becker 1999). Furthermore, there is a correlation between reproductive performance and age or breeding experience, so LRS can be significantly affected by the frequency of changing mates and the age or experience of these mates (Ludwig 2010). These traits of individual quality are thus subjected to sexual selection, which allows individuals to obtain high-quality mates and the possibility to increase LRS. Indeed, female terns show a slightly higher (although not significant) average breeding success over lifetime than male terns (unpublished data).

As mentioned above, the lack of overdispersion at clutch level may conceal sex ratio adjustments taking place within rather than between clutches. Sex manipulation according to hatching order seems common in other species (Husby *et al.* 2006; Dijkstra, Riedstra, *et al.* 2010), also in terns (female-bias of a-chicks in roseate terns, Szczys *et al.* 2001; and of c-eggs in common terns, Fletcher:2004aa), but it was not found here. There is an apparent tendency of the 3rd egg in 3-egg clutches to be female-biased in poorer years (Fig. 2b, chapter 5; page 127). However, detailed inspection of the interactions of the stepwise model (“Seasonal variation in sex ratio”, chapter 5, page 126), showed no significant trends between year quality and hatching order in 3-egg clutches. In fact, on average c-chicks had a similar sex ratio (Figure 12a).

The occurrence of sex-biased sequences that may result in favorable sex-combinations within the brood (if the first egg is male, is the second more likely to be male or female? and the third?) was also preliminary studied. Neither 2- nor 3-egg clutches showed any hint of this possible adjustment (unpublished data). In any case, it should be noted that we do not

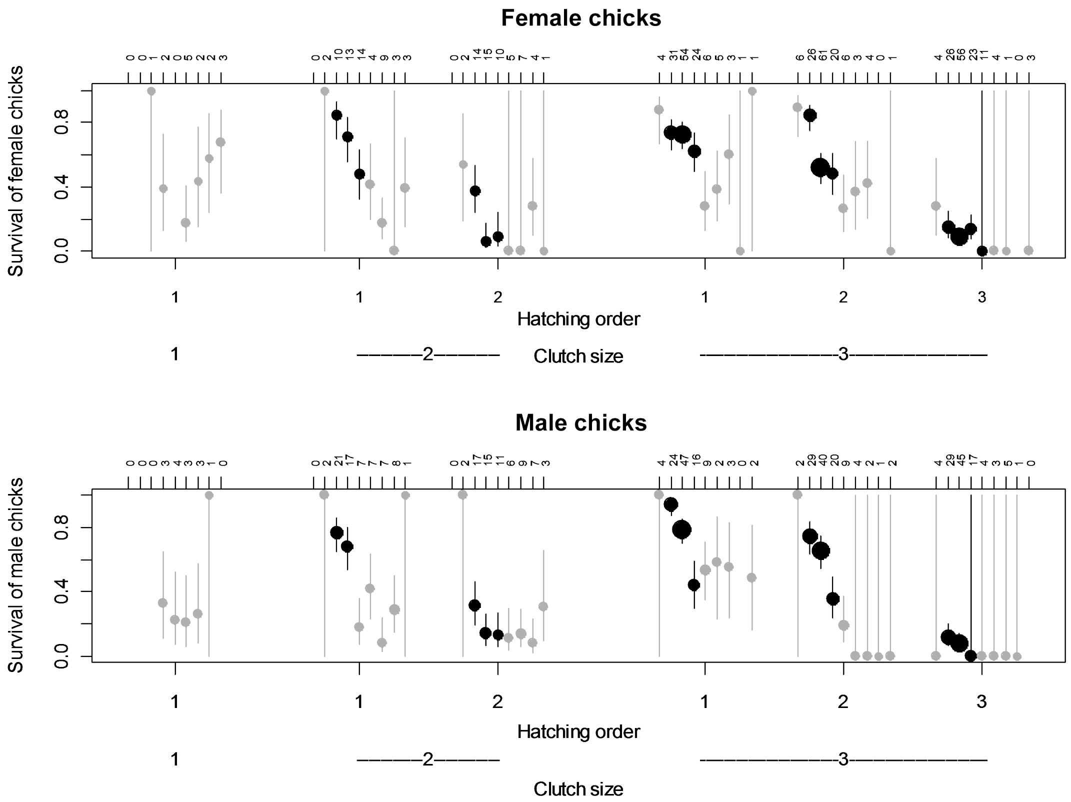


Figure 13: Offspring survival by sex in relation to clutch size, hatching order and laying date ($n = 1032$). Within blocks, laying date pentads are shown from left to right (a few combinations have no sample and therefore no dot). Means and SE were estimated from a GLMM with binomial error structure and logit link, including laying date as a fixed factor and mother and year identity as random effects. Estimates (mean ± 1 SE) are backlinked to the probability scale for display. Point sizes are proportional to sample sizes, and classes with sample size less than 10 are shown in grey in order to emphasize the larger-sample estimates in black.

exactly know how early during development biased sex ratios occur. Although our dataset covered a wide range of years (7) and broods (389), a shortcoming of the study is the lack of information on pre-hatching mortality and sex ratio of unhatched embryos, so that the conclusions raised here do not concern the real primary sex ratio (at fertilization) but the hatching sex ratio.

Earlier studies on common terns found female predominance at fledging and a sex-specific mortality of male chicks (Szczys *et al.* 2001; K. L. Fletcher and Hamer 2004; González-Solís, Becker, Wendeln, and Wink 2005; Becker, Ezard, *et al.* 2008). The data presented in this thesis, over a longer period and more robust against sporadic temporal relationships, did not confirm these results. However, fledging SR showed some changes with respect to hatching SR (Figure 12), which suggests certain differential mortality between both life stages. Indeed, factors like clutch size, hatching order and seasonality play an important role in offspring survival (Becker and Finck 1985). However, sons and daughters were equally affected (Figure 13; Table 3, chapter 5, page 129). Similarly, the influence of parental factors on offspring mortality was hardly sex-specific (Table 4, chapter 5; page 129), although some trends may be worth of further investigation (maternal body mass and arrival date might affect more the survival of sons).

The global picture of this thesis provides some relevant summarizing ideas. Active parental mechanisms of sex ratio bias (e.g. differential investment during the period of parental care), either related to SSD or to environmental and quality factors, do not seem to take place throughout growth. Instead, the adjustment of primary sex ratio is suggested as the main way of manipulation, maybe due to the lower investment energy required at this stage. This adjustment would occur in response to different allocation components influenced by SSD, life-history characteristics and condition factors, which may act in opposing directions. Chromosomal sex determination may still act as a constraint, but it can be overcome by consistent selection. If female birds are able to regulate sex determination mechanisms, they might be mainly in control of key factors in sexual and natural selection. However, it is important to remind that sex allocation theory has mostly assigned the sole control of sex ratio adjustment to parents, while also gametes and offspring themselves are likely to exert influence (Trivers 1974; Parker 1985; Pen 2006), not to dismiss the “sex ratio distorters”. These heritable elements that modify the SR to promote their own transmission (West 2009), are much more common than traditionally thought and may be another source of sex modification at the genetic level. All these simultaneous and potentially dissimilar selection pressures may lead to a compromise between strategies (Eshel and Sansone 1991) and thus reduce the sex ratio biases, either at individual or at population level (Beukeboom *et al.* 2001; Seger and Stubblefield 2002; Komdeur and Pen 2002).

Conclusions

Inter-specific analyses of sex ratio and vulnerability

1. Overall inter-specific avian HSR (reported until 2005) tends to be male-biased, while FSR is closer to parity. The strength of the trends is dependent on the SSD type.

2. Across bird species, hatching and fledging sex ratios are influenced by the degree of SSD, probably associated with differential production or rearing costs between sons and daughters. Dimorphic species show a higher proportion of the smaller sex, but the effect is particularly strong in species with female-biased SSD. Parents adjust offspring sex ratio by differential production, possibly the less costly mechanism, rather than by sex-specific mortality. These results support the existence of a certain parental control of primary sex ratio.

3. The degree of SSD is correlated with offspring sex-biased vulnerability, expressed as a greater mortality and mass reduction of the larger sex. The survival and viability costs involved in achieving a larger body size support the size-dependent explanations of vulnerability. However, they should be combined with sex-dependent explanations, as growing large is mainly disadvantageous when coupled with the male-phenotype.

4. Life-history traits such as mating system and type of parental care seem to modulate the relationships between sex ratios, sex-specific mortality and SSD. Sexual plumage dimorphism, age at first breeding or length of the fledging period may also regulate sex proportions and juvenile mortality. This reflects the complex matrix of factors that are probably acting on avian sex ratio evolution and questions the validity of Fisher's theory in this context.

5. Ideally, comparative analyses of sex ratio in birds should be restricted to studies with molecular-sex determination of the offspring (unhatched embryos included) and where mortality from hatching to fledging of sons and daughters is strictly monitored in the same broods.

Intra-specific analyses: The case of the common tern

1. Sexual dimorphism of chicks before fledging is present in some phenotypic traits, as well as in strategies of resources allocation during growth. These strategies include

sexual differences in the use of limited resources, in the performance of the immune system and in the intra-brood competition ability. However, sons and daughters are analogous in many other traits and suffer similar mortality rates. Developmental trade-offs may be optimized differently but advantageously by both sexes to attain comparable levels of condition and viability by the end of the growing period.

2 The subtle sexual differences found may indicate similar costs of raising males and females, and an absence of sex-specific parental investment after birth. However, the results of this thesis underline:

   i the potential of parents to affect the development of their offspring by differential supply of particularly important resources, such as carotenoids

   ii the influence of parental quality on the offspring sexual differences

   These influences might involve a certain differential reproductive effort from the parents; whether this is reflecting a difference in the reproductive value of sons and daughters is not clear.

3 Population-wide patterns in the common tern colony at the Banter See showed parity sex ratios and no sex-specific mortality. The situation would fit to a species where production/raising costs of sons and daughters are similar, which is consistent with the slight sexual dimorphism, and where the mechanisms of sex-specific nestling mortality are not triggered by size or sex factors.

4 The male-attractiveness and to some extent the environmental sex-allocation hypotheses were supported, since more sons hatched in pairs with heavier adult males and in better years. This might be a good allocation strategy if sons are indeed the more costly sex and its reproductive value is more strongly condition-dependent than that of daughters. The proportion of daughters unexpectedly increased with maternal experience and quality, maybe as the consequence of a "sex-number" trade-off. Otherwise, the production of high quality daughters may involve unrecognized fitness returns.

5 The lack of offspring sex-biased mortality in relation to parental factors suggests that sex ratio adjustments after birth (for instance, through selective feeding) may be too costly compared with biases at the production level. Alternatively, it may also indicate limitations of the parents in recognizing the sex of the progeny.

Spanish Section

Introducción

Antes pensaba que la tendencia de producir el mismo número de machos y hembras, cuando era ventajosa para las especies, era consecuencia de la selección natural, pero ahora veo que todo el problema es tan complejo que es preferible dejar su solución para el futuro.
— C. Darwin, 1874
The Descendent of Man and Selection in Relation to Sex

Sospecho que la opinión de Darwin ha tocado la fibra sensible de prácticamente todos los investigadores que han tratado de dar sentido a la exasperante literatura relativa a la adaptatividad para los padres de sesgar la razón de sexos hacia hijos o hijas.
— I. C. Hardy, 1987

Contexto

Históricamente, el estudio de la razón de sexos comenzó con la observación de que, en la mayoría de las especies de animales con sexos separados, nacían tantos machos como hembras. Aunque Darwin inició la discusión de las posibles explicaciones (incluso esbozó un argumento evolutivo, Darwin 1871), pronto se dio cuenta de que la cuestión era problemática para su teoría de la selección natural (Darwin 1874).

La raíz de este interés, sin embargo, radica en una pregunta anterior: dado que existen distintos sexos, ¿cómo se caracterizan? Machos y hembras son a menudo diferentes en morfología y comportamiento, siendo la anisogamia, es decir, el tamaño y movilidad de sus gametos, la diferencia esencial. Las hembras producen gametos grandes e inmóviles, los óvulos, mientras que los machos producen gametos pequeños y móviles, los espermatozoides; también se diferencian en los costes de producción. Esto implica que, en general, el éxito reproductivo de un macho está limitado por el número de hembras con las que puede aparearse, mientras que el éxito reproductivo de la hembra está limitado por su fecundidad. En este contexto, los machos compiten entre sí por aparearse con el mayor número posible de hembras, mientras que las hembras deben elegir al macho que asegure la procreación de una buena descendencia (Williams 1966; Trivers 1972). La selección sexual resultante tiene importantes consecuencias evolutivas y explica muchas de las diferencias entre los sexos. Las diferencias sexuales y su varianza son la base material de las diferencias en el éxito reproductivo relativo de machos y hembras, que entre otros aspectos, determina la asignación por sexo (SA, en sus siglas en inglés; véase el Cuadro 1 para descripción de conceptos básicos).

Cuadro 1. CONCEPTOS

Asignación por sexo (SA): proporción de recursos destinados a la reproducción masculina y femenina. Más concretamente, la división del esfuerzo reproductivo de los padres en la producción de hijos e hijas.

Razón o proporción de sexos (SR): número relativo de machos y hembras en una población. Se calcula como el número de machos dividido entre el número total de individuos de la población, y se expresa como proporción de machos en tanto por uno o tanto por ciento:

$$\mathrm{SR} = ♂ \,/\, (♀ + ♂)\ [\times 100]$$

Ejemplo. En una población de 620 individuos,
$\mathrm{SR} = 0.50$ (o 50 %) → ♂ (310) = ♀ (310) – paridad exacta, equilibrio
$\mathrm{SR} = 0.65$ (o 65 %) → ♂ (403) > ♀ (217) – ratio sesgada hacia machos ($\mathrm{SR} > 0.5$)
$\mathrm{SR} = 0.43$ (o 43 %) → ♂ (267) < ♀ (353) – ratio sesgada hacia hembras ($\mathrm{SR} < 0.5$)

Según el momento en que se mide la razón de sexos:
Razón primaria de sexos: en sentido estricto, la proporción de machos en el momento de la concepción (determinada en embriones). Concepto en muchos casos equivalente al de
Razón de sexos en la eclosión (HSR): proporción en el momento del nacimiento, determinada en pollos recién eclosionados.
Razón secundaria de sexos o ***en volantones (FSR)***: determinada en pollos volantones (emancipación). Muchos autores, sin embargo, consideran secundaria a la razón de sexos en el momento de la eclosión, para distinguirla de la de concepción.
Razón terciaria u ***operacional de sexos (OSR)***: proporción de individuos adultos reproductores.

Las proporciones variarán si los sexos difieren en sus tasas de mortalidad en las distintas etapas del desarrollo. Por ejemplo, si la tasa de mortalidad es mayor para los machos, una razón primaria equilibrada se convertirá en una razón secundaria sesgada hacia las hembras (Cuadro 1b, *Ejemplo 2*). Este es el concepto de **Mortalidad específica por sexo** o **Mortalidad diferencial (DM)**. La mortalidad diferencial durante el crecimiento se expresa como la diferencia entre la razón de sexos en la eclosión y en volantones, y se calcula como: FSR−HSR.

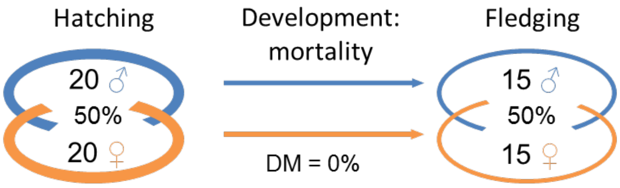
Valor reproductivo de un individuo: su contribución genética en la población futura. Mide la contribución a largo plazo al acervo génico. En algunos casos equivale a una medida de éxito a corto plazo como el reclutamiento, pero por lo general una medida tan simple no es suficiente.

Éxito reproductivo (anual/vitalicio) de un individuo: producción relativa de descendencia fértil (en un año/a lo largo de la vida). En la práctica es a menudo un cálculo del número de descendientes producidos por el individuo.

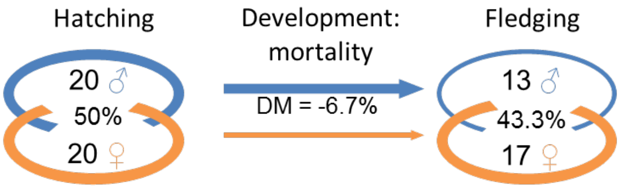
Cuadro 1b. PARA FAMILIARIZARSE CON SEX RATIOS

Estos supuestos pueden ayudar a aclarar conceptos:

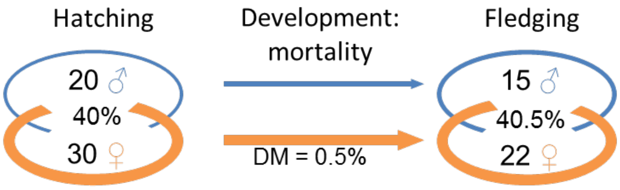
Ejemplo 1. En una población nacen 20 machos y 20 hembras (HSR equilibrado = 50 %). El mismo número de cada sexo (5 machos, 5 hembras) muere durante el crecimiento. En la emancipación, tendríamos de nuevo FSR = 50 %. Aunque obviamente existió cierta mortalidad (10 individuos de 40, un 25 %), no hubo mortalidad diferencial: DM = 50 − 50 = 0 %.



Ejemplo 2. Población donde nacen 20 machos y 20 hembras (HSR equilibrado = 50 %). Durante el desarrollo, 7 machos y 3 hembras mueren. La razón secundaria FSR = 13/30 = 43.3 % (sesgada hacia hembras). Mientras que la mortalidad de la descendencia fue del 25 % (10 individuos de 40), la mortalidad diferencial fue DM = 43.3 − 50 =-6.7 %. El valor absoluto indica, en porcentaje, cuánto muere un sexo más que el otro, y el signo negativo indica una mayor mortalidad masculina.



Ejemplo 3. Una población donde nacen 20 machos y 30 hembras (HSR sesgado hacia las hembras = 40 %). Durante el desarrollo, 5 machos y 8 hembras mueren, por lo que FSR = 40.5 % (sesgado hacia hembras). La mortalidad diferencial = 0.5 (el signo positivo indica una mayor mortalidad femenina). En este caso, incluso aunque murieron más hembras que machos, el FSR sigue sesgado hacia las hembras (pero en otros casos se podría invertir el sesgo).



La teoría de la asignación por sexo es uno de los grandes éxitos de la biología evolutiva. Empleada para examinar una amplia gama de temas, ha atraído la mayor atención como el área de investigación que considera cómo se produce la división de recursos entre hijos e hijas en especies con sexos separados. La primera consecuencia de esta división es inmediatamente evidente en el número de individuos de cada sexo que nacen, como expuso Darwin. Esto se denomina proporción o razón de sexos (SR, en sus siglas en inglés; véase el Cuadro 1). Debido a las dificultades prácticas de establecer valores a la asignación por sexo (¿cómo tratar el carácter multidimensional de los recursos? ¿cómo incluir los costes de futuras reproducciones? ¿qué y cómo medir en el campo o el laboratorio?), para estudiar alguno de sus aspectos se utiliza a menudo la razón de sexos, como concepto relacionado o incluso equivalente en algunos casos (Frank 1990).

Estos dos temas (la razón de sexos/asignación por sexo, basadas en las diferencias sexuales) constituyen por tanto el objeto de estudio de esta tesis.

Teoría de la razón de sexos y la asignación por sexo

Asignación por sexo a nivel poblacional

El principio básico que explica por qué es frecuente encontrar una razón de sexos equilibrada en las poblaciones, después de ser descrito en un primer intento por Darwin (Darwin 1871), fue desarrollado matemáticamente por Dusing (Düsing 1883, 1884) y después resumido por Sir Donald A. Fisher (Fisher 1930).

El principio de Fisher, uno de los "argumentos más célebres en biología evolutiva" (Edwards 1998), muestra que la selección natural favorece una inversión parental idéntica en ambos sexos. La idea se describe con una metáfora económica: los padres asignan fracciones de sus limitados recursos reproductivas a hijos e hijas, y de cada sexo reciben ciertos rendimientos, medidos como contribución genética a las generaciones futuras. En una población con una razón de sexos sesgada, el sexo más escaso tiene mejores perspectivas de apareamiento, y los padres con una tendencia genética a producir más de este sexo "raro" logran un mayor número promedio de nietos. La tendencia a producir el sexo escaso se vuelve más generalizada en la población, y por lo tanto disminuye la desigualdad de número de cada sexo. Esta selección dependiente de la frecuencia devuelve la razón de sexos a la igualdad, independientemente de cuál sea el sexo más escaso.

Cuando los beneficios y los costes (recursos invertidos) asociados a cada sexo son iguales, los padres invierten por igual y la razón de sexos de una población está en equilibrio, lo que implica una estrategia evolutivamente estable (ESS, en sus siglas en inglés; Maynard Smith y G. R. Price 1973). Cuando los beneficios y los costes asociados a cada sexo son diferentes, la estrategia evolutivamente más estable sigue siendo invertir cantidades iguales de recursos en ambos sexos. Esto implica que el SR de una población va estar sesgado hacia el sexo "más barato", el que necesite una menor cantidad de inversión. Por ejemplo, si producir un macho cuesta dos veces más que producir que una hembra, se producirán el doble de hembras que de machos ($SR = 0.33$), para igualar la inversión global en los sexos (Figura 14).

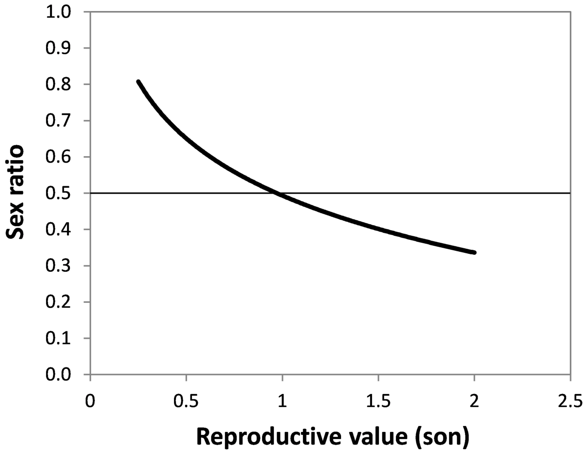


Figura 14: Resultado clásico de Fisher. Razón de sexos en una población, para situaciones en las que el valor reproductivo de los hijos es menor, igual o superior al de las hijas. Asumiendo un valor reproductivo medio de las hembras de 1.0, el valor reproductivo medio de los machos sería el número promedio de apareamientos que obtendrán, multiplicado por el valor medio reproductivo de las hembras. Esto viene dado por [(nº hembras / nº machos) x 1.0]. P.ej., si hay 100 hijos y 100 hijas, el valor reproductivo de machos = 1 y el de hembras = 1, y por lo tanto el SR sería igual (de West 2009).

El argumento, verbal en un principio y posteriormente formalizado matemáticamente (Shaw y Mohler 1953), comprende tres aspectos clave: (1) la eficacia biológica (valor reproductivo) de machos y hembras es igual (cada descendiente tiene una madre y un padre, y así machos y hembras hacen una análoga contribución genética a la siguiente generación); (2) la medida correcta de eficacia biológica es el número relativo de nietos, y (3) a nivel global de la población hay una inversión total idéntica en cada sexo, pero no necesariamente el mismo número de machos y hembras. La predicción de Fisher aplica a la razón de sexos secundaria (en especies con cuidado parental, la inversión total se considera hasta el final del periodo de cría) pero en el caso de que hubiera una mortalidad diferencial de cada sexo durante el desarrollo, también aplica a la razón primaria de sexos (véase página 166).

El escenario de Fisher conlleva una serie de supuestos implícitos que investigaciones posteriores han aclarado y flexibilizado (Williams y Williams 1979; Maynard Smith 1980; Frank 1990; Godfray 1994). Estos nuevos modelos procedían de observaciones empíricas que no podían ser explicadas por la teoría de Fisher. La investigación moderna del SR comenzó con Hamilton (Hamilton 1967), quien observó un fuerte sesgo femenino en poblaciones de insectos. Su hipótesis, la *Competencia Local por Pareja* (LMC, en sus siglas en inglés), muestran que cuando en un grupo local los hermanos compiten directamente entre sí por un número limitado de parejas, se favorece que los padres inviertan más en hijas. Una idea similar (Clark 1978) define la hipótesis de la *Competencia Local por Recursos* (LRC, en sus siglas en inglés), después de observar mayor número de machos en la descendencia de primates: cuando uno de los sexos es filopátrico y el otro se dispersa antes del apareamiento, la competencia entre los individuos del sexo filopátrico por los limitados recursos locales puede favorecer que los padres inviertan más en el sexo dispersivo. En general, la contribución importante de estos modelos a la teoría de asignación por sexo es la idea de que la competencia familiar puede afectar a la selección del SR. La selección, por tanto, favorecería una razón de sexos sesgada hacia el sexo que compite menos, o por el contrario, hacia el sexo filopátrico cuando éste mejora las condiciones para los parientes (*Mejora Local de Recursos*, LRE; Clark 1978; p.ej. "ayudantes-en-el-nido").

Asignación por sexo a nivel familiar

La teoría de Fisher explica la inversión en sexos total de la población y predice el SR medio de la población, pero no aclara cómo se deberían asignar los recursos a nivel individual (Williams y Williams 1979). Por ejemplo, cada familia podría asignar a ambos sexos por igual, o la mitad de las familias podría estar invirtiendo solamente en hijos y la otra mitad sólo en hijas. Kolman (Kolman 1960) demostró que en una población en equilibrio de Fisher, esta distribución dentro de las familias (= varianza de la razón de sexos) no está afectada por la selección natural, puesto que la eficacia biológica depende sólo de la asignación total de la familia y no de cómo se divide este total. Sin embargo, al igual que el modelo de Fisher, esta teoría se basa en un supuesto principal: la relación entre costes y beneficios es lineal para ambos sexos (a inversión doble, doble beneficio).

Por el contrario, los cambios en la inversión pueden no ser (y a menudo no lo son) directamente proporcionales a los cambios en los beneficios. Una serie de modelos predicen una asignación por sexo adaptativa como consecuencia de rendimientos específicos de cada sexo (p.ej. Trivers 1972; Maynard Smith 1980; Charnov 1982; Frank 1990). El punto de partida fue la hipótesis de la "dominación materna"de Trivers y Willard, que sugería que si se cumplían las siguientes condiciones,

I la disponibilidad de alimento varía y afecta a la condición materna (hay madres en condiciones relativamente mejores que otras)

II la condición materna está correlacionada con la condición de descendencia (madres en mejor condición producen descendencia de mayor calidad, dando lugar a adultos de mayor calidad)

III los hijos obtienen un mayor beneficio biológico de esas mejores condiciones y calidad que las hijas,

entonces las madres en buenas condiciones deberían sobreproducir hijos, y aquellas en condiciones relativamente peores, hijas (Trivers y Willard 1973).

Los estudios clásicos de Clutton-Brock *et al.* (Clutton-Brock y col. 1981, 1986) con el ciervo rojo *(Cervus elaphus)* aportaron el primer respaldo convincente a la hipótesis, que inicialmente fue desarrollada para especies poligínicas. Dos supuestos principales subyacen: (1) las

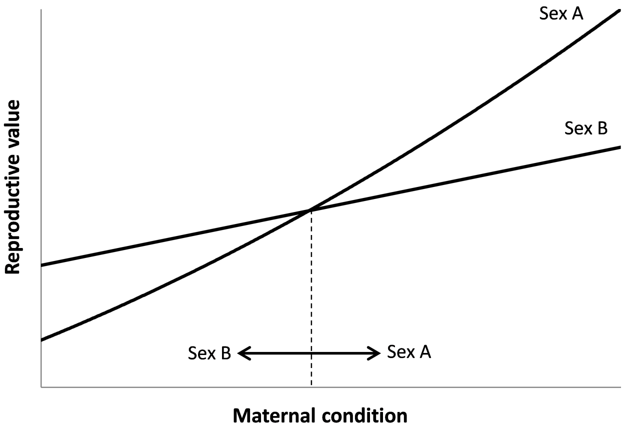


Figura 15: Hipótesis de Trivers-Willard. El sexo que obtiene mayor valor reproductivo debido a una mejor condición materna (sexo A) debe ser producido en condiciones relativamente buenas y el otro sexo (sexo B) bajo condiciones relativamente peores. Actualmente se aplica a toda una variedad de índices además de la condición maternal (adaptado de West 2009).

funciones que describen la relación entre la inversión y los rendimientos son diferentes y no lineales para cada sexo; y (2) las familias varían en la cantidad de recursos disponibles que pueden asignar.

En su libro *La teoría de la asignación por sexo*, Eric L. Charnov desarrolló el trabajo de Trivers-Willard mostrando cómo el mismo principio podía aplicarse a una amplia gama de taxones y en general más ampliamente: aparte de las condiciones parentales, cualquier parámetro socio-ambiental que previsiblemente pueda afectar de forma desigual a la eficacia biológica de hijos e hijas favorecería a los padres a invertir más en el sexo que presente mayor eficacia (Charnov 1982). Actualmente, la idea de que la selección favorece la asignación condicional si las condiciones ambientales influyen diferencialmente en la eficacia biológica de los sexos se denomina en general *hipótesis de Trivers-Willard* (Figura 15).

Otro avance importante fue la comprensión de las consecuencias que el ajuste individual de la razón de sexos tiene a nivel de población. El modelo de Charnov demostró que la selección dependiente de la frecuencia sigue siendo una característica clave en la asignación por sexo. Sin embargo, cuando no haya linealidad en la relación coste-beneficio ya no es esperable una inversión idéntica en cada sexo. Esto significa que aunque la hipótesis de Trivers-Willard se refiere al ajuste de SR a nivel familiar, tiene consecuencias a nivel poblacional: conduce a sesgos en la razón de sexos de la población, aunque su dirección y magnitud pueden ser difíciles de predecir (Frank 1990, 1998). Actualmente no hay ninguna teoría que indique con qué frecuencia la predicción de Fisher es cualitativamente incorrecta cuando se aplican las suposiciones de Trivers y Willard (Komdeur y Pen 2002).

En cualquier caso, estos modelos fomentaron enormemente la investigación del tema, lo cual ha contribuido a hacer de la hipótesis de Trivers-Willard y sus extensiones una de las áreas más productivas del estudio de SA.

Mecanismos de ajuste de la asignación por sexo

La variación en SR como consecuencia de la habilidad parental para invertir recursos diferencialmente se obtiene por uno de estos mecanismos (o ambos):

1. Manipulación del SR primario (antes del nacimiento)
En oposición a Trivers y Willard (Trivers y Willard 1973), quienes sugirieron una mayor mortalidad postnatal de un sexo como mecanismo para generar variación, Myers (J. H. Myers 1978) propuso que los padres podrían ser capaces de ajustar la razón primaria de sexos. Su modelo hace hincapié en ajustes que maximicen el número de crías, más que el valor reproductivo promedio de cada una. Si asumimos, como todos los modelos anteriores, que la asignación por sexo está controlada por cromosomas autosómicos (no sexuales), entonces el control parental sobre el sexo de la descendencia se convierte en una herramienta altamente valiosa para el ajuste adaptativo.

¿Poseen los animales mecanismos que les permitan este control? La capacidad para establecer el sexo de la descendencia depende del sistema de determinación de las especies. En algunos reptiles y peces, con determinación ambiental del sexo (ESD, en sus siglas en inglés; p.ej. pH o temperatura de incubación), las madres pueden ejercer cierto control a través de su comportamiento de puesta (la temperatura de incubación podría también influir en la razón de sexos en aves, por medio de mortalidad diferencial; Eiby y col. 2008; Göth y Booth 2005; véase el Cuadro 2). En organismos haplodiploides, p.ej. insectos, los huevos fertilizados (diploides) se convierten en hembras y los huevos no fertilizados (haploides) en machos, lo cual da a la madre un control muy preciso sobre el sexo de cada descendiente (Bull 1983).

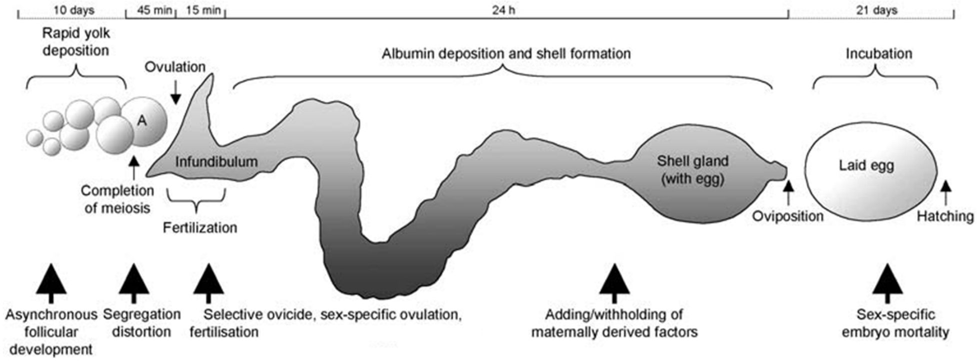
Por el contrario, la determinación genética o cromosómica del sexo (CSD, en sus siglas en inglés) de organismos como mamíferos y aves ha sido considerada tradicionalmente como un severo obstáculo para ejercer este control (Williams y Williams 1979; Bull y Charnov 1988; Frank 1990). El proceso aleatorio de la segregación mendeliana induce a prever una razón primaria de sexos cercana a la paridad, tal y como se esperaría de una distribución binomial. Sin embargo, aunque tanto la teoría de Fisher como la determinación cromosómica predicen un SR equilibrado, Fisher no predice una varianza binomial de la razón de sexos.

En la última década esta idea ha cambiado (pero véase Uller, Pen y col. 2007), gracias a estudios (sobre todo en aves) que mostraron patrones repetibles de ajuste del SR y evidencias significativas de control del sexo de la descendencia (Komdeur, Daan y col. 1997; West y Sheldon 2002; West 2009). Todavía es necesaria cierta precaución antes de asumir que una razón de sexos sesgada implica control adaptativo (también puede haber explicaciones no adaptativas). Sin embargo, ahora parece claro que la restricción del CSD puede ser superada y que los vertebrados poseen mecanismos de manipulación del sexo, aunque estos continúan siendo parcialmente desconocidos (véase Cuadro 2). La manipulación activa del SR primario podría ser la manera más eficiente de ajustar la razón de sexos de la descendencia, ya que minimiza la inversión en el sexo no deseado (Alonso-Álvarez 2006).

2. Manipulación del SR secundario (después del nacimiento)
En especies con cuidados parentales existen muchas posibilidades de inversión postnatal diferente para cada sexo, ya que la asignación y la mortalidad juvenil son potencialmente controladas por los padres. Las estrategias de los padres pueden incluir el infanticidio selectivo y diferencias en la defensa, refugio o alimentación de cada sexo (Bortolotti 1986; Lessells 2002; Leech, Rowe y col. 2006; Cameron-MacMillan y col. 2007; Mainwaring y col. 2011), que pueden conducir a una mortalidad o éxito reproductivo distintos.

Cuadro 2. POSIBLES MECANISMOS DE MANIPULACIÓN DEL SEXO EN AVES

En aves, la combinación de CDS y heterogamia en las hembras proporciona a las madres un gran potencial para influir en el sexo de la descendencia, aunque los mecanismos empleados son todavía poco conocidos. Las hembras, el sexo heterogamético (ZW), producen gametos Z ('macho') y W ('hembra') mientras que los machos (sexo homogamético, ZZ) contribuyen con esperma Z. Hay buenas revisiones del tema y de las evidencias disponibles (Pike y Petrie 2003; Alonso-Álvarez 2006; Rutkowska y Badyaev 2008); aquí sólo se explican brevemente los mecanismos más probables:



(Figura de Pike y Petrie 2003)

Fase de desarrollo folicular

1. **Desarrollo folicular asincrónico:** una tasa de crecimiento diferente de los folículos ♂ y ♀ podría alterar el orden de ovulación y permitir un control flexible del SR en relación a la secuencia de puesta.
2. **Atresia diferencial:** proceso de degeneración y reabsorción de folículos pre-meióticos antes del estado de madurez.

Fase de la meiosis

3. **Impulso meiótico:** selección del cromosoma sexual en la primera división meiótica; el cromosoma no deseado sería desterrado al cuerpo polar y el preferido sería asignado al óvulo.

Fase de pre-ovulación y ovulación

4. **Ovicidio o reabsorción selectivos:** aborto y posterior reabsorción del óvulo post-meiótico que contiene el cromosoma sexual no deseado.
5. **Ovulación diferencial:** unas horas antes de la ovulación, los niveles plasmáticos de algunas hormonas podrían inducir un desprendimiento no aleatorio de óvulos.

Fase de fertilización

6. **Fertilización diferencial:** regulación por parte de la madre de la fertilización de óvulos ♂ y ♀, a través de diferentes mecanismos (espermatozoides liberados selectivamente, limitación de la movilidad o fecundidad del esperma liberado, inhibición del desarrollo del cigoto. . .)

Poco antes o poco después de la puesta

7. **Mortalidad diferencial del embrión:** a través de la alteración de la composición del huevo (asignación diferencial de sustancias en la yema) o por manipulación directa de la temperatura de incubación.

Los mecanismos anteriores presentan dos factores comunes: (1) serían directamente mediados por hormonas maternas y (2) la hembra reproductora debe poder reconocer con precisión el sexo del óvulo. Existen evidencias positivas para ambos factores, a pesar del desconocimiento general (Groothuis y Schwabl 2008). Recientemente, Uller y Badyaev 2009 han sugerido, para aves, la existencia de sistemas que combinan CSD con factores ambientales que pueden adquirir una función de determinación del sexo.

Según la teoría de Fisher, la mortalidad diferencial durante el periodo de inversión parental conduce a una razón de sexos sesgada (Seger y Stubblefield 2002; West 2009). Si los hijos e hijas son igualmente costosos de producir pero los hijos son más propensos a morir, el coste promedio de un hijo es inferior al de una hija. Esto favorece un sesgo hacia los hijos en el SR primario (para igualar la inversión total). La razón de sexos al final del periodo de cuidado parental, sin embargo, puede acabar sesgada hacia las hijas debido a la mayor mortalidad de hijos.

Bajo principios no fisherianos, el SR en el nacimiento puede estar fijado, pero aún podría haber una enorme variación en la asignación a cada sexo, basada en mecanismos conductuales y fisiológicos modelados por la selección. Por tanto, la mortalidad diferencial debe ser siempre considerada como una posible explicación adaptativa si se encuentran sesgos en el SR secundario de una determinada población. Sin embargo, la mortalidad puede provenir simplemente de la mayor vulnerabilidad de uno de los sexos a los parásitos, hormonas o escasez de alimentos, o a su diferente habilidad para extraer recursos de los padres (Clutton-Brock 1991). En la práctica, la distinción entre estas explicaciones es complicada en muchos casos. Se ha propuesto también la teoría de que el sexo heterogamético es más susceptible a la mortalidad debido al estado hemicigótico de su cromosoma sexual (*Hipótesis de la heterogamia*; J. H. Myers 1978. En ese caso, la mortalidad en situaciones de estrés sería mayor para los machos en mamíferos y para las hembras en aves. Sin embargo, los patrones generales de mortalidad parecen estar sesgados hacia los machos tanto en aves como en mamíferos (Clutton-Brock y col. 1985), lo que sugiere que tanto la inversión parental diferencial como las distintas respuestas de cada sexo a las condiciones ambientales son explicaciones más probables que la heterogamia.

Manipular la supervivencia de las crías después del nacimiento podría ser costoso en términos energéticos, ya que supone una pérdida de recursos ya invertidos (J. H. Myers 1978; Maynard Smith 1980). Sin embargo, estos costes pueden ser pequeños si los de producción son asimismo bajos (Clutton-Brock 1991), o por lo menos inferiores a un determinado nivel bajo el cual el sesgo compense el coste. Por ejemplo, bajo ciertos supuestos, algunos modelos matemáticos calculan que el coste máximo que podría asumir un padre sería del 17 % del total de sus recursos (Pen y Weissing 2002).

¿Por qué estudiar la razón de sexos en aves?

La observación de sesgos en la razón de sexos de la descendencia ha fascinado a los investigadores desde hace muchas décadas. Hoy hay evidencias correlativas y experimentales de la influencia de los padres y de su control de la determinación del sexo en varios taxones del reino animal (insectos: Flanders 1965; Hardy 1994; mamíferos: Cameron 2004; reptiles: Radder 2007; aves: Sheldon y col. 1999; Nager, Monaghan, Griffiths y col. 1999; Pike 2005; peces: Conover y Voorhees 1990). Sin embargo, mientras que los invertebrados, cuyos sesgos pueden ser extraordinarios, han proporcionado algunas de las pruebas más consistentes de la teoría de SA (Ode y Hunter 2002), en las aves (y mamíferos) los ajustes son generalmente pequeños y su asignación por sexo es mucho más difícil de comprender (Komdeur y Pen 2002; Cockburn y col. 2002; West 2009).

Las estrategias vitales que caracterizan a las aves no encajan con una serie de suposiciones de los modelos clásicos. Aparte del mecanismo genético de determinación del sexo, poseen vidas longevas, baja fecundidad, cuidado biparental (el 81 % de las especies, aunque con diferentes grados y medios de control; Cockburn 2006), complejos mecanismos de ajuste de la inversión parental y compromisos entre episodios actuales y futuros de reproducción. Hasta hace relativamente poco, determinar el sexo de embriones o neonatos morfológicamente indistinguibles fue otro gran obstáculo para el estudio del SR en aves. Actualmente esta limitación ha desaparecido, en gran medida gracias al desarrollo de técnicas de sexado molecular fiables (Griffiths y col. 1998; Fridolfsson y Ellegren 1999). Aun así, la literatura de SR en aves se distribuye irregularmente a través de las especies y los patrones de asignación son conocidos por su inconsistencia (Palmer 2000; Krackow 2002; Ewen y col. 2004).

A pesar de estas dificultades, hay razones para interesarse por el estudio del SR en aves. En primer lugar, estudios empíricos han revelado patrones de asignación que dejan claro que las restricciones de la segregación mendeliana pueden ser superadas. En segundo lugar, las complejas sociedades de las aves incumplen las asunciones de apareamiento aleatorio y generaciones no coexistentes que subyacen en los modelos de inversión igualitaria (Fisher

1930). En tercer lugar, criar una nidada supone una inversión muy fuerte para sólo un número relativamente pequeño de descendientes, lo que hace de la elección entre hijos e hijas una cuestión especialmente sensible. Desde el punto de vista teórico, la investigación del SR entra en contacto con muchos aspectos de la biología animal, y proporciona perspectivas sobre características de comportamiento y ecología evolutiva; por ejemplo, sobre la solidez de las fuerzas de selección sexual.

Desde la perspectiva de las ciencias aplicadas, el conocimiento de los factores que influyen en el SR posee también gran valor. Los programas de conservación pueden beneficiarse de la manipulación de condiciones individuales o ambientales para alterar la razón de sexos de la descendencia, con la finalidad de ayudar al crecimiento de la población (Lenz y col. 2007; Lawrence y col. 2008); p.ej., induciendo a las madres a sobreproducir hijas (Wedekind 2002).

El kakapo (*Strigops habroptilus*), una especie de loro en peligro de extinción, ejemplifica la importancia de los estudios de SA para la conservación (Clout y col. 2002). La especie, lek-poligínica y con un dimorfismo sexual en tamaño considerable (machos más grandes que las hembras), muestra un SR de adultos altamente sesgado hacia los machos. Parte de los esfuerzos de conservación incluían la provisión de alimento suplementario para fomentar la cría. Sin embargo, tal como predice la hipótesis de Trivers-Willard, esto provocó que las hembras suplementadas produjeran un exceso del sexo con mayor valor reproductivo, machos (Clout y col. 2002). La inclusión de la teoría de SA ayudó a diseñar un nuevo programa de alimentación que solucionó el problema (Robertson y col. 2006). Otro ejemplo con connotación práctica es la investigación de los cambios en la razón de sexos relacionados con los contaminantes ambientales. La exposición al mercurio, DDT y otros organoclorados inclina la razón de sexos de los pollos hacia la producción de hembras en varias especies (Fry y Toone 1981; Erikstad y col. 2011; Bouland y col. 2012). La explicación puede proceder de la teoría de Trivers–Willard o de causas no adaptativas, pero esta tendencia puede conducir a la reducción del tamaño efectivo de la población. Esto sería especialmente alarmante para especies en peligro de extinción, como muchas aves marinas, con tamaños de población ya pequeños y expuestas al mercurio a través de su dieta de pescado (Bouland y col. 2012).

Modelos adaptativos de SA en aves: Evidencias

En la actualidad, algunas extensiones adicionales de las teorías clásicas hacen posible la construcción de modelos más realistas para aves, aunque todavía existe mucha simplificación. Por ejemplo, J. H. Myers 1978 y Williams y Williams 1979 abordaron el problema parental de cómo dividir los recursos entre cada descendiente, también conocido como el “compromiso entre tamaño, sexo y número” (véase también Carranza 2004; Carranza y Polo 2012).

Las revisiones de la década de los 80 afirmaban que la variación de SR en aves era escasa, de magnitud menor y con poco significado adaptativo (Charnov 1982; Clutton-Brock 1986; Bull y Charnov 1988). Desde entonces el número de estudios de SR ha aumentado considerablemente. Muchos de ellos presentan evidencias de variaciones significativas, algunas apoyadas por explicaciones adaptativas. Sin embargo, las pruebas realmente convincentes de sesgos adaptativos continúan siendo escasas. Cinco clases de modelos principales, acompañados por diversos grados de evidencia, pretenden explicar los patrones adaptativos de SR en aves (Hardy 1997; Cockburn y col. 2002; Komdeur y Pen 2002). Todos ellos están basados en alguno de los factores potencialmente capaces de influir en la razón de sexos (Cuadro 3).

Cuadro 3. FACTORES RELEVANTES PARA EL ESTUDIO DE SA EN AVES

Cualquier parámetro socio-ambiental que afecte previsiblemente a la eficacia biológica de hijos e hijas de manera diferente es relevante para el estudio de SA. Las aves, debido a sus complejas estrategias vitales y a la imprevisibilidad del ambiente, presentan muchos factores de posible influencia en la evolución de la razón de sexos:

Sistema de apareamiento (p.ej., grado de poliginia, endogamia local; LMC)
Esperanza de vida (compromisos entre reproducción actual y futura)
Diferencia sexual en filopatría (LRC)
Ayudantes en el nido (LRE)
Condición y calidad parental (Trivers-Willard)
Tipo y cantidad de cuidado parental (Trivers-Willard)
Factores ambientales (calidad del territorio, disponibilidad de alimento, estación)
Dimorfismo sexual en tamaño
Caracteres sexuales secundarios (hipótesis del Atractivo; Trivers-Willard)
Tamaño de la familia (compromiso entre sexo, número y orden de eclosión de pollos)

Hipótesis de Fisher

A primera vista, la teoría viene apoyada por el hecho que la motivó originalmente: muchas aves presentan una razón de sexos relativamente equilibrada (Clutton-Brock 1986; Gowaty 1991; Ewen y col. 2004). La demostración empírica es más problemática: un SR equilibrado no es propiamente una prueba para la teoría hasta que se acompaña de la evidencia de la igualdad de costes de producción para cada sexo. Medir el valor reproductivo o el coste de hijos e hijas es extremadamente difícil, ya que están involucrados diversos aspectos de eficacia biológica que interactúan entre sí. Una variante especializada, la *Hipótesis Homeostática* (los padres responden a la escasez de un sexo produciendo más individuos de ese sexo) permanece cuestionada, teórica (Leigh 1970) y empíricamente (Bensch y col. 1999). Un problema adicional en las aves es que el CSD conduce también a una expectativa de igualdad numérica en la razón de sexos (véase página 164). Postma y col. 2011 intentaron distinguir entre ambas posibilidades usando 14 años de datos de SR del gorrión melódico (*Melospiza melodia*) y no encontraron pruebas de la existencia de ninguna variación, bien genética o inducida por el medio ambiente.

En consecuencia, parece haber una falta de estudios adecuados en aves para probar la teoría de Fisher, que hasta el momento no ha sido abordada satisfactoriamente.

Hipótesis del ambiente social

Esta serie de hipótesis incluye la Competencia Local por Pareja (LMC), Competencia Local por Recursos (LRC) y Mejora Local de Recursos (LRE) (para sus predicciones, véase página 161). Los factores aquí relevantes son la existencia de competencia familiar por las oportunidades de apareamiento y los sesgos en filopatría-dispersión o en el comportamiento de ayuda entre miembros de la familia.

La teoría LMC es un área muy productiva de la SA, pero su importancia en aves, que viven normalmente en poblaciones estables, es muy limitada.

La LRC en aves fue explorada por Gowaty (Gowaty 1993), que comparó especies paseriformes (donde las hembras se dispersan más) con especies de anátidas (donde los machos se dispersan más) y encontró una tendencia en la dirección esperada (las aves paseriformes favorecían la producción de hijas y las anátidas la producción de hijos). Sin embargo, la controversia sobre los datos y métodos utilizados (P. J Weatherhead y Montgomerie 1995) y los resultados negativos cuando se incluyeron datos de otros órdenes (Palmer 2000), indican que este patrón puede ser poco fiable. No obstante, estudios más recientes están explorando el tema (Hjernquist y col. 2009).

La hipótesis LRE (ayudantes en el nido) se sustenta en pruebas más consistentes. Se han encontrado patrones de ajuste del SR en la dirección esperada (grupos que carecen de ayudantes producen más crías del sexo que ayuda) en varias especies (revisadas por West y Sheldon 2002; Griffin y col. 2005; West, Shuker y col. 2005). Otras especies no muestran este comportamiento (Bednarz y Hayden 1991; Legge y col. 2001; Koenig y col. 2001; Doutrelant y col. 2004), pero Griffin y col. 2005 demostraron que el grado de ajuste del SR está relacionado con la variación en el beneficio que proporcionan los ayudantes.

Basado en estas teorías, el caso del carricero de Seychelles (*Acrocephalus sechellensis*) es probablemente el ejemplo más claro e importante de ajustes del SR; no sólo entre las aves, sino entre todos los vertebrados. Aparte de la llamativa razón de sexos que presenta, este caso ilustra cómo múltiples factores pueden operar simultáneamente en los patrones de asignación por sexo (LRE, LRC y también influencias ambientales; Komdeur 1996; Komdeur, Daan y col. 1997) y proporciona pruebas explícitas de la naturaleza adaptativa de la variación del SR (Komdeur 1998).

Hipótesis de Trivers-Willard

Incluso en sentido estricto (la inversión en ambos sexos depende de la condición materna, considerada en especies poligínicas), la teoría proporciona un buen modelo para las aves: las situaciones de (1) variación inter-familiar en los recursos parentales y (2) falta de linealidad en los rendimientos marginales en respuesta a la inversión, son probables en muchas especies de aves. Además, algunos estudios preliminares eliminaron la poliginia como pre-requisito para los efectos Trivers-Willards (Burley 1981, 1986). Desde entonces, la influencia de la condición materna ha sido profusamente investigada y apoyada experimentalmente (R. Kilner 1998; Nager, Monaghan, Griffiths y col. 1999; Whittingham y Dunn 2000; Velando 2002; Alonso-Álvarez y Velando 2003; Pike 2005). Argumentos similares se han aplicado a una variedad de factores (teoría en sentido amplio): fecha de puesta/estacionalidad, orden de puesta y eclosión, tamaño del huevo, tamaño de la puesta, calidad del territorio, disponibilidad de alimento, número de ayudantes, edad, calidad y experiencia parental y duración del vínculo de la pareja, entre otros (para revisiones, véanse Hasselquist y Kempenaers 2002; Alonso-Álvarez 2006; West 2009).

Una extensión de la teoría que ha suscitado mucho interés es la *Hipótesis del Atractivo* (Burley 1981; para el modelo matemático, Pen y Weissing 2000). Las hembras ajustarían el SR en respuesta al atractivo o calidad del compañero, sobreproduciendo hijos cuando están emparejadas con machos atractivos o de buena calidad. Estos padres darían lugar a descendencia de mayor calidad, al pasar buenos genes o a través de una atención paterna de mayor calidad. Puesto que en aves la variación en el éxito reproductivo es mayor generalmente entre los machos (Møller y Ninni 1998), los hijos se beneficiarían más que las hijas de un padre de mayor calidad. Algunos estudios experimentales apoyan esta hipótesis (Ellegren y col. 1996; Von Engelhardt y col. 2004; Pryke, Rollins y col. 2011) mientras que otros no (Rutstein y col. 2005). Sin embargo, parece haber una tendencia ampliamente repetible y significativa hacia la producción de hijos cuando la pareja es un macho más atractivo (West y Sheldon 2002; Delhey y col. 2007; West 2009).

A pesar de la fuerza de las tendencias de Trivers-Willard en muchas especies, extraer patrones comunes para las aves sigue siendo complejo. Muchos estudios han encontrado inconsistencias en la dirección o directamente ausencia de ajustes incluso cuando las condiciones a priori los hacían esperables (P. J. Weatherhead 1985; Sheldon 1998; Hartley y col. 1999; Zann y Runciman 2003; Budden y Beissinger 2004). Dos meta-análisis sobre el tema llegaron a conclusiones diferentes (West y Sheldon 2002; Ewen y col. 2004). Algunos autores han argumentado que la variación en el grado de ajuste del SR entre especies y poblaciones, sin embargo, puede explicarse por la variación en la fuerza de la selección (K. E. Arnold y col. 2003) y por la baja detectabilidad a nivel de nido (Rosivall 2008).

El respaldo definitivo para la teoría de Trivers-Willard debe provenir de la demostración de las consecuencias del ajuste del SR (p.ej., demostrando que la condición materna afecta diferencialmente al valor reproductivo de hijos e hijas) como han hecho algunos estudios experimentales (Nager, Monaghan, Griffiths y col. 1999; Badyaev y col. 2002; Saino, Romano y col. 2010). Al mismo tiempo, es necesario realizar estudios que exploren los factores relevantes en especies y poblaciones concretas, con vistas a explicar la amplia variabilidad taxonómica en el grado de ajuste del SR.

Hipótesis de los costes de reproducción

Estos argumentos alternativos reúnen algunas de las premisas de Trivers-Willard y LRC, pero difieren en la perspectiva: el énfasis está puesto en el valor reproductivo y eficacia biológica de la madre, y no en los de su descendencia (J. H. Myers 1978; Wiebe y Bortolotti 1992). Las hembras en condiciones desfavorables podrían ser reacias a invertir en el sexo con mayores exigencias para minimizar el riesgo de fracaso y/o para aumentar las posibilidades de reproducción posterior. Algunas especies sesgan su producción hacia machos o hembras en función de la jerarquía materna: las hembras "primarias" (que son ayudadas por los machos en el aprovisionamiento) producen más hijos; las hembras secundarias (que no lo son) producen más hijas (Nishiumi 1998; Westerdahl y col. 2000). Sin embargo, la semejanza en la razón de sexos esperada por estas hipótesis y por Trivers-Willard hace difícil poder distinguir entre ellas.

Hipótesis de la reducción de nidada

En lugar de considerar las interacciones entre padres e hijos, estas hipótesis se centran en las interacciones que tienen lugar dentro de la nidada. Ciertas combinaciones de sexos podrían evitarse porque conducen a situaciones de fraticidio, o podrían verse favorecidas si así se consigue una reducción eficiente de la pollada. Han sido estudiadas principalmente en rapaces (p.ej., Bortolotti 1986; Dzus y col. 1996), donde se han encontrado secuencias de producción sesgadas. Sin embargo, hasta el momento no está claro si las desventajas de ciertas combinaciones sexuales conducen a la manipulación del SR primario o simplemente a la existencia de una mortalidad específica por sexo.

Nota de uso del lenguaje

Frases como "los individuos son seleccionados para maximizar su éxito reproductivo", no significan que los animales maximicen conscientemente su éxito reproductivo, ni que sean conscientes de la relación entre comportamiento y éxito reproductivo o las consecuencias de la selección natural. Este lenguaje se utiliza para evitar la repetición de largas oraciones que detallan cómo funciona la selección natural –p.ej.: los individuos que tienen mayor éxito reproductivo proporcionan una mayor contribución genética a la siguiente generación, y por lo tanto la selección natural favorecerá a los genes que provocan en los individuos un comportamiento que maximice su éxito reproductivo (de West 2009).

Análisis inter-específico de la razón de sexos y vulnerabilidad

Relación entre SR y SSD

La teoría de Fisher predice que cuando los individuos de un sexo son más costosos, la razón de sexos al final del periodo de cuidado parental debería estar sesgada hacia el sexo "más barato", para que la inversión global en los sexos sea igual. Esto significa que los patrones de asignación por sexo dependen, al menos parcialmente, del coste relativo de producir hijos e hijas. En muchos organismos el coste de producción de machos y hembras es probablemente muy similar. Sin embargo, hay especies que presentan notables diferencias en el tamaño corporal de machos y hembras (dimorfismo sexual en tamaño, SSD en sus siglas en inglés).

Aunque la idea ha sido cuestionada (Teather y P. J. Weatherhead 1994), normalmente se asume que en estas especies el sexo más grande tiene mayores exigencias de energía durante el desarrollo (Slagsvold y col. 1986; Richner 1991; Magrath y col. 2007), lo que puede incluso afectar a la condición de los padres (Cameron-MacMillan y col. 2007). Además, estudios comparativos revelan una correlación positiva entre el grado de SSD y la cantidad de comida/energía requerida por los sexos (Anderson, Budde y col. 1993; Krijgsveld y col. 1998). El sexo más grande necesitará una mayor inversión parental (es más "caro") y cuanto más dimórfica es la especie, tanto mayor la mayor diferencia en inversión para cada sexo.

Si las diferencias de tamaño entre hijos e hijas en especies con SSD reflejan los costes diferenciales de los sexos (Krijgsveld y col. 1998; Magrath y col. 2007), se podría predecir una razón de sexos poblacional sesgada hacia el sexo más pequeño. Hasta el momento, las evidencias de influencia del SSD en la razón de sexos son imprecisas: algunas especies presentan los patrones esperables (más hijas en especies con machos más grandes o más hijos en especies con hembras más grandes; Howe 1977; Wiebe y Bortolotti 1992; Griffiths 1992; Torres y Drummond 1997), pero otras especies no (Newton y Marquiss 1979). Las revisiones comparativas no aclaran el panorama: Clutton-Brock *et al.* concluyeron que había pocas pruebas de variación (Clutton-Brock y col. 1985; Clutton-Brock 1991), Slagsvold *et al.* mostraron una razón secundaria de sexos sesgada hacia el sexo más pequeño (Slagsvold y col. 1986), mientras que Dijkstra *et al.* determinaron que entre pollos volantones el sexo más pequeño era más escaso (Dijkstra, Daan y Pen 1998).

Desde otra perspectiva, un mayor tamaño puede conceder beneficios selectivos, pero es probable que también lleve aparejado un coste en forma de mayor mortalidad u otro tipo de vulnerabilidad (p.ej., un peor estado fisiológico). Estas diferencias entre sexos pueden aumentar aún más en situaciones de condiciones desfavorables, escasez de alimentos o estrés ambiental, y a menudo los machos (generalmente el sexo más grande) se ven más afectados (Clutton-Brock y col. 1985; Roskaft y Slagsvold 1985; Griffiths 1992). Se han propuesto dos teorías para explicar la mayor vulnerabilidad general de los machos: (1) los mayores requerimientos nutricionales del sexo más grande (**vulnerabilidad dependiente del tamaño**) o (2) factores asociados al sexo (**vulnerabilidad dependiente del sexo**). Estos últimos incluyen la heterogamia (página 166) y la *hipótesis del fenotipo masculino*: independientemente del tamaño del macho, pertenecer al sexo masculino conlleva efectos negativos. Por ejemplo, un aumento de la testosterona podría influir negativamente en otros aspectos del desarrollo como la inmunocompetencia (Zuk 1990; Fargallo y col. 2002; Müller, Dijkstra y col. 2003).

Los análisis comparativos de Clutton-Brock (Clutton-Brock y col. 1985) demostraron que las diferencias en vulnerabilidad (evaluada como mortalidad juvenil) eran proporcionales al grado de SSD. Sin embargo, estudios subsiguientes encontraron patrones opuestos (Nager, Monaghan, Griffiths y col. 1999; Nager, Monaghan, Houston y col. 2000; Hörnfeldt y col. 2000) o directamente no encontraron diferencias sexuales en el desarrollo y mortalidad juvenil en especies con SSD (Westerdahl y col. 2000; Råberg y col. 2005). Además, un tamaño grande puede ser ventajoso en situaciones de fuerte competencia entre hermanos, siendo los individuos del sexo más pequeño los que sufren entonces mayor mortalidad o efectos fisiológicos negativos (Anderson, Reeve y col. 1993; Råberg y col. 2005).

Análisis y especies modelo

Los resultados anteriores ofrecen un panorama algo confuso de las relaciones que existen entre el SSD, la vulnerabilidad específica por sexo y el ajuste del SR en aves. Una posible solución a este problema es usar la variación entre diferentes taxones como fuente de datos, es decir, utilizar un análisis comparativo inter-específico. Este tipo de enfoque ofrece resultados interesantes y ampliamente aplicables, porque supera las particularidades concretas de cada especie y evita el problema de falta de independencia entre especies (Mayhew y Pen 2002).

Se requieren, por tanto, estudios comparativos con el fin de examinar la variación del alcance o la precisión del ajuste de SR (Sheldon 1998). Por ejemplo, sería posible comprobar las predicciones de Fisher, si la razón de sexos de la descendencia se correlaciona con la razón de la inversión parental (determinada por el grado de dimorfismo sexual en tamaño) en los dos sexos (**Capítulo 1**). Un resultado fisheriano podría distinguirse de uno puramente heterogamético (página 164), puesto que en las especies con marcados costes sexuales de producción, la hipótesis de igual inversión de Fisher predice un sesgo numérico, mientras que la heterogamia predice igualdad numérica (Hardy 1997). Además, la mortalidad y la vulnerabilidad diferenciales pueden analizarse en relación al SSD y bajo diversos ambientes, entre una amplia gama de especies. Esto constituye una forma de determinar la validez general, la dirección y la intensidad de la hipótesis de vulnerabilidad dependiente del tamaño (**Capítulos 1 y 2**). El análisis comparativo permite también el análisis de otros caracteres y de sus diferentes grados de variación, sobre los que la selección natural ha trabajado a lo largo de periodos de tiempo que ningún estudio observacional o experimental puede reproducir (Mayhew y Pen 2002). Por lo tanto, otros múltiples rasgos vitales de las especies, que posiblemente influyen en la SA y más específicamente en las relaciones entre SSD y SR (Cuadro 3, página 169) puede incluirse en los análisis (**Capítulo 1**).

El análisis comparativo puede complementarse con un enfoque a nivel de especie (cada especie considerada como un dato independiente), ya que éste proporciona una interpretación biológica y visual de las tendencias más accesible, al eludir las suposiciones restrictivas de los métodos comparativos (T. Price 1997; Martins 2000, página 175).

El uso de aves como especies modelo beneficia el estudio de estas cuestiones, gracias a algunas de sus características. En primer lugar, las hembras son el sexo heterogamético (véase página 165). En segundo lugar, entre las aves hay especies monomórficas, especies con SSD sesgado hacia el macho (macho más grande que la hembra) y otras (rapaces, búhos y algunas aves marinas) con SSD sesgado hacia la hembra (hembra más grande que el macho) (Székely y col. 2007). En ambas direcciones, varias especies y familias muestran dimorfismos en tamaño extremadamente pronunciados. Esta amplia gama de grados de dimorfismo hace de las aves excelentes taxones para el análisis comparativo entre especies. Por último, las aves tienen un corto periodo de crecimiento determinado, por lo que la mayoría de ellas alcanza el tamaño adulto poco después de la emancipación. El periodo de desarrollo es un momento crucial en términos de requerimientos energéticos, especialmente en caso de crecimiento dimórfico. Por lo tanto, el efecto del sexo en las diferencias en requerimientos nutricionales, condición física o supervivencia puede observarse fácilmente antes de que la descendencia (altricial) sea independiente de sus padres.

Metodología general

El mayor obstáculo con que se enfrentan las pruebas comparativas entre especies es la falta de independencia entre taxones. Es probable que especies emparentadas sean similares porque heredan caracteres de un antepasado común y por lo tanto, no toda la variación en los caracteres de las especies es el resultado de eventos evolutivos independientes (Felsenstein 1985; Harvey y Pagel 1991). Los métodos estadísticos que tratan a las especies como puntos independientes no son totalmente válidos, ya que pueden sobreestimar la cantidad de variación evolutiva independiente e inflar artificialmente el significado de cualquier prueba (un problema conocido como “sobre-conteo filogenético”, Mayhew y Pen 2002).

El método de contrastes independientes basado en Felsenstein 1985 es una de las técnicas que se ocupan de este problema. El método calcula puntuaciones llamadas “contrastes”: las diferencias entre dos taxones hermanos en cada punto de bifurcación o rama (“nodo”) del árbol filogenético. Los contrastes representan la variación adquirida desde que los taxones compartieron un ancestro y son filogenéticamente independientes (Figura 16). Como todas los métodos, requiere una serie de asunciones: un modelo Browniano y evolutivo vagamente parsimonioso; y tasas iguales de cambio evolutivo por unidad de longitud de rama en todas las ramas de la filogenia. Los contrastes pueden analizarse paramétricamente por regresión lineal, que debe ser forzada a través del origen, ya que el cambio en la variable dependiente en respuesta al cambio cero en la variable independiente debe ser también cero (Garland y col. 1992). Así, el valor esperado de la pendiente es igual a la verdadera relación entre los caracteres comparados en ausencia de efectos filogenéticos (Pagel 1993).

Un análisis comparativo requiere la recolección de dos tipos de datos: estimaciones de los

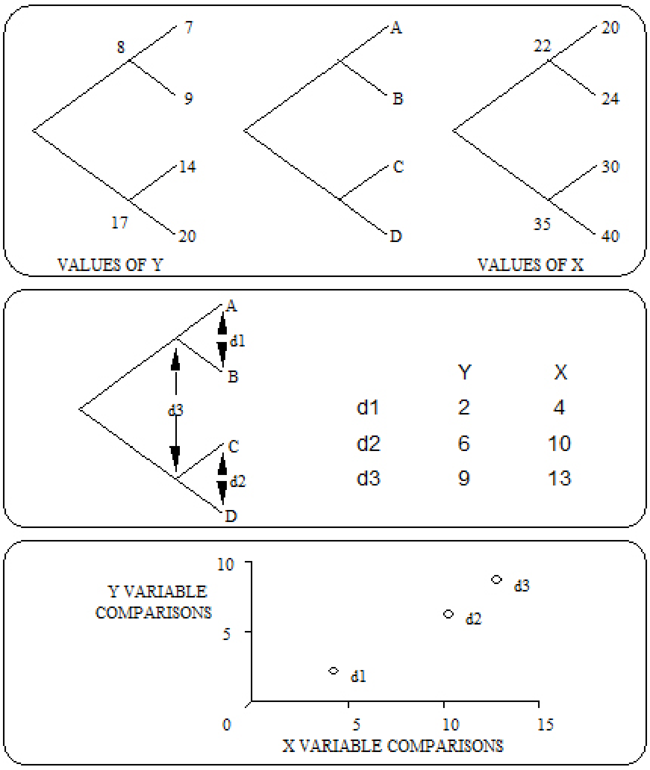


Figura 16: Modelo simplificado de comparaciones independientes (de Harvey y Pagel 1991 y Guía del usuario de CAIC). Valores de dos caracteres, X e Y, para cada una de las cuatro especies, A – D. Los nodos superiores se calculan como el valor medio de los nodos inferiores. Las especies A y B divergen en el nodo superior, por lo que la diferencia entre ellas (d1) debe haber evolucionado desde entonces (igual con d2). Estos dos conjuntos de diferencias son independientes. Además, la diferencia entre los nodos superiores (d3) aporta una tercera comparación independiente. Si las comparaciones de la variable Y se representan frente a las comparaciones de la variable X, se puede ver si el cambio evolutivo en los dos caracteres está correlacionado.

caracteres para cada especie e información filogenética sobre las relaciones entre especies.

Los datos de las especies se obtienen generalmente de estudios previamente publicados (lo que limita a la literatura disponible). Es necesario establecer unos criterios de inclusión de estudios con el fin de reducir los errores de medición, y también resumir las características de la especie en un solo dato. La razón de sexos, por ejemplo, es sensible a las condiciones locales. El nivel apropiado para la comparación podría ser por tanto la población, en lugar de la especie. Sin embargo, esto es apropiado sólo si el resto de variables relevantes también se ha estimado dentro de cada población, lo que a menudo no sucede (Harvey y Nee 1997). Por consiguiente, se deberá calcular un valor promedio de las distintas poblaciones (Mayhew y Pen 2002). Para asegurar la inclusión de resultados correctos en esta tesis, se utilizaron criterios específicos (1) para evaluar los métodos de cada estudio (tamaño de la muestra, diferencias de medición, métodos de determinación del sexo...); (2) para estimar el grado de

SSD de las especies y la masa de los volantones; (3) para recopilar los factores de estrategia vital; y (4) para el cálculo de los valores promedio de SR en caso necesario.

La información filogenética que poseemos de las especies mejora constantemente: en la actualidad, varios proyectos pretenden definir el árbol de la familia de las aves con estimaciones de longitud de la rama (p.ej. proyecto Árbol de la Vida `http://tolweb.org/Neornithes/15834`, Hackett y col. 2008). Con la información disponible en el momento de elaboración de este trabajo, y al igual que otros estudios que habían aplicado este método para el estudio de la diversidad en aves (Promislow y col. 1992; Owens y Bennett 1994), se usó una filogenia molecular (Sibley y Ahlquist 1990) basada en experimentos de hibridación de ADN (Figura 17a). Esta filogenia fue el primer intento completo de reconstruir el cladograma de toda una clase de organismos basado en datos moleculares, y estudios posteriores confirmaron muchas de sus conclusiones (Mooers y Cotgreave 1994). Asume una topología filogenética por encima del nivel de familia y múltiples ramificaciones entre géneros dentro de las familias y entre especies dentro del género, con todas las longitudes de rama establecidas en longitudes iguales (Owens y Hartley 1998). Sin embargo, ha sido también objeto de controversia (Sarich y col. 1989) debido a sesgos o inexactitudes. Por ello, se usó una segunda filogenia basada en la clasificación de Cracraft 1981, que utiliza caracteres morfológicos tradicionales como el esqueleto (Figura 17b). El uso de diferentes cladogramas permitió evaluar la medida en que los resultados eran dependientes de las diferentes reconstrucciones de la historia evolutiva aviar. Sin embargo, los extensos análisis de los Capítulos 1 y 2 demuestran que ambos modelos de filogenia conducen a resultados cualitativamente similares.

De un número de programas similares derivados de Felsenstein 1985, CAIC (Análisis Comparativo por Contrastes Independientes, Purvis y Rambaut 1995b) es el más popular y el utilizado en este trabajo de tesis. Si la filogenia es completamente conocida y todas las variables son continuas el programa aplica estrictamente el método de Felsenstein. Pero si parte de la filogenia es incierta y contiene politomías (nodos con más de dos ramas) que expresan la ignorancia de la verdadera estructura (como en el caso de las aves), CAIC las trata como politomías suaves (Pagel 1992). CAIC fue utilizado en esta tesis para analizar tanto variables distribuidas de forma continua (como el periodo de incubación) como datos categóricos discretos (p.ej. presencia de ayudantes), ya que también el análisis de contrastes de variables categóricas está implementado (Burt 1989) –para obtener más información sobre los algoritmos y análisis utilizados por CAIC, véase Purvis y Rambaut 1995a–.

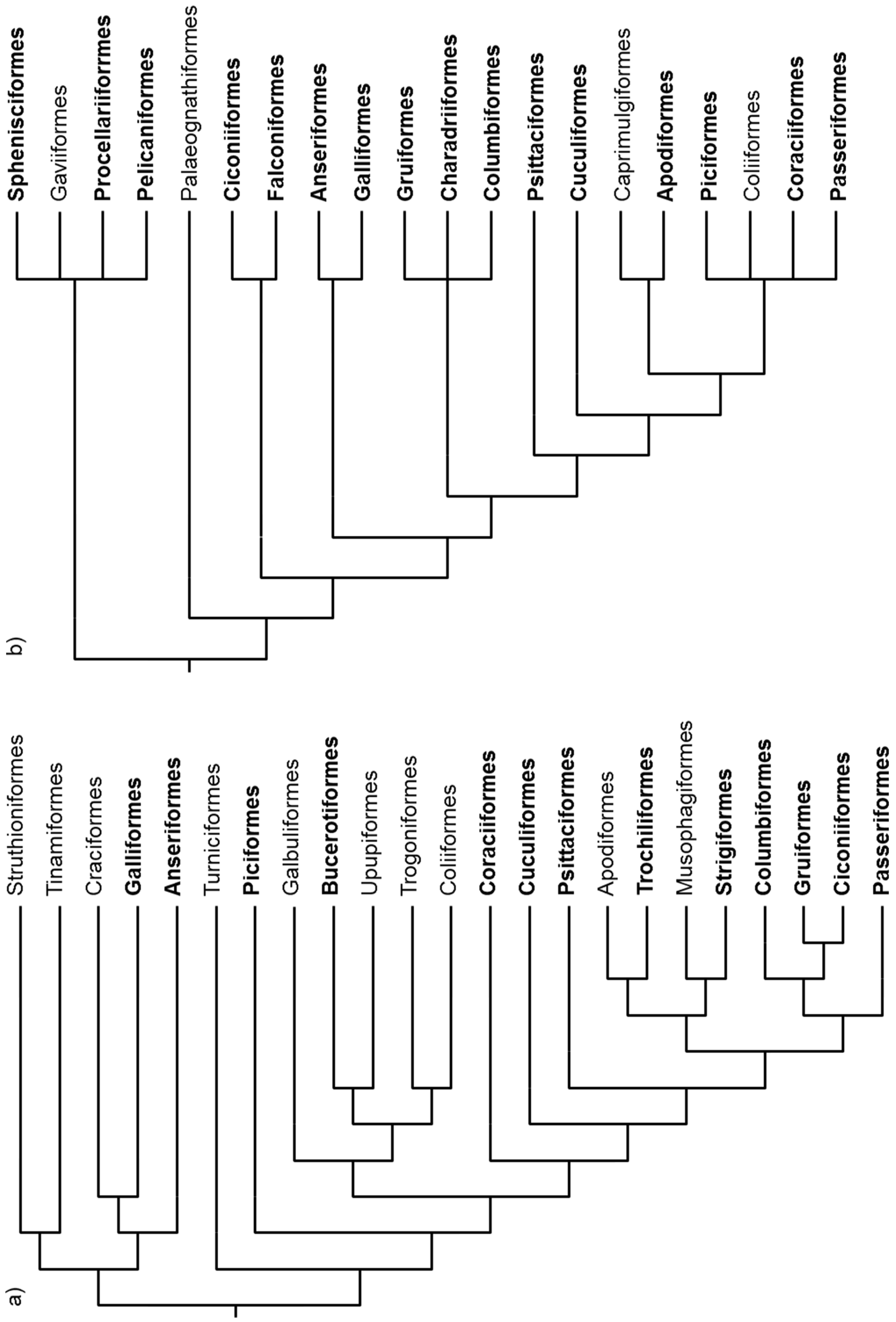


Figura 17: Cladogramas utilizados para los análisis comparativos: a) molecular (Sibley y Ahlquist 1990), b) morfológica (Cracraft 1981). Resaltados en negrita los órdenes incluidos en el estudio.

Análisis intra-específico: El caso del charrán común

Especie modelo

Esta parte de la tesis se centra en el charrán común (*Sterna hirundo*), un ave marina migratoria de pequeño tamaño. Se caracteriza morfológicamente por sus alas puntiagudas, cola bifurcada, caperuza negra, delgado pico rojizo con punta negra y patas rojizas (Figura 18b, pág. 185). Con un promedio en masa de 110–140 g y en longitud de 32–39 cm (Becker y Ludwigs 2004), es una especie ligeramente dimórfica. En adultos, las diferencias por sexo son significativas para el pico (6 %), la distancia pico-cabeza y la longitud del tarso (todas ellas mayores en los machos), y los machos adultos pesan entre un 1–3 % más (K. L. Fletcher y Hamer 2003; Becker y Ludwigs 2004). Además, los machos volantones presentan un 3 % más de masa que las hembras en ese mismo periodo (Becker y Wink 2002). No hay dimorfismo sexual en el plumaje ni parece existir en otros rasgos físicos.

Para estudiar el charrán común a la luz de la teoría de SA, son necesarios ciertos conocimientos previos de su estrategia vital, así como de todas las posibles diferencias intersexuales en características comportamentales y ecológicas (Cuadro 3, página 169).

El charrán común muestra las características típicas de las aves marinas: alta supervivencia en adultos, madurez tardía, filopatría notable, baja tasa reproductiva anual y largos periodos de crianza de los pollos. Los charranes viven hasta 30 años, su tasa de supervivencia de adultos está alrededor del 90 % y la tasa de mortalidad específica por edad parece ser independiente del sexo (Nisbet y Cam 2002; Ezard y col. 2006; Becker y Zhang 2011). La mayoría de los individuos procrea a la edad de 3 años, después de pasar al menos un año de prospección en la colonia (Ludwigs y Becker 2002; Dittmann y Becker 2003; Dittmann, Zinsmeister y col. 2005). La fecha de llegada a la colonia y la edad de reclutamiento están positivamente vinculadas (Becker, Dittmann y col. 2008). Las hembras parecen alcanzar la madurez ligeramente antes: su edad de reclutamiento es significativamente diferente (0,4 años antes que los machos, Ludwigs y Becker 2002) y se reproducen por primera vez a una edad más temprana (12 % de las hembras comienzan a los 2 años de edad, por tan solo el 2 % de los machos, Ludwigs y Becker 2005). La explicación podría provenir del hecho de que los machos son más territoriales y requieren mayores habilidades de búsqueda de comida, debido a la alimentación de cortejo (Ludwigs y Becker 2002). A pesar de una filopatría natal relativamente alta (Nisbet y Cam 2002; Becker y Ludwigs 2004), los adultos jóvenes presentan cierta dispersión diferencial por sexo: las hembras son más propensas a explorar otras colonias y emigrar (Dittmann, Zinsmeister y col. 2005; Becker, Ezard y col. 2008). Sin embargo, se estima que la razón de sexos en individuos reproductores está cerca de la paridad (Nisbet y Cam 2002). La productividad media varía entre 0 – 2.4 volantones por pareja (éxito promedio = 0.8, Becker 1998) y algunos componentes del rendimiento reproductor disminuyen estacionalmente (González-Solís, Wendeln y col. 1999; Becker, Wendeln y González-Solís 2001). Por otro lado, los charranes comunes mejoran su rendimiento reproductor con la edad, aunque a partir de los 14 años de edad se observa un ligero declive (senectud) (Rebke y col. 2010).

El charrán común cría en colonias y presenta un sistema de apareamiento monógamo que probablemente favorece las parejas concordantes en edad y fecha de llegada a la colonia (Ludwigs y Becker 2005; Ludwig y Becker 2008). Suelen establecer vínculos de pareja de larga duración (sin ayudantes) y demuestran una alta fidelidad, por lo que las cópulas y paternidad extra-pareja son infrecuentes (González-Solís, Sokolov y col. 2001). Sin embargo, se ha estimado una tasa de divorcio interanual del 25 % (González-Solís, Becker y Wendeln 1999; González-Solís, Becker, Jover y col. 1999). Las puestas contienen 2–3 huevos (a veces 1 ó 4), sin diferencias específicas de sexo en el volumen (K. L. Fletcher y Hamer 2004). Las puestas de reemplazo después del fracaso de la primera son bastante comunes (entre 10.6 % y 22.4 % en algunos años) y no suponen ninguna desventaja para la supervivencia o futura reproducción de los padres (Becker y Zhang 2011). Las segundas puestas "reales" (después de completar con éxito una primera) son raras y se presentan sólo en temporadas excepcionalmente favorables (Becker y Ludwigs 2004). Los pollos semi-precociales nacen de forma asincrónica y a partir de ese momento dependen del intenso cuidado parental proporcionado por ambos adultos hasta la emancipación (edad de emancipación: entre 22–29 días). Ni las tasas de crecimiento ni la edad de emancipación parecen ser dependientes del sexo (Becker y Wink 2003).

Una característica importante del charrán común es la existencia de diferencias sustanciales de calidad entre los individuos. La calidad en aves ha sido descrita a través de distintos parámetros, que incluyen la capacidad para producir huevos grandes (Bolton 1991) o puestas grandes (Coulson y Porter 1985), para criar al principio de la temporada (Sydeman y Eddy 1995), la cantidad de alimento proporcionado a la descendencia (Nisbet 1973) o la condición corporal (Weimerskirch 1992). La masa corporal, por ejemplo, es una medida de condición en el charrán común y está vinculada a la calidad individual y al éxito reproductivo (Wendeln y Becker 1999; Becker, Wendeln y González-Solís 2001). De hecho, la masa corporal de pollos post-volantones correlaciona positivamente con la probabilidad de supervivencia, de retorno y de reclutamiento (Ludwigs y Becker 2006; Braasch, Schauroth y col. 2009). Además, algunos indicios sugieren que la masa corporal puede ser un rasgo altamente heredable (Ludwigs y Becker 2006). En el charrán común, la calidad individual y eficacia biológica se miden también a través de la edad, la fecha de llegada a la colonia y la fecha de puesta, la experiencia y éxito reproductivo, y la longevidad. En general estos rasgos están intercorrelacionados: los individuos de mayor edad llegan antes a la colonia, ponen antes puestas con mayor número de huevos y crían con éxito un mayor número de volantones (Ezard y col. 2007; Becker, Dittmann y col. 2008). Por tanto, el carácter "tamaño de puesta de 3 huevos" se utiliza comúnmente como índice representativo de los indicadores de alto nivel de calidad y cuidado parental (J. M. Arnold y col. 2004; González-Solís, Becker, Wendeln y Wink 2005).

Con respecto a factores ambientales, el charrán común es muy dependiente de las condiciones ecológicas impuestas por el medio marino, donde la abundancia y disponibilidad de recursos alimenticios es generalmente impredecible en el tiempo y el espacio (Dänhardt y Becker 2011).

La teoría de SR aplicada al charrán común

La ausencia de un gran dimorfismo sexual en tamaño en el charrán común podría hacer pensar que hay pocas razones para esperar un ajuste del SR o unos costes o mortalidad diferenciales entre los sexos. Sin embargo, dos indicios desafían esta idea. En primer lugar, las evidencias previas de manipulación del SR en otras especies monomórficas en tamaño y con cuidado bi-parental (Lessells y col. 1996; Bradbury y Blakey 1998; Cordero y col. 2001; Whittingham, Valkenaar y col. 2002; Andersson y col. 2003; Husby y col. 2006; Dowling y Mulder 2006; Dijkstra, Riedstra y col. 2010; Zielinska y col. 2010). En segundo lugar, algunas de las características de estrategia vital y comportamiento de esta especie (a partir de la descripción anterior) tienen el potencial de actuar como presiones selectivas que conduzcan a la evolución de las estrategias de asignación por sexo.

Específicamente:

1. Los adultos de charrán muestran variaciones inter-individuales en los recursos que pueden invertir, relacionadas con su edad, condición y calidad. Esto implica diferencias en el atractivo individual, importantes en el momento del apareamiento, alimentación de cortejo o cambio de pareja.
2. Los factores medioambientales influyen considerablemente en la reproducción y en la tasa de mortalidad. Por otra parte, algunos aspectos del rendimiento reproductor (p.ej., la masa del huevo) disminuyen estacionalmente.
3. La existencia de un cierto SSD en los adultos y los pollos, incluso siendo leve, puede implicar diferencias en los costes o la probabilidad de mortalidad de cada sexo.
4. El tamaño de puesta está relacionado con la calidad de los padres, lo que implica la probable existencia de compromisos entre el número, sexo y orden de eclosión de la descendencia.
5. Existen ligeras diferencias sexuales en los patrones de filopatría y reclutamiento, que pueden afectar al nivel de competencia local entre familiares.

Además, estudios previos de la especie sugieren que los rendimientos de la eficacia biológica de machos y hembras no son iguales (Becker y Wink 2003; K. L. Fletcher y Hamer 2004; Ludwigs y Becker 2006; Braasch, Schauroth y col. 2009; González-Solís, Becker, Wendeln y Wink 2005). Estos estudios sugieren que los hijos son más sensibles a condiciones adversas y/o a padres de peor calidad, y en general que hay factores dependientes de la condición que afectan diferencialmente a la descendencia: hijos en buenas condiciones podrían tener mayores posibilidades de reclutamiento y apareamiento en comparación con hijos en peores condiciones, mientras que el éxito de las hijas podría ser menos dependiente de la condición.

Los factores expuestos anteriormente, en conjunción con el cuidado biparental, podrían conducir a un ajuste adaptativo del SR en el charrán común bajo los supuestos de Trivers-Willard, hipótesis del Atractivo, condiciones ambientales y/o hipótesis LRC. Otras complejidades añadidas (tamaños de puesta mayores de uno, conflicto entre los padres sobre el cuidado parental, compromisos con reproducciones futuras, etc.) hacen que las predicciones sobre la existencia de ajustes y su direccionalidad sean difíciles de establecer (Frank 1990). Sin embargo, en el charrán común algunos posibles escenarios en el ajuste del SR a nivel familiar podrían seguir estos patrones (adaptados de Hasselquist y Kempenaers 2002):

1. **Factores de calidad: la calidad difiere entre individuos**

   Machos de calidad alta | Machos de calidad baja:

   a) Calidad heredada por el hijo:

   - Supervivencia similar de hijos e hijas: SR sesgado - ♂ | SR sesgado - ♀
   - Menor supervivencia de hijos: Sin sesgo | SR sesgado - ♀

   b) Calidad no heredada o heredada independientemente del sexo:

   - Misma eficacia - costes de hijos e hijas: Sin sesgo
   - Mayor coste - eficacia de hijos: SR sesgado - ♂ | SR sesgado - ♀

   Hembras de calidad alta | Hembras de calidad baja:

   - Misma eficacia - costes de hijos e hijas: Sin sesgo
   - Mayor coste - eficacia de hijos: SR sesgado - ♂ | SR sesgado - ♀

2. **Factores estacionales**

   El rendimiento reproductor disminuye con la fecha de puesta:

   - Costes similares de hijos e hijas: Sin sesgo
   - Mayor coste de hijos: SR sesgado - ♀ (según avanza la fecha de puesta)

   La fecha de puesta afecta a la edad de primera reproducción/probabilidad de reclutamiento:

   - Hijas maduran antes: SR sesgado - ♀ al principio de la temporada
   - Hijos maduran más tarde: SR sesgado - ♂ al principio de la temporada

3. **Factores medioambientales: la competencia local difiere entre sexos**

   Años con buenas condiciones | Años con peores condiciones:

   - Mayor coste o menor supervivencia de hijos: SR sesgado - ♂ | SR sesgado - ♀
   - Supervivencia y coste similar de hijos e hijas: Sin sesgo

4. **Factores de puesta: la competitividad juvenil difiere entre sexos**

   Buenas condiciones de cría | Peores condiciones de cría:

   - Hijos son más agresivos: Sin sesgo | SR sesgado - ♀

     (primeras posiciones de la puesta)
   - Hijos son menos competitivos: SR sesgado - ♀ (primeras posiciones)

Basándose en estos factores, es posible evaluar una serie de expectativas específicas (**Capítulo 5**). Este tipo de análisis correlativo requiere un conjunto extenso de datos: por un lado, de determinación del sexo de los pollos, y por otro, información detallada sobre las familias: condición y calidad parental, fechas de puesta, estimación de las condiciones ambientales, orden de eclosión, y supervivencia de la descendencia hasta la emancipación. A continuación es recomendable comprobar si la varianza en la razón primaria y secundaria de sexos de la población difiere de la que sería esperable bajo una distribución binomial del SR. El último paso consistiría en investigar si estas proporciones de sexos están asociadas a los factores de posible influencia (Hasselquist y Kempenaers 2002). El mismo proceso debe llevarse a cabo para investigar la existencia de mortalidad diferencial por sexo en la descendencia y su correlación con los factores en estudio.

Sin embargo, la evaluación de modelos de SA requiere conocer previamente si y cómo las condiciones ambientales y parentales afectan al valor reproductivo relativo de hijos e hijas (Leimar 1996, pero véase Pen y Weissing 2000). Es importante investigar si un sexo es más costoso de producir que el otro, por ejemplo, porque presenten diferencias sexuales en crecimiento, tasa de metabolismo, capacidad competitiva, condición fisiológica o en la resistencia a enfermedades/parásitos (Burness y col. 2000; Saino, Ambrosini y col. 2002; Banbura y col. 2008). A través de la exploración de: (1) la existencia de dimorfismo sexual, además de en tamaño, en otros rasgos fenotípicos de los pollos; (2) su asociación con las condiciones medioambientales y de cría; y (3) su correlación con factores parentales, se podrían obtener indicios de la existencia de inversión parental diferencial en los sexos (Saino, Ambrosini y col. 2002; **Capítulo 3**). Además, uno de los sexos puede ser más "caro" de producir aun cuando machos y hembras sean similares en tamaño u otras características fenotípicas. Esto ocurriría si, por ejemplo, hijos e hijas desarrollan diferentes estrategias de crecimiento para maximizar la condición (Becker y Wink 2003) y así mejorar su capacidad competitiva. Una forma de comprobar esta posibilidad es explorar experimentalmente las diferencias potenciales entre los sexos en cuanto a su rendimiento en un compromiso fisiológico-inmunológico que tiene implicaciones para la calidad de la descendencia y el aprovisionamiento parental (**Capítulo 4**).

Metodología general y lugar de estudio

El trabajo de campo realizado para la segunda parte de esta tesis se llevó a cabo en la colonia del lago Banter (Figura 18a), situada en la zona costera-portuaria de Wilhelmshaven (Alemania). Anualmente, entre 90 y 530 parejas de charranes comunes (1992–2008) crían en las 6 islas idénticas del lugar. Allí se desarrolla desde hace 20 años un estudio integral que investiga diversos aspectos de la estrategia vital y la demografía de la especie (Becker, Wendeln y González-Solís 2001). Desde 1992, cada pollo nacido en la colonia ha sido anillado y marcado con un transponder pasivo por vía subcutánea antes del momento de emancipación. Esto permite la identificación individual de cada charrán durante toda su vida. Además, todas las aves marcadas desde 1998 han sido molecularmente sexadas usando métodos PCR estándar, y antes de 1998 los individuos reproductores fueron sexados mediante observaciones de comportamiento. Un sistema de antenas y balanzas localizadas en lugares estratégicos registra automáticamente la identidad y el peso de las aves marcadas. Por otro lado, los progenitores de cada puesta son identificados mediante la colocación de una antena alrededor del nido durante la incubación (Figura 18b). Este enfoque permite el registro de parámetros tales como edad, fecha de llegada a la colonia, masa corporal y otras características individuales (producción total de pollos volantones, número de intentos de cría, etc.). Los controles de la colonia cada dos días proporcionan datos pormenorizados sobre cada nido: tamaño de la puesta, fecha y orden de puesta, fecha y orden de eclosión, y seguimiento de los huevos y los pollos hasta la emancipación (métodos detallados en Becker y Wendeln 1997; Becker, Wendeln y González-Solís 2001; Becker, Dittmann y col. 2008). Por otra parte, la recopilación de datos específicos para esta tesis incluyó mediciones en los pollos de varios atributos morfológicos a diferentes edades, así como muestreos de sangre, plumas, parásitos y la toma de medidas del color y el estado inmunológico (Figura 18c).

Figura 18: a) El área del lago Banter en Wilhelmshaven, Alemania (de `http://www.photos-aus-der-luft.de`) y la colonia (foto de M. M. Benito); b) Charrán común (*Sterna hirundo*) en un posadero equipado con una antena (foto de B. Metzger) y nido rodeado por una antena (foto de M. M. Benito); c) Metodología: medidas del grosor de la membrana interdigital, proteínas en plasma y color del pie (fotos de B. Metzger y R. Nagel).

Objetivos

El *sex ratio* (SR) o razón de sexos es un tema complejo, afectado no sólo por procesos de determinación sexual sino también por dinámicas ecológicas y particularidades de cada especie. Hasta ahora, su estudio en aves ha proporcionado pocas respuestas consistentes. El objetivo principal de esta tesis era profundizar en los posibles factores implicados en el ajuste de la proporción sexual, usando a las aves como modelos de estudio.

La tesis se estructura en dos secciones. Cada sección aborda temas específicos, en general relacionados con la asignación por sexo y la razón de sexos, pero también de interés general en biología evolutiva.

La **primera sección** Análisis inter-específico de la razón de sexos y vulnerabilidad (Capítulos 1 y 2) se centra en el dimorfismo sexual en tamaño (SSD, en sus siglas en inglés) como factor clave en la regulación de la razón de sexos entre la descendencia. El objetivo principal de esta sección era evaluar cómo el grado de SSD en una amplia gama de especies de aves influye en los patrones de sex ratio, y determinar la validez de las teorías sobre la vulnerabilidad dependiente del tamaño.
Los objetivos específicos eran:

- Proporcionar una revisión inter-específica de la literatura dedicada al sex ratio y relacionada con el SSD (Capítulo 1)
- Evaluar si el SSD está asociado a sesgos en la razón de sexos, en diferentes momentos vitales (eclosión y emancipación) (Capítulo 1)
- Evaluar la sensibilidad de la descendencia (mortalidad y efectos fisiológicos negativos en la condición física) en relación al SSD (Capítulos 1 y 2)
- Evaluar la influencia de otras características de las especies en estas relaciones entre sex ratio y SSD (Capítulos 1 y 2)

La **segunda sección** Análisis intra-específicos: El caso del charrán común (Capítulos 3, 4 y 5) se centra en un ave marina, el charrán común *Sterna hirundo* (Linnaeus, 1758). Su ligero dimorfismo sexual en tamaño pone de relevancia otras características de la especie que también podrían estar involucradas en la creación de patrones de asignación por sexo. El objetivo principal de esta sección era investigar, a través de estudios observacionales y experimentales, los factores que podrían influir en la razón de sexos en esta especie.
Los objetivos específicos eran:

- Describir el dimorfismo sexual en el fenotipo y en las estrategias de desarrollo de los pollos de charrán común, ya que este dimorfismo podría estar relacionado con diferencias en el valor reproductivo o coste de cada sexo (Capítulos 3 y 4)
- Obtener indicios de una posible asignación diferencial por parte de los padres, mediante la exploración de estas diferencias fenotípicas en relación a rasgos parentales (Capítulo 3)
- Examinar la razón de sexos y la mortalidad por sexo tanto a nivel poblacional como individual (Capítulo 5)
- Evaluar la influencia de factores ambientales, de estacionalidad y de condición y calidad parental en los patrones de razón de sexos y mortalidad a nivel individual (Capítulo 5)

Resultados globales y Discusión

La existencia de los dos sexos, su origen y sus diferencias es un complejo fenómeno biológico que todavía no tiene una explicación universal (Maynard Smith 1980; Charnov 1982). El estudio científico de la asignación por sexo se centra en la variación en la cantidad y calidad de machos y hembras y en sus causas próximas y últimas. Los mecanismos de asignación por sexo, aunque han sido aplicados con éxito en numerosos taxones, son aún poco conocidos en vertebrados. En esta tesis se investigaron las causas de variación en la asignación por sexo en aves, centradas en el papel del dimorfismo sexual en tamaño y otras características de las estrategias vitales, y asociadas a diferencias en el valor reproductivo de hijos e hijas.

Asignación por sexo y dimorfismo sexual en tamaño

Razón de sexos y dimorfismo sexual en tamaño

En esta sección se presentan los resultados del análisis de un modelo a nivel poblacional, que hace predicciones acerca de la razón de sexos (SR) bajo el supuesto de que el ajuste facultativo se verá favorecido cuando los beneficios en eficacia biológica compensen los costes (West, Reece y col. 2002). Buenos ejemplos de estos análisis implican especies en las que es posible estimar la intensidad de la selección para dicho ajuste, por ejemplo especies con dimorfismo sexual en tamaño (SSD; West y Sheldon 2002).

En especies con SSD, los padres tendrían que invertir más en la producción y cría del sexo más grande, ya que se supone que éste tiene mayores necesidades (Slagsvold y col. 1986; Nicolaus y col. 2009; Kalmbach, Griffiths y col. 2009; Kristensen y col. 2013). La teoría de Fisher expone que la razón de sexos al final del periodo de cuidado parental estará sesgada hacia el sexo más pequeño y más barato, de manera que la inversión poblacional total en ambos sexos sea igual. La inversión de recursos en los dos sexos puede igualarse mediante el ajuste de la razón de sexos en el nacimiento (primaria, HSR) o durante el periodo de cuidado parental (secundaria, FSR). Bajo el supuesto de que la descendencia de especies con mayor dimorfismo en tamaño presentará una mayor diferencia sexual en la demanda (Clutton-Brock y col. 1985; Magrath y col. 2007), es esperable que estas diferencias en los costes de reproducción debidas al SSD se correspondan con la tasa de inversión en machos y hembras, y por lo tanto, que se correlacionen con los sesgos en la razón de sexos en distintas etapas de la vida.

Los análisis comparativo y a nivel específico presentados en el Capítulo 1 corroboran parcialmente esta predicción, ya que muestran que el grado de SSD influye en la razón

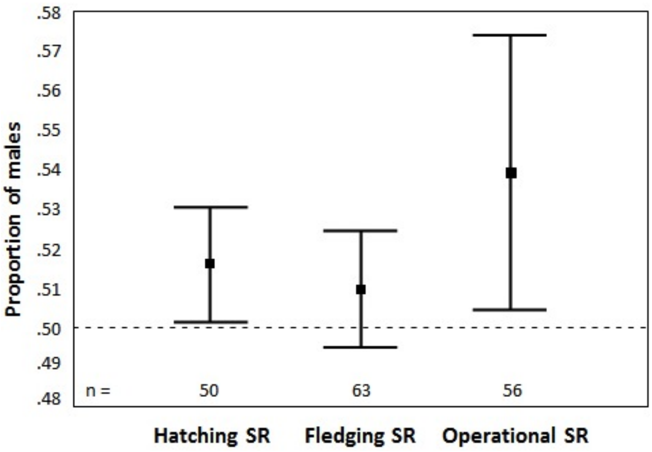


Figura 19: Valores interespecíficos medios de la razón de sexos (95 % IC) en distintas etapas de la vida. La razón de sexos está calculada como proporción de machos.

de sexos en recién nacidos y en volantones. A pesar del débil efecto global, las tendencias fueron consistentes cualitativamente y en la dirección esperada. Las especies dimórficas mostraron una tendencia general a sesgar el SR primario hacia el sexo más pequeño (más barato), en ambos sentidos del SSD: hubo mayor proporción de hijas en especies donde los machos son relativamente más grandes y viceversa (Fig. 1a y Tabla 1, capítulo 1; pág. 44). A nivel de especies sin control de filogenia, la media inter-específica de la razón primaria de sexos mostró un predominio significativo de machos (Figura 19). Sin embargo, las tendencias dependieron del tipo de SSD: especies monomóficas y con machos más grandes mostraron un SR más cercano a la paridad (Fig. 1a, Apéndice S5, capítulo 1; pág. 66), mientras que las desviaciones fueron particularmente notables en las especies con hembras más grandes. Los patrones en el momento de la emancipación (Fig. 19; Fig. 1b y Tabla 1, capítulo 1 –pág. 44–; Fig. 1b, Apéndice S5, capítulo 1 –pág. 66) fueron similares pero suavizados respecto a los del nacimiento.

Los escasos análisis comparativos previos que existen sobre razón de sexos en especies de aves con SSD no han mostrado evidencias consistentes de sesgos en el SR primario a nivel de población (Clutton-Brock 1986; Pen, Weissing y col. 2000). Se ha encontrado una razón secundaria de sexos sesgada hacia el sexo más pequeño en dos estudios (Slagsvold y col. 1986; Pen, Weissing y col. 2000). Sin embargo, otro reveló el patrón opuesto: más individuos del sexo más grande entre los volantones (Dijkstra, Daan y Pen 1998). Los resultados presentados en esta tesis incluyen datos nuevos de muchas especies (especialmente de especies con SSD sesgado hacia hembras, que eran escasas) y uso de técnicas relativamente nuevas (de determinación del sexo y de análisis). En efecto, los resultados fueron más claros cuando se controlaron las relaciones filogenéticas y se usaron datos restringidos a los estudios moleculares, que utilizan técnicas de sexado más fiables y permiten la determinación del sexo muy temprano en el desarrollo.

Otros estudios previos de especies concretas han mostrado razones primarias de sexo significativamente sesgadas, especialmente entre especies con gran dimorfismo en tamaño (p.ej., Dijkstra, Daan y Buker 1990; Velando 2002; Kalmbach, Nager y col. 2001; Øigarden y Lifjeld 2013). Esto no es sorprendente, ya que los ajustes más extremos y precisos de la razón de sexos se esperan en especies en las que los beneficios del ajuste facultativo de SR son altos y los costes bajos, como es predecible si el grado de SSD se asocia con costes diferenciales de hijos e hijas (Komdeur 2012). Por lo tanto, el ajuste general de la razón primaria de sexo, particularmente en especies con SSD sesgado hacia las hembras, puede ser una manera de igualar la inversión entre sexos con diferentes costes. Sin embargo, no se encontraron asociaciones significativas entre el SSD y el HSR (ni FSR) en los similares análisis comparativos del Capítulo 2 (Tabla 13.1, pág. 75). A pesar de que la discrepancia puede provenir del número relativamente pequeño de especies usadas en el Capítulo 2 (15 contra 50), no se puede afirmar tajantemente que la desproporción numérica en presencia de SSD sea el resultado de una inversión equivalente en ambos sexos.

El alcance de la influencia que tiene la variación de la razón de sexos primaria y secundaria en la razón de sexos terciaria se desconoce en gran medida. Como proporción de machos y hembras preparados para reproducirse, la razón de sexos terciaria u operativa (OSR, Cuadro 1, pág. 158) influye en gran medida en la intensidad de los mecanismos de competencia reproductiva en una población, y por lo tanto también en la intensidad de selección sexual (Kvarnemo y Ahnesjoe 2002). El OSR de 56 especies de aves también fue analizado para esta tesis (datos en los Apéndices A y B, pág. 233 y 235). El valor inter-específico medio mostró una proporción significativa de machos, más elevada que en cualquier otra etapa de la vida (Figura 19).

Debido a la aceptación general del argumento de Fisher, las razones terciarias de sexo han sido consideradas aproximadamente 1:1 en las poblaciones de aves silvestres. Sin embargo, Mayr (Mayr 1939) y revisiones posteriores (Payevsky 1993; Donald 2007) llegaron a la conclusión de que las razones de sexos no equilibradas son comunes entre aves adultas, con machos superando en número a las hembras por un promedio del 33 % (aunque también pueden influir factores filogenéticos). Se ha argumentado que estas diferencias se deben, más que a razones de sexos sesgadas en la descendencia, a mayores tasas de mortalidad femenina entre las etapas de independencia y madurez sexual (Donald 2007). Sin embargo, los valores de la razón terciaria de sexos suelen ser difíciles de obtener y pueden estar severamente sesgados según el tipo de método de conteo. Mientras que el OSR se refiere estrictamente a la proporción de hembras y machos en edad de reproducción en una población en un momento dado (medida en zonas de reproducción; Kvarnemo y Ahnesjoe 2002), la mayoría de los estudios utilizados para esta tesis midieron la razón de sexos en adultos durante la temporada de cría *y* post-cría, en zonas de invernada o durante la migración. Incluso cuando aparentemente la medida del OSR es correcta, es posible cierta inexactitud si los sub-adultos o inmaduros presentes en las zonas de cría pertenecen principalmente a un sexo. Por lo tanto, si bien la tendencia que aquí se presenta coincide con otros estudios, hay que ser conscientes de la validez limitada de estos datos.

El análisis a nivel de especies de la relación entre el grado de SSD y la razón de sexos en

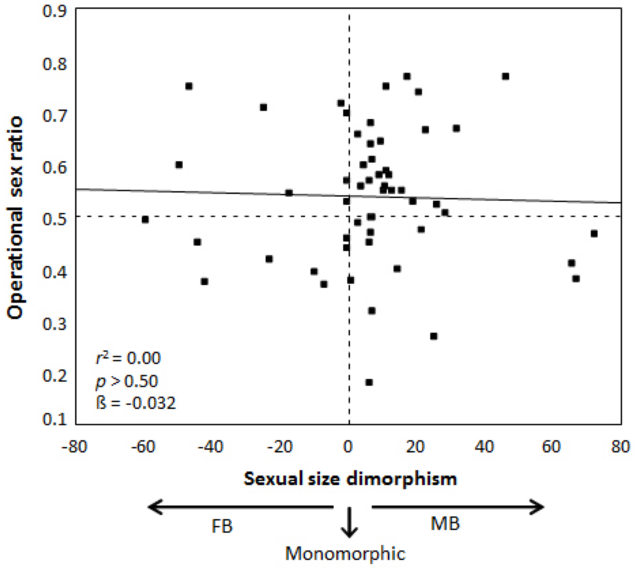


Figura 20: Relación entre SSD (índice de Storer) y la razón operacional de sexos. FB, especies con SSD sesgado-♀, MB, especies con SSD sesgado-♂.

adultos no reveló ningún patrón significativo (Figura 20). Esto puede indicar que los costes del dimorfismo incurren principalmente durante el crecimiento juvenil, y sugiere que la sugerida mayor mortalidad femenina después de la emancipación es el resultado de otros factores más que del tamaño. La heterogamia, las diferencias sexuales en el comportamiento de forrajeo, dispersión postnatal, depredación o esfuerzo reproductivo (Promislow y col. 1992; Owens y Bennett 1994; Awkerman y col. 2007), y el tipo de sistema de apareamiento (Breitwisch 1989), se postulan como los principales factores determinantes del desequilibrio de la razón de sexos en adultos. Una mayor proporción de machos adultos en la población podría crear la suficiente varianza en su éxito de apareamiento como para impulsar la evolución de ornamentos sexuales (Dearborn y col. 2001) y del SSD. En cualquier caso, este resultado, aunque coincidente con observaciones previas (Donald 2007), debe ser confirmado por un análisis controlado filogenéticamente.

Vulnerabilidad de la descendencia y dimorfismo sexual en tamaño

La variación de la razón secundaria de sexos respecto a la paridad podría provenir simplemente de un sesgo inicial del SR en el nacimiento, pero también de una supervivencia diferencial de los sexos entre la eclosión y el momento de emancipación. Muchos estudios han mostrado una mayor sensibilidad de uno de los sexos (revisado en Jones y col. 2009), aunque no está claro qué factores son los responsables de un comportamiento diferencial de los hijos e hijas que finalmente pueda conducir a una mortalidad específica por sexo.

La vulnerabilidad diferencial puede ser el resultado de una manipulación parental activa de las condiciones de la descendencia después del nacimiento. Se han encontrado cuidados parentales diferentes para cada sexo en varias especies (R. M. Kilner 2006; Griggio y col. 2009; Mainwaring y col. 2011; Leech, Rowe y col. 2006). De acuerdo con la teoría de Fisher, una mortalidad diferencial durante el periodo de inversión parental favorece una asignación por sexo diferencial. Por tanto, en los casos en que la manipulación adaptativa esté involucrada, la razón primaria de sexos se correlacionaría con las tasas de mortalidad. En especies donde la mortalidad femenina es mayor, por ejemplo, la razón primaria de sexos sería sesgada hacia las hembras. Aunque no fue analizado específicamente en esta tesis, el predominio de machos en la eclosión en todo tipo de especies (pero especialmente en aquellas con hembras más grandes), así como la comparación entre los patrones de HSR y FSR (Fig. 1, Apéndice S5, capítulo 1; pág. 66) no parece confirmar esta posibilidad.

Con mayor probabilidad, las diferencias sexuales en mortalidad y en general en vulnerabilidad reflejan las diferencias entre sexos de los patrones de crecimiento. El análisis comparativo de Clutton-Brock (recopilado en Clutton-Brock 1991) encontró mayor mortalidad masculina en especies donde el macho es más grande, lo que también se ha encontrado en casos particulares (en los dos sentidos de SSD: Griffiths 1992; Krijgsveld y col. 1998; Torres y Drummond 1997; Bortolotti 1986; Kalmbach, Furness y col. 2005). Sin embargo, también se han encontrado resultados opuestos (p.ej. Nager, Monaghan, Houston y col. 2000; Hörnfeldt y col. 2000) o directamente ausencia de mortalidad diferencial en especies con SSD pronunciado (Westerdahl y col. 2000; Råberg y col. 2005). Los resultados presentados aquí (usando datos de razón de sexos estrictamente controlada en las mismas puestas) mostraron claramente la existencia de una mortalidad específica por sexo correlacionada negativamente con el dimorfismo en tamaño (Fig. 2b y Tabla 1c, capítulo 1 –pág. 44, 46–; Tabla 13.1 y Fig. 13.1, capítulo 2 –pág. 75, 76–). El sexo más grande sufre una mayor mortalidad: los hijos presentan una mortalidad relativamente mayor en las especies con machos más grandes y viceversa, y la desventaja aumenta al incrementarse el dimorfismo. La vulnerabilidad en volantones, medida como el cambio de masa bajo diferentes condiciones, también está asociada con el grado de SSD (Tabla 13.1 y Fig. 13.2, capítulo 2; pág. 75, 79): el sexo más grande sufrió una reducción mayor de la masa cuando las condiciones de cría eran pobres. Los cambios mínimos de masa entre los descendientes de especies monomórficas sugieren que en ausencia de SSD ningún sexo presenta más vulnerabilidad que el otro.

En conjunto, estos resultados indican que tanto machos como hembras pagan un coste, en forma de mortalidad o eficacia biológica reducida, para lograr un mayor tamaño final. Las teorías de vulnerabilidad dependiente del tamaño sugieren dos mecanismos divergentes: (1) una mayor vulnerabilidad del sexo más grande cuando las condiciones de cría son peores, debido a sus mayores necesidades nutricionales o (2) una mayor vulnerabilidad del sexo más pequeño (Oddie 2000; Råberg y col. 2005; Rowland y col. 2007) debido a la ventaja del sexo más grande en la competencia entre hermanos (Hipkiss y col. 2002). Debido a que tanto el argumento de Fisher como el primer mecanismo predicen una razón secundaria de sexos desviada hacia el sexo más pequeño y sugieren el mismo mecanismo para lograrlo (mayor mortalidad del sexo más grande), sería difícil distinguir la verdadera causa de la mortalidad diferencial. En cualquier caso, el tamaño parece de hecho el principal motivo de mortalidad, ya que las hijas de especies con hembras más grandes experimentan desventajas similares a las de los hijos de especies con machos más grandes. Los efectos dependientes del tamaño, en concreto debidos el aumento de la sensibilidad del sexo más grande, son posiblemente la causa de la vulnerabilidad diferencial.

Sin embargo, otras evidencias añaden más complejidad. También se han propuesto explicaciones de vulnerabilidad independiente del tamaño (p.ej., la "hipótesis del fenotipo masculino"), tras la observación en aves de una mayor vulnerabilidad general de los machos. En estos resultados, la mortalidad global de la descendencia fue también ligeramente mayor para los machos (Fig. 2b, capítulo 1 –pág. 46–; Fig. 13.1, capítulo 2 –pág. 76–), al igual que el valor promedio de mortalidad en el análisis comparativo del Capítulo 2. Es más, análisis posteriores del cambio de masa en volantones mostraron que la tendencia general anteriormente descrita dependía del tipo de SSD: mientras que las diferencias de masa en hembras parecen ser independientes del SSD, en los machos están fuertemente correlacionadas con el grado de dimorfismo (Fig. 13.3, capítulo 2; pág. 80). Esto sugiere que la supervivencia de la descendencia se ve afectada negativamente por otros rasgos sexuales aparte del tamaño.

Por lo tanto, a pesar de que ser más grande conlleva costes de viabilidad y de supervivencia durante el crecimiento, parece ser una desventaja sobre todo cuando va ligado al fenotipo masculino. El perfil hormonal de los machos es la causa probable de esta vulnerabilidad (Olsen y Kovacs 1996). Los altos niveles de testosterona y otras hormonas necesarias para la diferenciación sexual masculina podrían influir negativamente en otros aspectos del desarrollo (Fargallo y col. 2002; Møller, Sorci y col. 1998; Müller, Groothuis y col. 2005). Nuestros resultados corroboran un reciente meta-análisis, que concluyó que los patrones sexuales de vulnerabilidad dependen de los efectos combinados de SSD y otros factores, y no del tamaño o factores de sexo por sí solos (Jones y col. 2009).

En cualquier caso, si las diferencias en mortalidad encontradas en este y otros estudios no se deben a decisiones parentales, podrían moderar la intensidad de las desviaciones del SR en las poblaciones, porque una mayor mortalidad inicial del sexo más grande reduciría las diferencias en los costes de producción de machos y hembras (Komdeur 2012).

Características de estrategia vital en la relación SR-SSD

La razón de sexos ha sido estudiada con frecuencia en especies con SSD porque esta característica es considerada un factor determinante en la variación en SR. Sin embargo, las aves poseen estrategias vitales que añaden una capa de complejidad a la asignación por sexo (Cuadro 3, pág. 169). La teoría de Fisher se basa en ciertas suposiciones que podrían no tener cabida en este contexto, ya que estos factores pueden afectar de forma no lineal a los retornos de eficacia biológica de los hijos e hijas. Los caracteres de estrategia vital seleccionados para este análisis fueron la presencia de dimorfismo sexual en plumaje, colonialidad, sistema de apareamiento, presencia de ayudantes, tipo de migración, distribución de las áreas de cría, ubicación del nido, periodo de incubación y periodo hasta la emancipación, tamaño de puesta, secuencia de eclosión, modo de desarrollo, edad de primera reproducción y tasa de

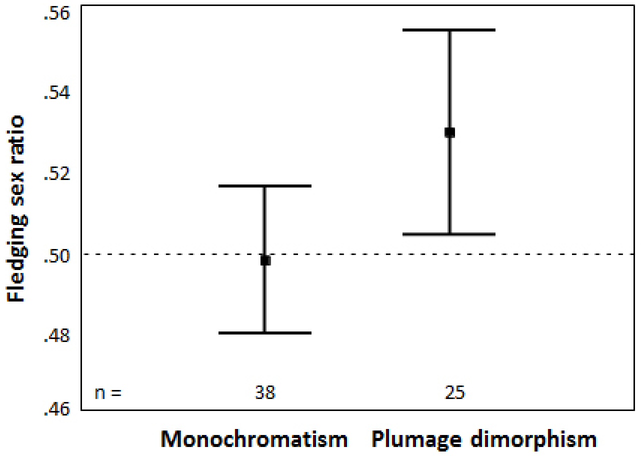


Figura 21: Valor medio interespecífico de la razón de sexos (95 % IC) según la presencia o ausencia de dimorfismo sexual en plumaje

supervivencia en adultos (definiciones y datos en los Apéndices S1 y S4, capítulo 1; pág. 51, 61–65). Estos atributos han sido considerados por diferentes modelos de SA como potenciales moduladores de variación adaptativa en la razón de sexos.

Una vez controlados los factores de estrategia vital, las relaciones entre la razón primaria y secundaria de sexos y el dimorfismo en tamaño no mostraron patrones significativos. La importancia del SSD en la regulación del SR podría verse disminuida debido a la oposición o mayor influencia de otras características de las especies. Las especies dicromáticas, por ejemplo, mostraron una mayor proporción de machos entre los volantones (Figura 21) independientemente del grado de SSD. El dicromatismo en el plumaje es una señal de selección sexual, incluso en especies monógamas (Webster y col. 2007), y una mayor varianza en el atractivo de la pareja podría motivar a las hembras a invertir diferencialmente en su descendencia (*hipótesis del atractivo*). Dado que la relación no aparece también en el momento de eclosión, podría esperarse que el dicromatismo influyera en la mortalidad diferencial entre ambas etapas de la vida. Sin embargo, éste no es el caso, lo que sugiere que el dimorfismo en plumaje podría estar intercorrelacionado con otros factores.

Un desarrollo altricial de los pollos y el aumento de la edad de primera reproducción también se asociaron con un número mayor de machos volantones; además, la tasa de mortalidad masculina disminuyó en especies con periodos más largos hasta la emancipación (Tabla 2a-b, capítulo 1; pág. 45). Estas tendencias fueron independientes del grado o tipo de SSD.

Sin embargo, otras características de estrategia vital mostraron un efecto conjunto sobre la razón de sexos *y* SSD (base de datos de estudios moleculares; Tabla 2c, capítulo 1, pág. 45): se encontraron más machos entre los volantones de especies altriciales y una menor mortalidad masculina en las especies monógamas. El sistema de apareamiento de la especie y el modo de desarrollo de la descendencia conllevan diferentes esquemas de cuidado parental. Los pollos precoces pueden mostrar diferentes requerimientos energéticos entre los sexos, pero dado que se alimentan ellos mismos, son probablemente mucho menos exigentes con los padres que los pollos altriciales, que dependen en gran medida del cuidado parental. Por otro lado, en especies polígamas los hijos pueden sufrir más presión, ya que hay mayor varianza reproductiva entre los machos y su éxito reproductivo está fuertemente influido por el tamaño corporal (Nishiumi 1998). Las influencias de SSD y los factores de estrategia vital, sin embargo, mostraron signos opuestos en ambos casos. Esto demuestra la complejidad de estas relaciones y la dificultad que entrañan las predicciones. En algunos casos, los efectos pueden actuar todos en la misma dirección, lo que haría posibles ciertas predicciones explícitas (p.ej. el carricero de Seychelles, Komdeur 1998). En otros casos, como los presentados aquí, los factores podrían estar actuando en direcciones divergentes, y en ausencia de una estimación cuantitativa de la importancia de cada efecto, es difícil saber qué pauta esperar. Sin embargo, estos análisis son útiles para hacer predicciones relativas (comparativas) acerca de las variaciones en el SR poblacional o en el grado de ajuste del SR entre especies, más que lo que ocurra en una especie en particular (Griffin y col. 2005; West 2009).

La evidencia, aunque heterogénea, de que las características de estrategia vital afectan a las proporciones de sexos, a la mortalidad y al papel modulador del SSD en éstas, apunta hacia un escenario de "asignación condicional por sexo" más que a un escenario de "inversión igualitaria". Dado que varios de sus supuestos no se cumplen, la validez del argumento de Fisher para los sesgos de SR relacionados con SSD a nivel poblacional es aún incierta.

El valor del método comparativo para el análisis de la razón de sexos

Aunque los métodos comparativos han recibido una atención creciente en los últimos años y son firmemente alentados (West 2009), últimamente los estudios entre especies han vuelto a sus orígenes. Varios estudios sugieren que los análisis a nivel de especies pueden ser más adecuados para algunas formas de análisis comparativo que los contrastes evolutivamente independientes (Martins 2000; T. Price 1997).

El rigor de los estudios cuantitativos se basa en los datos que los sustentan. En estudios comparativos, éstos varían ampliamente en cuanto a precisión, cantidad y métodos de recolección. Una de las críticas frecuentes al análisis comparativo es que la variación intra-específica es demasiado grande como para permitir comparaciones significativas entre las especies. Sin embargo, cuando el rango de taxones estudiados es amplio, la variación entre especies es generalmente mayor que la variación dentro de las especies. Así, aunque puede haber variación entre los individuos dentro de una población, y en todas las poblaciones de la misma especie, esta variación es a menudo pequeña en comparación con la variación entre especies o niveles taxonómicos mayores. La figura 22 muestra que éste es el caso de la razón de sexos, especialmente para el FSR; la mayor variación del HSR podría deberse al mayor número de especies con tan solo 2 valores obtenidos. Junto con los resultados de los Capítulos 1 y 2, esto confirma que al menos en algunos casos, el enfoque a nivel de especies y el filogenético pueden conducir a resultados consistentemente similares, por lo que ambos tipos de análisis comparativo son valiosos y complementarios.

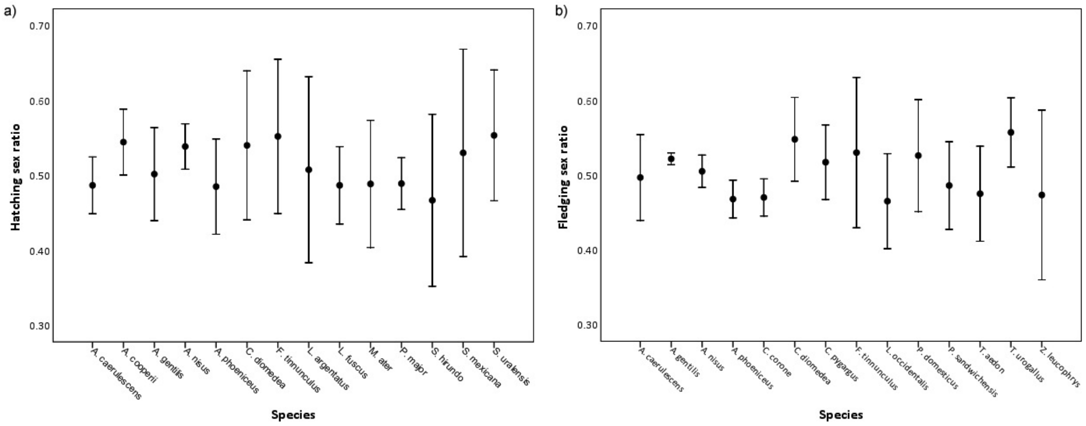


Figura 22: Valores medios (95 % IC) de la razón de sexos a) primaria y b) secundaria para especies de las que obtuvo más de un valor.

Asignación por sexo en el charrán común

Para comprobar las teorías de la asignación por sexo en una especie concreta, es importante abarcar tres aspectos: investigar la variación en la razón de sexos a diferentes niveles dentro de la población, investigar la diferencia en los costes de producción de hijos e hijas (tal vez afectados por factores ambientales y parentales) e investigar si la razón de sexos se ajusta de acuerdo a estos factores (Hasselquist y Kempenaers 2002).

Atributos sexuales de la descendencia

Cuando se investiga la variación en las proporciones de sexos, los factores con posibles efectos diferenciales en la eficacia biológica de cada sexo deben ser identificados (véase Komdeur y Pen 2002). Hasta ahora la atención se ha centrado en las diferencias sexuales en tamaño como generadoras de variación en SR, pero el charrán común es sólo ligeramente dimórfico en tamaño (machos alrededor de un 3 % más grandes). Según los resultados de la primera parte, las poblaciones de charrán común mostrarían una ligera prevalencia de machos en la eclosión y una vulnerabilidad ligeramente sesgada hacia los machos. La combinación de ambos mecanismos darían lugar a una razón secundaria de sexos también ligeramente sesgada hacia machos; en cualquier caso, todas las proporciones estarían cerca de la paridad.

Sin embargo, aparte del tamaño, la calidad del ambiente externo y social, la calidad de los padres, el número y la asimetría entre la descendencia, etc. son también factores probablemente influyentes (Sheldon 1998). Por tanto, es importante estudiar si un sexo es más costoso de producir que el otro, por ejemplo, porque tiene una mayor tasa metabólica o mayores exigencias alimentarias (Teather y P. J. Weatherhead 1988; Krijgsveld y col. 1998), crece más rápido (Teather 1987), hace más llamadas de petición o demuestra mayor agresividad (Ayala y col. 2007). Cualquier dimorfismo sexual descrito en los pollos de charrán común podría implicar una diferencia entre los sexos capaz de modificar su valor reproductivo relativo. Por otra parte, cualquier diferencia sexual en cuanto a la sensibilidad indicaría que existen efectos de poseer un fenotipo masculino o femenino sin la influencia de diferencias considerables en tamaño corporal.

Los pollos de charrán común (en estos estudios en concreto, pollos-a o primeros pollos) muestran dos notables excepciones a la falta generalizada de dimorfismo sexual en la mayoría de sus características morfológicas, fisiológicas e inmunológicas (Tabla 1, capítulo 3; pág. 91). Poco antes de la emancipación, hijos e hijas presentan diferente longitud de la distancia pico-cabeza y diferente CMI (respuesta inmune celular), ambas mayores en los machos (Capítulo 3). La longitud pico-cabeza es también mayor en machos adultos y post-volantones que en hembras (Becker y Wink 2002). Por el contrario, la masa corporal, la única característica dimórfica que se había encontrado previamente en pollos de charrán común, no difirió entre sexos en este estudio. La razón puede provenir de la ausencia de suficiente poder estadístico para detectar diferencias sutiles justo en el comienzo del proceso de divergencia, puesto que la masa comienza a desviarse de una manera dependiente de sexo a partir de los 18 días de edad (Becker y Wink 2003).

El dimorfismo aparece también en el desarrollo específico por sexo de algunas estrategias de crecimiento (Capítulos 3 y 4). Los compromisos diferenciales durante el crecimiento de los pollos macho parecen implicar que los recursos se asignan preferentemente a la condición corporal (Capítulo 4) y a la función inmune (Capítulo 3; véase también Cucco y col. 2006; Soler y col. 2003), tal vez para compensar los efectos perjudiciales del fenotipo masculino (Blas y col. 2006). Precisamente la alta concentración de hormonas inmunosupresoras en machos se invoca a menudo como la causa de que en general su actividad inmune esté reducida respecto a la de las hembras (Møller, Sorci y col. 1998; Roberts y col. 2004; pero véase Hasselquist, Marsh y col. 1999). Además, cuando son expuestos experimentalmente a peores condiciones (puestas ampliadas, limitación de alimento, aumento de la competencia), los machos sufren generalmente un mayor descenso de CMI (Fargallo y col. 2002; Chin y col. 2005; Dubiec y col. 2006). Los pollos macho de charrán común, por el contrario, mostraron aquí respuestas inmunes más fuertes que las hembras, especialmente en puestas de 3 huevos y en situaciones de competencia con hermanos durante el periodo final del desarrollo (Fig. 3 y Tabla 2, capítulo 3; pág. 94, 97).

Por tanto, cuando hay amplia disponibilidad de recursos debido a buenas condiciones ambientales o parentales - simuladas a través de la suplementación experimental de un recurso limitado en la naturaleza (Capítulo 4) y medidas a través del tamaño de puesta como indicador de calidad parental (Capítulo 3)–, los hijos parecen priorizar el desarrollo de características tales como tamaño corporal y CMI. Esto les permitiría alcanzar el ligero dimorfismo en tamaño que presentan en el momento de emancipación. También podría indicar mayor viabilidad y tasas superiores de supervivencia, ya que una CMI alta está relacionada positivamente con atributos individuales como la masa corporal (Alonso-Álvarez y Tella 2001), las probabilidades de supervivencia (González y col. 1999) y el estado nutricional (Lochmiller y col. 1993). Los pollos machos investigados en el estudio experimental de carotenoides, sin embargo, no mostraron respuestas inmunes más elevadas que las hembras, tal vez debido a un insuficiente poder estadístico (menor tamaño muestral), a interacciones con el metabolismo de carotenoides o a influencias del año (Fig. 3a, capítulo 4; pág. 113).

Si aparentemente los hijos tienen mejores perspectivas, ¿por qué su fenotipo y mortalidad son similares a los de las hijas? La falta de asociaciones entre el sexo, el fenotipo y la supervivencia de los pollos (Capítulos 3 y 4) podría significar, a pesar de los resultados anteriores, que hijos e hijas no difieren en sensibilidad ante las condiciones ambientales y de cría. Sin embargo, otra explicación sería que los pollos hembra poseen sus propias estrategias de crecimiento respecto a la asignación de recursos, que les permitirían equilibrar las situaciones de competencia y maximizar el acceso al cuidado parental. Para lograr un tamaño y una condición similares a las masculinas, las hembras podrían reducir la inversión en la función inmune en favor de otras características (Capítulos 3 y 4). Corroborando esta interpretación, los pollos hembra asignaron más recursos a la coloración de la piel (Fig. 2 y Fig. 4, capítulo 4; pág. 112, 114). Esta señal visual podría ser una ventaja potencial para la obtención de atención parental, aunque parece acarrear un pequeño coste en sistema inmune. Esta estrategia en el compromiso entre coloración y respuesta inmune, junto con la evidencia de que las hembras resisten mayores periodos de competencia (Tabla 4, capítulo 3; pág. 98), podrían constituir patrones óptimos si el subdesarrollo del sistema inmune en la etapa juvenil puede compensarse más adelante (Birkhead, F. Fletcher y col. 1999).

Aparte del ya mencionado leve SSD, evidencias previas de la especie señalan a los machos como el sexo más vulnerable en condiciones adversas o con padres de mala calidad y por lo tanto, el sexo más costoso de la descendencia. Los hijos sufren mayor mortalidad post-natal cuando son criados por padres jóvenes y ligeros (González-Solís, Becker, Wendeln y Wink 2005) o en ciertas posiciones de eclosión (K. L. Fletcher y Hamer 2004). Además, las puestas mixtas parecen ventajosas para los machos (Becker y Wink 2003) mientras que en puestas que contienen únicamente machos el segundo pollo muestra peores condiciones (Braasch, Palme y col. 2011). Los resultados de esta tesis respecto a las pautas que siguen los machos en los compromisos de crecimiento podrían implicar (1) que los machos se benefician más de las buenas condiciones, (2) que su valor reproductivo es más dependiente de la condición que el de las hembras y (3) que bajo ciertas condiciones, también pueden tener ventaja en las situaciones de competencia. A partir del conjunto de evidencias, sin embargo, no se puede concluir que hijos e hijas muestren grandes diferencias en los costos de producción/cría, al menos basadas en rasgos fenotípicos.

Al ser una especie semi-altricial, los pollos de charrán común dependen de la cantidad y calidad de cuidado parental que reciben. Por lo tanto, sería esperable que algún factor parental contribuya a un cierto dimorfismo fenotípico entre hijos e hijas. Los padres de calidad superior probablemente invierten más recursos en su descendencia que otros padres, y sus crías pueden responder diferencialmente según su sexo a esa mayor disponibilidad o calidad de recursos (Metcalfe y Monaghan 2001; Hegyi y col. 2011). Por otro lado, los padres pueden ajustar el nivel de recursos asignados a sus hijos e hijas si esto resulta en diferentes

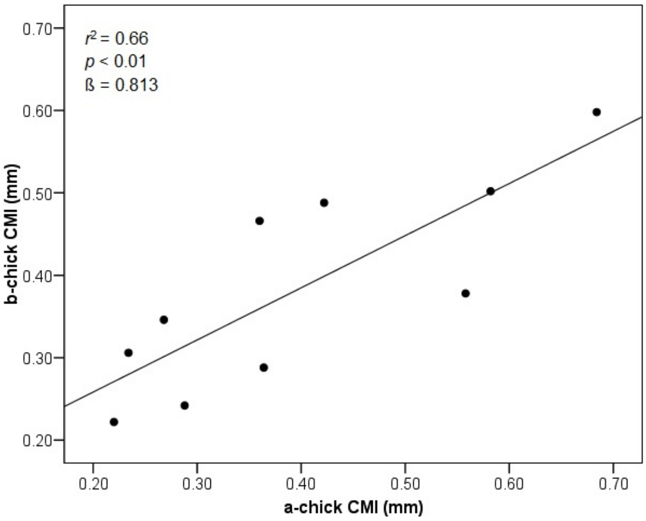


Figura 23: Valores del aumento absoluto en grosor de la membrana interdigital (mm) después del test de PHA, en pollos hermanos de charrán común ($n = 10$ nidos). a-chick: primer pollo eclosionado; b-chick: segundo pollo eclosionado.

valores reproductivos y así en diferentes beneficios para los padres (Frank 1990).

Sin embargo, factores parentales tales como la edad, fecha de llegada a la colonia y masa corporal no están claramente asociados con los rasgos fenotípicos ni con la mortalidad de los pollos, por lo menos diferencialmente para cada sexo (Fig. 2 y Tabla 3, capítulo 3; pág. 95, 96). Aun así, la respuesta inmune de los pollos macho se relacionó positivamente con la calidad de los padres –medida a través del tamaño de puesta; ver pág. 180– (Fig. 3, capítulo 3; pág. 97). Esta asociación fue especialmente notable en los hijos que compartieron nido con hermanos durante la etapa final de su desarrollo. Esto indica por un lado, la ventaja masculina en situaciones de fuerte competencia (a partir de los 14 días de edad, Klaassen y col. 1992) y por otro, la alta calidad de los padres que consiguen criar con éxito más de un pollo volantón (véase Apanius y Nisbet 2006). La varianza fenotípica en la respuesta inmune tiene un componente genético importante (Drobniak y col. 2010), como confirma la relación significativa encontrada entre la respuesta inmune de hermanos, en los pocos nidos donde se muestrearon dos pollos (Figura 23). Las condiciones de cría (tamaño de la nidada, presencia de hermanos), como reflejo de la calidad del cuidado o condición parental, desempeñan por tanto un papel crucial en el buen estado del sistema inmune (Hõrak y col. 1999).

En general, estos resultados sugieren que la inversión parental después del nacimiento no es marcadamente específica para cada sexo. La estrategia vital utilizada por las aves marinas podría consistir no en maximizar la descendencia criada en una temporada concreta, sino en llevar a cabo un gran número de intentos reproductivos que permitan criar más pollos (Forbes y Mock 2000). Otra posibilidad es que la inversión parental diferencial sí tenga lugar, pero se produzca con el fin de lograr un estado fenotípico similar para hijos e hijas; especialmente si, como sugieren algunos modelos de inversión parental, los padres están seleccionados para favorecer a la descendencia de un sexo concreto y las madres al otro

(Lessells 1998). Por último, las diferentes vías de desarrollo encontradas en los hijos e hijas de charrán común podrían reflejar patrones impuestos a los pollos por padres estratégicos, ya que hasta la emancipación, el acceso a recursos importantes depende exclusivamente del aprovisionamiento parental.

En resumen, los resultados de esta tesis ponen de relieve:

1. el potencial de los padres para influir en el desarrollo de la descendencia, a través del suministro diferencial de recursos especialmente importantes como los carotenoides
2. la influencia de la calidad parental en las diferencias sexuales, aunque pequeñas, de la descendencia.

Aunque estas influencias sugieren la existencia de un esfuerzo reproductivo diferencial por parte de los padres, y ciertas formas en que la asignación parental podría afectar diferencialmente a la descendencia, no está claro si esto refleja una diferencia en el valor reproductivo de hijos e hijas.

Patrones poblacionales e individuales de la razón de sexos

La teoría de la asignación por sexo hace predicciones sobre la razón de sexos numérica, pero también sobre su varianza estadística (véanse páginas 162 y 164). En organismos con CSD, lo esperable sería una varianza binomial, pero bajo ciertas circunstancias (p.ej., en situaciones de Trivers-Willard) la selección puede favorecer una sobredispersión (varianza mayor que la binomial) (Frank 1990). En consecuencia, la varianza del SR en la población es de interés porque puede indicar control parental de la asignación por sexo (Krackow 2002).

El análisis del ajuste de la razón de sexos sobre una gran muestra de nidos de charrán común (Capítulo 5) reveló que los patrones poblacionales (en la eclosión y emancipación; Fig. 1, capítulo 5, pág. 125; pág. 126) estaban ligeramente sesgados hacia las hembras pero no eran significativamente distintos de la paridad. Aunque más hijas que hijos murieron durante el desarrollo, no se encontró mortalidad diferencial significativa (Tabla 3, capítulo 5; pág. 129). Análogamente, la identidad de la madre no explicó la variación en la razón de sexos o en la mortalidad. Hasta el momento, estos resultados encajarían teóricamente con la situación de una especie casi monomórfica, con similares costes de producción y cría de hijos e hijas, y en la cual los mecanismos de mortalidad no muestran efectos de heterogamia o fenotipo masculino. Otros estudios en especies con leve SSD han encontrado resultados similares (Leech, Hartley y col. 2001; Fair y O. B. Myers 2002; Ramsay y col. 2003; Pagliani y col. 1999), también en el charrán (González-Solís, Becker, Wendeln y Wink 2005; K. L. Fletcher y Hamer 2004). Además, la falta de sobredispersión en el modelo de datos agregados a nivel de puesta podría indicar que la asignación por sexo en esta población es aleatoria, tal y como se esperaría de un muestreo puramente binomial. Todas estas evidencias sugieren que no existe una manipulación de la razón de sexos en esta población.

Sin embargo, de esos análisis sólo podemos inferir la dispersión de varianza para ese determinado nivel de agregación. Si los ajustes de la proporción de sexos, por ejemplo, ocurren dentro de la misma puesta y no entre ellas, no necesariamente aparecerá una sobredispersión. Además, los mecanismos de incremento de varianza parecen estar ampliamente generalizados (Krackow 2002), y sus efectos pueden operar en direcciones opuestas, por lo que la distribución resultante no diferiría de una binomial. Por lo tanto la ausencia de desviación en una muestra particular no puede considerarse una evidencia de la ausencia de factores que afectan la variación de la razón de sexos (Edwards 1960) o de la inexistencia de manipulación a nivel individual (Hasselquist y Kempenaers 2002). Por ejemplo, el carricero de Seychelles, siendo uno de los ejemplos más claros de asignación adaptativa, no muestra sesgos en la proporción de sexos de la población (Komdeur, Daan y col. 1997; Komdeur 1998). Algunos autores sugieren que los modelos de estrategias evolutivamente estables no hacen predicciones claras sobre la razón de sexos a nivel de población, incluso en casos de ajuste facultativo (Cockburn y col. 2002; Pen y Weissing 2000).

Los análisis a nivel individual en los charranes del lago Banter corroboraron parcialmente algunas de las hipótesis de asignación por sexo (en el sentido de Trivers-Willard) basadas en características de la estrategia vital de la especie. La proporción de machos en la eclosión se correlacionó con la masa del padre y mostró cierta influencia de la calidad ambiental del año (Tabla 1 y 2, Fig. 1 y 3b, capítulo 5; pág. 125, 126, 128), aunque no estuvo influida por la estacionalidad dentro de la temporada (Figura 24).

La influencia de estos dos factores era de hecho esperable. Los factores ambientales influyen fuertemente en el éxito reproductivo de las aves y también en la razón primaria de sexos (Suorsa y col. 2003; Merking y col. 2012; Pryke y Rollins 2012). Las pequeñas aves marinas como los charranes, con dieta especializada, áreas de forrajeo pequeñas y presupuesto energético ajustado son particularmente sensibles a cambios locales en el suministro de alimentos y a condiciones climáticas adversas (Furness y Tasker 2000). Por otra parte, la condición del charrán común, medida a través de la masa corporal, es una característica individual estable e independiente de la edad que tiene importantes efectos sobre la reproducción (Wendeln y Becker 1999; Wendeln, Becker y Wagener 1997). Por tanto la condición del macho adulto, independientemente de la condición o calidad de la hembra, parece ser un incentivo para asignar más recursos hacia los hijos.

Estos resultados parecen confirmar la hipótesis del atractivo y hasta cierto punto la hipótesis ambiental. Esto sería una buena estrategia de asignación si los hijos son realmente el sexo más costoso, si su valor reproductivo es más dependiente de la condición que el de las hijas (Addison y col. 2008; Zielinska y col. 2010) o si los hijos se benefician más de unas mejores condiciones de cría, como sugieren los resultados del análisis fenotípico.

Hasta el momento, las observaciones confirmaron una asignación diferencial por sexo en la dirección esperada. La asociación negativa entre el tamaño de puesta y la experiencia materna con el número de hijos en la eclosión, sin embargo, no concuerda con la predicción de una mayor asignación hacia machos con un aumento de calidad en los padres. Las madres con mayor experiencia reproductiva, que mayoritariamente ponen 3 huevos, produjeron un mayor número de hijas (Fig. 3a y Tabla 2, capítulo 5; pág. 128). Hay varias explicaciones posibles para esta observación. La primera de ellas sería la existencia de senectud reproductiva, pero en el

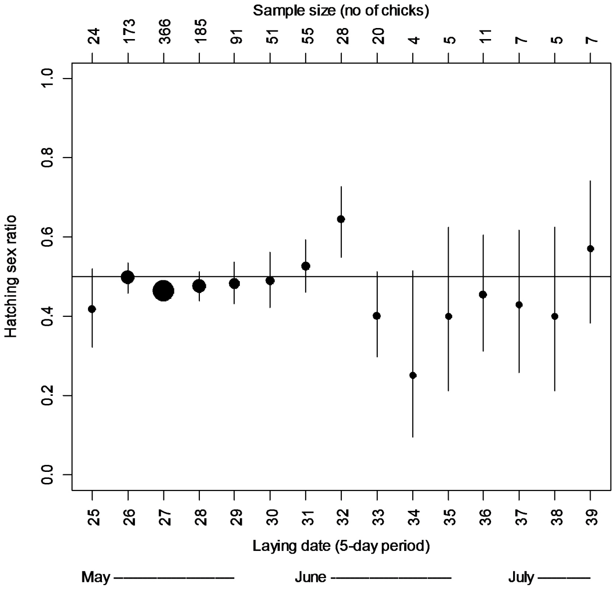


Figura 24: Razón de sexos en la eclosión según la fecha de puesta (en grupos de 5 días, 1 = 1 – 5 Enero; años 2002 – 2008). Medias y ES estimados de un GLMM con estructura de error binomial y enlace logit, incluyendo fecha de puesta como factor fijo e identidad de la madre y año como efectos aleatorios. Las estimaciones (media ± 1 ES) están enlazadas a la escala de probabilidad. Los tamaños de los puntos son proporcionales al tamaño muestral.

charrán común, especialmente en hembras, ésta se ha encontrado sólo después de un periodo de rendimiento creciente que dura hasta los 14 años de edad (Rebke y col. 2010). También pueden ocurrir cambios fisiológicos con la edad que alteren la frecuencia de gametos, pero falta apoyo empírico para esta idea (Martin y col. 1995). Este resultado puede ser también la consecuencia de un compromiso entre el número de crías, su sexo y sus probabilidades de supervivencia. Las madres experimentadas son también individuos de alta calidad, de más edad y con fechas de llegada tempranas, que ponen tres huevos y crían más pollos que sobreviven hasta la emancipación. Sin embargo, podrían hallarse cerca de su límite de capacidad y necesitar equilibrar sus recursos (Bauch y col. 2010) o podrían intentar evitar futura competencia, puesto que es más probable que las hembras exploren otras colonias y emigren (Dittmann, Zinsmeister y col. 2005; Becker, Ezard y col. 2008).

Por otra parte, una mayor producción de hijas podría proporcionar una buena inversión terminal comparada con una menor cantidad de pollos en conjunto, o con los hijos, si los beneficios biológicos provenientes de las hijas poseen mayor fiabilidad. En caso de que la calidad se transmita principalmente desde la madre (y no el padre) a la descendencia, no todos los nietos tendrían el mismo valor. Para una madre de alta calidad, una hija sería más valiosa que un hijo debido a que la calidad relativa de ésta sería más alta que la media, y posteriormente sería heredada por la descendencia de su hija (Leimar 1996). Por lo tanto, la producción de hijas de alta calidad podría implicar beneficios para la eficacia biológica que aún no están claramente establecidos.

Globalmente, los efectos de ajuste en la razón de sexos del charrán común siguen direcciones diferentes, y por tanto podrían anularse entre sí, siendo la razón del equilibrio encontrado en los patrones poblacionales del SR.

En este contexto, una cuestión importante es la varianza que existe en el éxito reproductivo de machos y hembras adultos de charrán, lo que hasta el momento se desconoce. Teóricamente, en la mayoría de las aves marinas no deberían existir conflictos sobre el cuidado parental porque los intereses de los sexos son similares, y los miembros de la pareja dependen mutuamente para criar a la descendencia (Birkhead y Parker 1997). En consecuencia, la paternidad extra-pareja es rara (González-Solís, Sokolov y col. 2001). Además, no existe evidencia de que la supervivencia de los adultos esté ligada al sexo y se asume que la razón operacional de sexos está equilibrada (Ezard y col. 2006; Becker y Zhang 2011). Esto puede significar que hay pocas razones para esperar diferencias en la varianza del éxito reproductivo entre machos y hembras. Sin embargo, la estabilidad intra-individual y las grandes diferencias inter-individuales en la condición corporal que presentan los charranes podrían resultar en diferencias en el éxito reproductivo a lo largo de la vida (LRS; véase Cuadro 1, pág. 158) y en la eficacia biológica (Wendeln y Becker 1999). Además, existe una correlación entre el comportamiento reproductivo y la edad o la experiencia, tal que el LRS puede verse afectado significativamente por la frecuencia de cambio de compañero y la edad o experiencia de estos compañeros (Ludwig 2010). Estos rasgos de calidad individual están sujetos a la selección sexual, que permite a los individuos obtener compañeros de alta calidad y la posibilidad de aumentar su éxito reproductor. De hecho, las hembras de charrán muestran un promedio de éxito reproductivo a lo largo de la vida ligeramente superior (aunque no significativo) al de los machos (datos no publicados).

Como se mencionó anteriormente, la ausencia de sobredispersión a nivel de puesta puede ocultar ajustes de la razón de sexos que estén ocurriendo dentro de la puesta, y no entre ellas. La manipulación del sexo según el orden de eclosión parece común en algunas especies (Husby y col. 2006; Dijkstra, Riedstra y col. 2010), y también en charranes (sesgo hacia hembras en pollos-a de charrán rosado, Szczys y col. 2001; y en huevos-c de charrán común, K. L. Fletcher y Hamer 2004), pero no se encontró aquí. Hay una aparente tendencia a sesgar el 3er huevo hacia hembras en años con peores condiciones (Fig. 2b, capítulo 5; pág. 127). Sin embargo, una inspección detallada de las interacciones del modelo ("Variación estacional en la razón de sexos", capítulo 5; pág. 126), mostró la falta de tendencias significativas entre la calidad de año y el orden de eclosión en nidos de tres huevos. De hecho, en promedio los pollos-c mostraron una razón de sexos similar (Figura 25a).

Para investigar la aparición de secuencias sesgadas de producción que puedan resultar en combinaciones sexuales favorables dentro del nido (si el primer huevo es macho, ¿tiene el segundo mayor probabilidad de ser macho u hembra? ¿y el tercero?) se realizó un estudio preliminar. Ni los nidos de dos huevos ni los de tres mostraron ningún indicio de este posible ajuste (datos no publicados). En cualquier caso, debemos recordar que no sabemos exactamente en qué momento del desarrollo se producen los sesgos en la razón de sexos. Aunque nuestros datos cubren una amplia gama de años (7) y nidos (389), una limitación

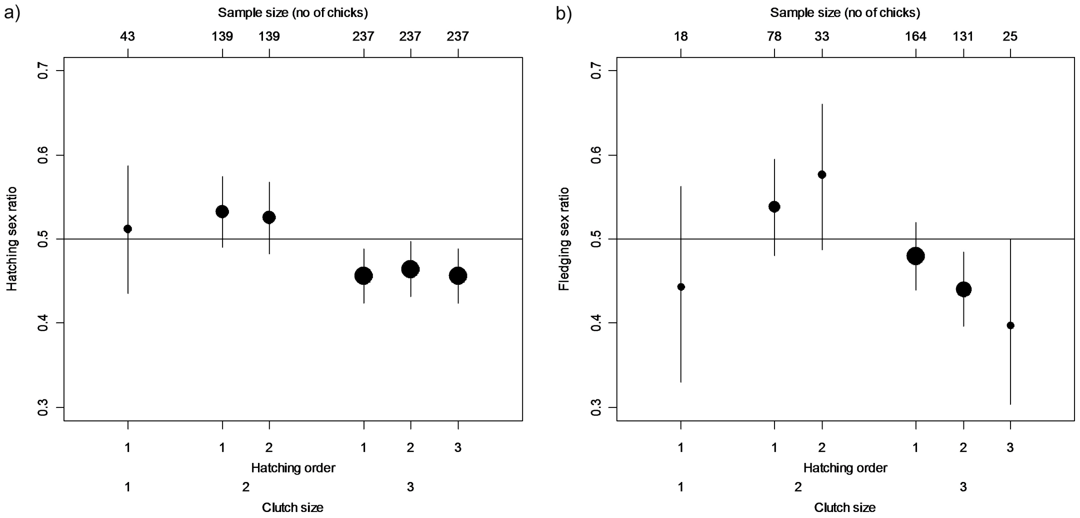


Figura 25: Razón de sexos a) en eclosión y b) en emancipación, en relación al tamaño de puesta y orden de eclosión. Medias y ES estimadas de un GLMM con estructura de error binomial y enlace logit, incluyendo orden de eclosión como factor fijo e identidad de la madre como efecto aleatorio. Las estimaciones (media ± 1 ES) están enlazadas a la escala de probabilidad.

del estudio es la falta de información sobre mortalidad pre-eclosión y el SR de embriones no eclosionados. Por tanto, las conclusiones planteadas aquí no se refieren tanto a la razón primaria de sexos "estricta" (en el momento de la fertilización) sino a la razón de sexos en el momento de eclosión.

Algunos estudios previos en el charrán encontraron un predominio de hembras entre los volantones y una mortalidad sesgada hacia los pollos macho (Szczys y col. 2001; K. L. Fletcher y Hamer 2004; González-Solís, Becker, Wendeln y Wink 2005; Becker, Ezard y col. 2008). Los datos presentados en esta tesis, que abarcan un periodo más largo y por tanto son más robustos contra relaciones temporales esporádicas, no confirman estos resultados. No obstante, el SR en la emancipación mostró algunos cambios respecto al de la eclosión (Figura 25), lo que sugiere una cierta mortalidad diferencial entre ambas etapas. De hecho, factores como el tamaño de puesta, el orden de eclosión y la estacionalidad desempeñan un papel crucial en la supervivencia de la descendencia (Becker y Finck 1985). Sin embargo, hijos e hijas se vieron igualmente afectados (Figura 26; Tabla 3, capítulo 5, pág. 129). Del mismo modo, la influencia de factores parentales sobre la mortalidad de los pollos no fue específica para cada sexo (Tabla 4, capítulo 5; pág. 129), aunque sería interesante indagar más sobre algunas tendencias (la fecha de llegada y la masa de la madre podrían afectar más a la supervivencia de los hijos).

El panorama general de esta tesis ofrece algunas conclusiones relevantes. Los mecanismos activos de los padres para sesgar la razón de sexos (p.ej., inversión diferencial durante el periodo de cuidado parental), bien relacionados con el dimorfismo en tamaño o con factores de calidad ambiental y parental, no parece que tengan lugar durante el periodo de

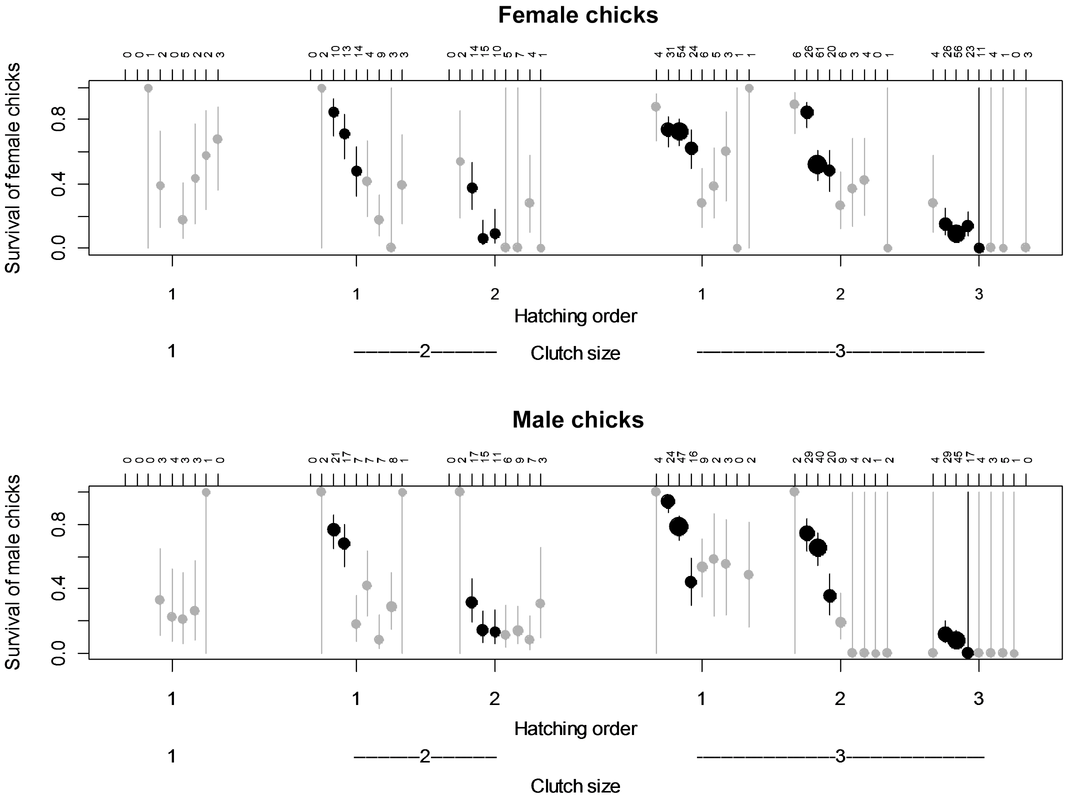


Figura 26: Supervivencia de machos y hembras en relación con el tamaño de puesta, orden de eclosión y fecha de puesta ($n = 1032$). Dentro de los bloques, la fecha de puesta (en grupos de 5 días) aparece de izquierda a derecha (algunas combinaciones no tienen muestra y por lo tanto no hay punto). Medias y ES estimadas de un GLMM con estructura de error binomial y enlace logit, incluyendo fecha de puesta como factor fijo e identidad de la madre y año como aleatorios. Las estimaciones (media ± 1 ES) están enlazas a la escala de probabilidad. Los tamaños de los puntos son proporcionales al tamaño muestral y las clases con tamaño muestral menor de 10 aparecen en gris para resaltar las estimaciones con muestras más grandes (en negro).

crecimiento. En cambio, el ajuste de la razón primaria de sexos aparece como la principal forma de manipulación, tal vez debido a la menor inversión energética necesaria para esta etapa. Este ajuste se realizaría en respuesta a diferentes componentes de asignación, que son influidos por SSD, características de la estrategia vital y factores de condición, y que pueden actuar en direcciones opuestas. La determinación cromosómica del sexo aún puede constituir un gran obstáculo, pero podría ser superado por selección consistente. Si las aves hembra son capaces de regular los mecanismos de determinación sexual, serían las principales responsables del control de factores clave en la selección natural y sexual. Sin embargo, es importante recordar que la mayoría de las teorías de asignación por sexo ha atribuido el control del ajuste de la razón de sexos únicamente a los padres. No obstante, es probable que los gametos y la propia descendencia también sean capaces de ejercer influencia (Trivers 1974; Parker 1985; Pen 2006), sin olvidar a los "distorsionadores de SR". Estos elementos hereditarios que modifican el SR para promover su propia transmisión (West 2009), son mucho más comunes de lo que se creía, y pueden ser el origen de modificaciones del sexo a nivel genético. Todas estas presiones simultáneas y potencialmente diferentes en la selección pueden conducir a un compromiso entre estrategias (Eshel y Sansone 1991) y así reducir los sesgos en la razón de sexos, ya sea a nivel poblacional o individual (Beukeboom y col. 2001; Seger y Stubblefield 2002; Komdeur y Pen 2002).

Conclusiones

Análisis inter-específico de la razón de sexos y vulnerabilidad

1 En aves, a nivel global inter-específico, la razón primaria de sexos (analizada hasta 2005) tiende a estar sesgada hacia los machos, mientras que la razón secundaria se acerca más a la paridad. La intensidad de estas tendencias depende del tipo de SSD.

2 Las razones de sexos primaria y secundaria están relacionadas con el grado de SSD, probablemente asociado con diferencias en los costes de producción o cría de hijos e hijas. Las especies dimórficas muestran una mayor proporción del sexo más pequeño, pero el efecto es particularmente notable en las especies con SSD sesgado hacia la hembra. Los padres ajustan la razón de sexos de la descendencia a través de una producción diferencial más que a través de una mortalidad diferencial, posiblemente por ser un mecanismo menos costoso. Estos resultados sugieren la existencia de cierto control parental de la razón primaria de sexos.

3 El grado de SSD está asociado con la vulnerabilidad diferencial de la descendencia, expresada a través de una mayor mortalidad y reducción de la masa en el sexo más grande. Los costes en supervivencia y viabilidad implicados en la consecución de un mayor tamaño son compatibles con las teorías de vulnerabilidad dependiente del tamaño. Sin embargo, deben combinarse con las teorías de vulnerabilidad dependiente del sexo, ya que crecer para ser más grande presenta desventajas sobre todo cuando va ligado al fenotipo masculino.

4 Algunas características de la estrategia vital, tales como el sistema de apareamiento y el tipo de cuidado parental parecen modular las relaciones entre SSD, razón de sexos y mortalidad diferencial. El dimorfismo sexual en plumaje, la edad de primera reproducción o la duración del período hasta la emancipación podrían también regular las proporciones sexuales y la mortalidad juvenil. Esto refleja la compleja matriz de factores que actúan en la evolución de la razón de sexos en aves y cuestiona la validez de la teoría de Fisher en este contexto.

5 Es recomendable que los análisis comparativos de SR en aves estén restringidos a estudios con determinación molecular del sexo en la descendencia (incluso en embriones no nacidos) y donde la mortalidad de ambos sexos entre el nacimiento y la emancipación sea estrictamente controlada en las mismas puestas.

Análisis intra-específico: El caso del charrán común

1. El dimorfismo sexual en pollos antes de la emancipación está presente en algunos rasgos fenotípicos, así como en ciertas estrategias de asignación de recursos durante el crecimiento. Estas estrategias incluyen diferencias sexuales en el uso de recursos limitados, en la función inmunológica y en la capacidad de competencia entre hermanos. Sin embargo, hijos e hijas son análogos en muchos otros rasgos y sufren tasas similares de mortalidad. Los compromisos durante el desarrollo pueden optimizarse de modo diferente pero ventajoso en ambos sexos, que podrían así alcanzar niveles comparables de condición y viabilidad al final del período de crecimiento.

2. Las diferencias sexuales encontradas en los pollos son sin embargo tenues, y podrían indicar que criar machos y hembras conlleva un coste similar y que después del nacimiento no existe una inversión diferencial por parte los padres. Sin embargo, los resultados de esta tesis ponen de relieve:
   - I El potencial que poseen los padres para influir en el desarrollo de la descendencia, a través del suministro diferencial de recursos especialmente importantes como los carotenoides
   - II La influencia de la calidad parental en las diferencias sexuales de la descendencia

   Estas influencias podrían implicar cierto esfuerzo reproductivo diferencial por parte de los padres, aunque no está claro que esto refleje una diferencia en el valor reproductivo de hijos e hijas.

3. Los patrones poblacionales en la colonia de charrán común del Banter See mostraron una razón de sexos equilibrada y ausencia de mortalidad diferencial. Esta situación se ajusta a una especie en la cual los costes de producción/cría de hijos e hijas son similares, consistentes con el ligero dimorfismo sexual, y donde los mecanismos de mortalidad no son dependientes del tamaño o de factores sexuales.

4. La hipótesis del macho atractivo y hasta cierto punto la hipótesis ambiental fueron corroboradas, ya que nacieron más hijos en parejas con machos más pesados y en años de buenas condiciones. Esto constituiría una buena estrategia de asignación por sexo si de hecho los hijos son el sexo más caro y su valor reproductivo depende más de la condición que el de las hijas. Sorprendentemente, la proporción de hijas aumentó con la experiencia y calidad maternal, tal vez como consecuencia de un compromiso entre el sexo y el número de la descendencia. Por otro lado, la producción de hijas de alta calidad podría conllevar beneficios para la eficacia biológica aún desconocidos.

5. La ausencia de relación entre mortalidad diferencial en la descendencia y factores parentales sugiere que el ajuste de la razón de sexos después del nacimiento (por ejemplo, a través de una alimentación selectiva) puede ser demasiado costoso en comparación con los sesgos a nivel de producción. Sin embargo, también podría indicar limitaciones de los padres a la hora de reconocer el sexo de su descendencia.

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Appendices

Appendix A Adult sex ratio and body masses database

			Body mass			
Species		**Adult SR**	Male	Female	Mean wt	**References (SR)**
Accipiter gentilis	European goshawk	0.75	712.0	1140.0	926.0	26
Accipiter nisus	European sparrowhawk	0.52	144.0	264.0	204.0	26
Aegolius funereus	Tengmalm's owl	0.60	101.0	167.0	134.0	19
Alectoris rufa	Red-legged partridge	0.55	516.0	439.0	477.5	31
Anas acuta	Pintail	0.74	856.0	693.0	774.5	17
Anas americana	Widgeon	0.58	792.0	719.0	755.5	17
Anas clypeata	Shoveller	0.50	636.0	590.0	613.0	17
Anas crecca	Green-winged teal	0.55	335.0	294.0	74.3	17
Anas discors	Blue-winged teal	0.59	353.0	315.0	334.0	17
Anas penelope	Eurasian wigeon	0.54	735.0	550.0	642.5	18
Anas platyrhynchos	Mallard	0.56	1142.0	1096.0	1119.0	17, 46
Aptenodytes patagonicus	King pinguin	0.56	16000.0	14300.0	15150.0	33
Archilochus colubris	Ruby-throated hummingbird	0.40	3.0	3.3	3.2	30
Aythya affinis	Lesser scaup	0.61	850.0	790.0	820.0	17
Aythya americana	Redhead	0.55	1100.0	990.0	1045.0	17
Aythya collaris	Ring-necked duck	0.50	730.0	680.0	52.6	17
Aythya valisineria	Canvasback	0.60	1248.0	1190.0	1219.0	17
Bucephala albeola	Bufflehead	0.67	433.0	313.0	373.0	17
Calidris minutilla	Least sandpiper	0.37	18.9	20.2	19.6	8
Catharus guttatus	Hermit thrust	0.75	32.1	28.6	30.4	40
Circus cyaneus	Northern harrier	0.38	346.0	527.0	436.5	26
Coturnix coturnix	European quail	0.72	103.5	105.1	104.3	35
Ephippiorhynchus senegalensis	Saddle-billed stork	0.47	6378.0	5947.0	6162.5	24
Erithacus rubecula	European robin	0.18	16.9	15.8	16.4	9
Falco tinnunculus	European kestrel	0.55	213.0	252.0	232.5	26
Ficedula albicollis	Collared flycatcher	0.49	12.9	12.5	12.7	14
Fregata minor	Great frigatebird	0.71	927.0	1183.0	1055.0	13

Species		Adult SR	Male mass	Female mass	Mean wt	References (SR)
Geopelia striata	Barred dove	0.57	56.0	56.0	56.0	38
Larus argentatus	Herring gull	0.48	1177.0	944.0	1060.5	7
Larus occidentalis	Western gull	0.27	1136.0	879.0	1011.0	22, 36
Malurus leucopterus	White-winged fairy-wren	0.45	7.0	6.6	6.1	34
Manorina melanocephala	Noisy miner	0.70	68.0	68.0	68.0	5
Manorina melanophrys	Bell miner	0.64	32.5	30.3	31.4	10
Manorina melanotis	Black-eared miner	0.65	52.5	47.6	50.1	16
Mimus polyglottos	Northern mockingbird	0.57	48.5	48.5	48.5	6
Molothrus ater	Brown-headed cowbird	0.67	48.9	38.8	43.9	45, 47
Molothrus bonariensis	Shiny cowbird	0.53	38.7	31.9	35.3	28
Neotis denhami	Stanley’s bustard	0.41	6350.0	3200.0	4775.0	1
Otis tarda	Great bustard	0.38	8883.0	4421.0	6652.0	2, 3
Oxyura jamaicensis	Ruddy duck	0.77	610.0	510.0	560.0	17
Parus major	Great tit	0.68	19.1	17.8	18.5	27
Passer domesticus	House sparrow	0.53	30.2	30.2	30.2	12
Petroica australis	New Zealand robin	0.53	29.5	29.5	29.5	4
Phalacrocorax carbo	Cormorant	0.40	2423.0	2085.0	2254.0	44
Phylloscopus trochilus	Willow warbler	0.38	8.9	8.8	8.9	32
Polemaetus bellicosus	Martial eagle	0.45	3174.2	4951.7	4230.0	24
Regulus calendula	Ruby-crowned kinglet	0.32	6.9	6.4	6.7	21
Sitta europaea	Nuthatch	0.66	24.3	23.5	23.9	27
Sterna dougallii	Roseate tern	0.44	110.0	110.0	110.0	41
Strigops habroptilus	Kakapo	0.77	2060.0	1280.0	1670.0	11, 43
Sula leucogaster	Brown booby	0.42	982.0	1233.0	1110.0	42
Tetrao tetrix	Black grouse	0.53	1290.0	988.0	1139.0	20
Tetrao urogallus	Capercaillie	0.47	4240.0	1985.0	3112.5	20, 23
Vidua macroura	Pin-tailed whydah	0.46	14.4	14.4	14.4	39
Zenaida macroura	Mourning dove	0.57	123.0	115.0	119.0	37
Zonotrichia leucophrys	White-crowned sparrow	0.58	30.7	27.1	30.0	15, 25, 29

Appendix B References for the adult sex ratio database

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