Manipulation of Primary Sex-Ratio: an Updated Review

Carlos Alonso-Alvarez
Unidad de Ecología, Instituto de Investigación en Recursos Cinegéticos (IREC-CSIC-UCLM-JCCM). Ronda de Toledo, s/n. 13005 Ciudad Real, Spain

ABSTRACT

Some bird species would be able to manipulate primary sex ratio, i.e. the proportion of male and female offspring at the laying time. Such trait could be considered being mostly under maternal control. Nevertheless, the exact mechanism involved remains undetermined. Knowledge of the mechanism would contribute to any assessment of its cost in terms of resources and/or time, which would be important to formulate predictions of those scenarios where it could have evolved. In fact, small costs of sex ratio control could overcome the adaptive value of adjusting the proportion of sexes in the progeny. Pike and Petrie (2003) published an excellent review on the potential mechanism involved. However, many new experimental and correlational evidences have appeared from that date, providing new and interesting perspectives. Thus, hormonal control is obtaining strong support from recent findings. The present review updates the current knowledge on this subject with emphasis on the question of whether sex ratio manipulation is a widespread trait among avian taxa. Finally, future directions for research, including not only those related to the mechanism per se, but also those linked to conditions necessary for the evolution of this trait, are presented. Thus, the necessity of determining fitness functions of male and female offspring and both parents, as well as the problem of if this trait is the result of a evolutionary constraint or strategy, are also addressed.

Keywords: birds, primary sex-ratio, proximate mechanisms, segregation distortion, sex allocation, sex-specific embryo mortality

1. INTRODUCTION

The possibility of an active maternal manipulation of sex ratio at the time of laying (the primary sex ratio) has attracted the attention of poultry researchers (e.g. Batellier et al., 2004), but mainly engaged the interest of evolutionary biologists (e.g. Hardy, 2002) due to its implications in the context of life history theory (Stearns, 1992). In fact, this is one of the few areas where we can hope a reasonable good fit between empirical data and the predictions of simple theoretical models (West et al., 2002). A recurrent problem has been the almost absolute ignorance about the proximate mechanisms involved. The knowledge of such mechanisms should contribute to understand the trade-off between costs and benefits faced by females when chose the sex of their offspring, thus allowing us to predict those scenarios where such adaptation could have evolved (Sheldon et al., 1998; Komdeur and Pen, 2002). Standard sex allocation models (the classic sex allocation theory: Charnov, 1982) assume that sex ratio manipulation is without cost to the individual in control. Although this could be reasonable for haplo-diploid species (e.g. insects), in species with chromosomal sex determination parents could need to selectively kill embryos or reabsorb them at some point during development, which would imply a loss of resources and/or time. When some cost is included in new analyses, the expected sex ratio is less biased than predicted by standard models (Pen, 2000; Komdeur and Pen, 2002). In fact, small costs could have important effects. Pen et al. (1999) showed that in European kestrels (Falco tinnunculus) a delay in the laying sequence caused by discarding and replacing only one egg of the undesired sex is sufficient to cancel out any advantage of manipulating the sex ratio. Therefore, the knowledge of the mechanism of primary sex ratio manipulation would contribute to

*To whom correspondence should be addressed: E-mail: carlos.alonso@uclm.es
adjust the real world to the theory (Hasselquist and Kempenaers, 2002; Komdeur and Pen, 2002).

The aim of the present review is to update and summarise current knowledge on primary sex-ratio manipulation in avian species. In 2003, Thomas Pike and Marion Petrie published an excellent review on the potential mechanisms involved including a bibliography up to around the middle of 2002 (Pike and Petrie, 2003). However, as molecular sexing techniques allowing sexing at early ages (Griffiths et al., 1998; Fridolfsson and Ellegren, 1999) have become very popular, studies on this subject have increased exponentially, revealing many significant evidences of primary sex ratio allocation in birds.

In this review, I will first describe the potential mechanisms involved in primary sex-ratio allocation, updating past knowledge by adding new references and perspectives to those provided by Pike and Petrie (2003). Second, I will summarise new correlational and experimental studies showing facultative primary sex ratio allocation among birds. Third, I will address the hypothesis that primary sex ratio manipulation is a widespread trait in birds. Finally, I will provide several ideas about the direction of future research.

2. POTENTIAL MECHANISMS OF PRIMARY SEX RATIO MANIPULATION IN BIRDS

The potential mechanisms involved in facultative sex ratio allocation can be presented following a temporal dimension, from follicular formation to egg laying, i.e. the time period when maternal control of primary sex ratio should have to occur. Moreover, as shown by Pike and Petrie (2003), I will also discuss new findings about sex-specific embryo mortality, though these could be also considered as related to secondary sex ratio (i.e. sex ratio at fledging age).

2.1 Follicular development

In birds, yolk-filled follicles enter in a period of rapid yolk development (RYD) 4–16 days before ovulation (Johnson, 1986, 2000). The sex of each oocyte is determined only 0.5–4 h before ovulation, during meiosis I (Olsen and Fraps, 1950; Romanoff, 1960; Johnson, 1996), although Ankney (1982) suggested that it could be earlier. Ovulation of each oocyte is produced with a 24–28 h interval in poultry (Johnson, 1986) although this can vary among bird species: 20–30 h in most passerines (Burley and Vadehra, 1989) and 40–44 h in domestic pigeons (Johnson, 1986; Pike, 2005). Pike and Petrie (2003) proposed two potential mechanisms acting during rapid follicular development: differential developing rate of follicles and follicular atresia.

2.1.1 Differential developing rate of follicles

In the first case, Pike and Petrie (2003) suggested that RYD could differ between those follicles that would finally lead to male and female gametes (following Ankney, 1982). If follicular growth rate is regulated by hormones (i.e. follicle stimulating hormone or FSH; Johnson, 2000), and these hormones show a decrease during season (e.g. Silverin et al., 1997), this could explain why in some species sex ratio changes throughout the season (e.g. Krebs et al., 2002; Andersson et al., 2003). A similar effect could apply to within clutch sex ratio, as described in other species (e.g. Rutstein et al., 2004; Lezalova et al., 2005). Thus, in large-clutch species the slower-growing sex could appear at the end of the sequence because latter eggs are produced when the level of circulating hormones has already dropped.

Badyaev et al. (2005) were able to demonstrate that pre-ovulation oocytes producing males or females can differ in their growth rates. Using house finches (Carpodacus mexicanus), they analysed the temporal pattern of yolk deposition by the measurement of the lipid layers present in the yolk (see description in Young and Badyaev, 2004) and also carrying out early embryo sexing (but see Arnold et al., 2003a for limits of early sexing techniques). Their results also challenged the traditional view that a strict hierarchical order of oocyte sequestration into the RYD phase exists, and that such order should be maintained at the time of ovulation because growth rate would not differ among oocytes (Figure 1A; King, 1973). Thus, it was suggested that differences in follicle growth rate could lead to changes in the ovulation pattern (Badyaev et al., 2005) and the original order of oocyte sequestration into RYD would not be respected at the time of ovulation (Figure 1B). This would allow a flexible control of sex ratio in relation to the laying sequence.

The question arises about how mothers can distinguish between male and female oocytes at pre-meiotic stages and so induce differential growth. Unfortunately, there is no clue about how such a mechanism could act. However, we could pose the problem in other terms:
how does the rate of RYD subsequently influence sex determination? Badyaev et al. (2005) found that female androgen and prolactin circulating levels during RYD correlated with offspring sex within the clutch: higher androgen and lower prolactin concentrations were present when the oocyte became a male. Since variation in follicle growth rate would mean different degrees of exposure to circulating hormones, male and female follicles with different growth rates would also accumulate varying concentrations of these substances in the yolk. As female plasma prolactin and androgen levels change throughout the laying period, male and female follicles would have been developed in different hormonal milieus (Badyaev et al., 2005, 2006a).

In summary, the different amount of hormones in pre-meiotic oocytes as consequence of different RYD periods could explain within-clutch sex-ratio bias. Interestingly, in peafowls (Pavo cristatus), domestic hens (Gallus gallus domesticus) and zebra finches (Taeniopygia guttata), male and female eggs seem to differ in their content of maternal androgens (Petrie et al., 2001; Müller et al., 2002; Rutstein et al., 2005a; but see also Eising et al., 2003; Pilz et al., 2005). The potential mechanism through which these hormones could affect sex determination is discussed further in Sections 2.2.1, 5.1 and 5.3.

2.1.2 Selective atresia

Atresia is the degeneration and resorption of one or more ovarian follicles before a state of maturity has been reached (e.g. Johnson, 2003). Pike and Petrie (2003) suggested that sex-specific atresia could act among follicles in pre-hierarchical phase (also Gilbert et al., 1983). Interestingly, atresia in birds is rarely undergone during hierarchical stage unless induced by a strong physiological stressor, e.g. fasting or water deprivation (Johnson, 2003; but see Beukeboom, 1988). If atresia is mainly present during pre-hierarchical stages, a sex-manipulation mechanism based on such process would save energy and avoid the costs associated to laying gaps, for example longer laying sequences could mean higher risk of predation (see Section 2.3).

The process of follicular atresia during the hierarchical stage seems to be controlled by hormones. Atresia can be experimentally induced by means of LH and progesterone injections during this period (Johnson and Leone, 1985 and Yoshimura et al., 1993, respectively). On the other hand, some substances such as growth hormone (GH) and growth factor IGF-1 seem to attenuate the process (Johnson, 2003). Interestingly, the change from pre-hierarchical to hierarchical stages (RYD phase) is also related to increases in hormone levels (i.e. FSH; Palmer and Bahr, 1992; Johnson, 2000). However, as in the case of differential RYD, the question about how females can identify the future sex of each follicle to induce selective atresia remains without an answer.

2.2 Segregation distortion

The most efficient mechanism, in terms of costs/benefits of sex ratio manipulation (Oddie, 1998; Komdeur et al., 2002), should be the selection of sex...
chromosomes at the first meiotic division. This would avoid the loss of energy and/or time associated with other mechanisms. Segregation distortion has been described in some insects (review in Hardy, 2002), but not in birds where the process would face at least two problems: meiotic constraints and the theoretical “parent–gamete conflict”.

2.2.1 Meiotic constraints

In birds, the female is the heterogametic sex producing gametes with either Z or W chromosomes (afterwards ZZ males or ZW females). The sex of the gamete is determined at meiosis I (Figure 2). In anaphase I, one centrosome is placed on a cell membrane protrusion, which would mean that it will form the first polar body (Swanson et al., 1981). Simultaneously, the other centrosome would be included in the secondary oocyte, which would produce the ovum (Figure 2). A hypothetical mechanism for gender manipulation could be that the chromosome of preferred sex could be actively assigned to the ovum and the rejected one would be sent to the polar body. However, Krakow (1999) refutes this because the centrosomes would be assigned to ovum or polar body before chromosome separation (Figure 2), which would be an absolutely random process. (citing Nicklas, 1997). He speculates on the possibility that centrosomes could move between poles just before separation of chromosomes, but he discards this possibility due to the potential risk of “non-disjunct haploid nuclei” (Krackow, 1999).

Following his arguments, the potential influence of yolk hormones on segregation distortion is also discarded because it would only constitute a mechanism regulating the correct timing of these meiotic processes, but not sex adjustment (citing Chandra, 1991).

Chandra (1991) suggested that yolk content may have facilitated the evolution of female heterogamety and the absence of “dosage compensation” in birds. In mammals, for instance, females inactivate one of their two X chromosomes so as to balance gene dosage with that of males. Male birds (ZZ), however, would carry a double dose of sex-linked genes relative to ZW females (Cock, 1964; Baverstock et al., 1982; Chandra, 1991). Therefore, sex-specific amounts of hormones in the yolk could attenuate these differences. Nevertheless, recent evidence suggests that dosage compensation is also present in avian species (McQueen et al., 2001; Ellegren, 2002; Nakagawa, 2004), which rather weakens the argument.

It must be realised that the biochemical parameters regulating chromosome segregation in meiosis are still poorly known (Petronczki et al., 2003). Nicklas (1997) describes how in some cases the attachment of both partner chromosomes to the same pole (a monopolar attachment error) is subsequently rectified. Microtubule motor proteins are able to regulate the link between each microtubule and the pole (Figure 2) and would be involved in such a rectification process (Endow, 1999). Although Nicklas (1997) emphasises the apparent random basis of these processes, the possibility of active sex-chromosome discrimination, and subsequent allocation of each chromosome to polar body or ovum, cannot be excluded. In summary, it is possible that some subtle mechanism is being overlooked.

Recent correlational and experimental findings are suggesting that yolk and circulating hormones could lead to segregation distortion. Yolk of male eggs can contain higher androgen levels than yolk of female eggs (Petrie et al., 2001; Müller et al., 2002; Rutstein et al., 2005a; but see Eising et al., 2003; Pilz et al., 2005). Pike and Petrie (2005a) show that female peafowls paired with males whose attractiveness was experimentally reduced produced more female embryos, had significantly higher yolk corticosterone, and tended to have lower yolk testosterone levels. In a similar study in peafowls, poor maternal condition, high plasma corticosterone and low plasma testosterone levels of peahens were associated with a female-biased primary sex ratio (Pike and Petrie, 2005a).

Fig. 2 Anaphase I of meiosis. Microtubules shorten, sister chromosomes are pulled apart by contracting fibres moving to opposite directions. Krakow (1999) suggests that centrosomes would be assigned to ovum or polar body before chromosome separation, which would be a random process.
2005b). Meanwhile, Veiga et al. (2004) showed that female spotless starlings (Sturnus unicolor), which received testosterone implants during egg formation, produced higher proportion of sons in their clutches. Similarly, female zebra finches injected with testosterone during egg laying tended to produce clutches with sex ratios skewed towards males (Rutkowska and Cichón, 2006). By contrast, von Engelhardt et al. (2004) showed no effect of 17-β oestradiol injections on primary sex ratio in the same species. In a more complete experimental design, Pike and Petrie (2006) manipulated circulating levels of testosterone, 17-β oestradiol and corticosterone in female Japanese quails (Coturnix coturnix japonica) by means of implants during the laying period. Corticosterone was the only hormone able to bias the primary sex ratio with corticosterone-treated females showing a higher proportion of females in their clutches. Finally, pharmacological doses of progesterone injected into female domestic chickens also induced a female biased primary sex-ratio (Correa et al., 2005). Correa et al. (2005) suggested that progesterone would be the best candidate to be involved in segregation distortion because follicular steroid production during meiosis I is mostly restricted to this hormone (Etches and Duke, 1984; see also Krackow, 1995). In summary, the available evidence seems to contradict the apparent constraints of meiosis in producing segregation distortion mechanisms.

2.2.2 Parent-gametic conflict

Reiss (1987) proposed that any mechanism involving suicidal activities of the unwanted sex chromosome should be selected against. After segregation of both haploid chromosome sets during meiosis I, some maternal signal should induce the unwanted haploid set to enter the first polar body. This implies that a gene on an autosome would have to determine the sex chromosome related to itself, promoting its suicide if the signal determines that it is from the wrong sex (Krackow, 2002). However, this would be only possible if the fitness of one sex is at least three times the fitness of the other sex. How could this be?

We could imagine that the unwanted sex is the male, and assume that there is an autosomal allele “A” which would act following parental necessities, whereas allele “a” does not. There would be four possible haplotypes: ZA, Za, WA, Wa, where ZA would eliminate itself. In such scenario, the A-allele would be transferred to a third of all zygotes and its expected fitness would be f/3, where f represents female fitness and m would be the male fitness; Krackow, 2002). However, if A does not react in agreement with maternal needs, its fitness would be: m/4 + f/4. Therefore, the allele would be spread in the population only when f/3 > (m/4 + f/4), i.e. f > 3m. Therefore, evolution of such an allele would be rare. Moreover, the demonstration of this argument requires the assessment of female and male fitness functions in the studied species, which is rarely addressed in sex ratio literature (see Section 5.5). Probably, the only example adjusted to these requirements would be the case of Seychelles warblers (Acrocephalus seychellensis; Komdeur et al., 1997, 2002). To conclude, the parent-gametic conflict could also apply to other stages of egg production (see below).

2.3 Selective re-absorption and ovulation

The short period between sex determination and ovulation would allow mothers to abort and subsequently reabsorb the post-meiotic ova with the unwanted sex-chromosome (proposed by Emlen, 1997). This mechanism would allow females to reabsorb ova until an ovum of the appropriate sex is produced. Emlen (1997) suggested that the abortion and re-absorption of a follicle would imply an approximate 24 h gap in the laying sequence due to the fact that the most mature follicle secretes hormone “inhibin”, which hampers the development and ovulation of other follicles (Chen and Johnson, 1996; Lovell et al., 2003). Hence, selective re-absorption should be mainly present in those species with small clutch sizes, which would reduce the costs inherent to delayed laying periods. Such costs would be a higher risk of predation and/or increased hatching asynchrony, which could affect chick survival (Emlen, 1997). Furthermore, it could suppose a cost in terms of energy. In fact, a developing follicle receives about 2 g of yolk proteins daily (Johnson, 2000). However, at least some part of this energy would be recycled by re-absorption. Unfortunately, the energetic efficiency of this process is unknown.

Nonetheless, the mechanism could have evolved only with the first egg of the laying sequence. This could avoid the cited costs (Arnold et al., 2001; Blanco et al., 2002; Neuhäuser, 2003). However, skewed sex ratio in subsequent eggs has been also reported (Badyaev et al., 2002; Rutstein et al., 2004).
Pike (2005) challenged Emlen (1997)’s hypothesis by proposing an alternative mechanism acting at least on second eggs. He suggested that the first egg could follow the absorption/ovulation pattern until the ova of the wanted sex arrived. Afterwards, rather than leave the sex of the second egg to chance, if the next follicle in the hierarchy is of the required gender then it would be ovulated as normal, but if it is not then it will be aborted and the next follicle, regardless of sex, ovulated in its place. Such a “selective abortion” model does not imply a gap in the laying sequence. Instead, the follicle placed at the following position to the aborted one could be immediately ovulated, although at the cost of a reduction in its yolk volume (see Figure 3). Such model was proposed as a post-hoc explanation perfectly adjusted to his findings in domestic pigeons (Pike, 2005), where the first egg coincided at 100% with the predicted sex, and the second one only met that in 75% of cases. The model could not apply to following eggs since a higher lost of egg volume would risk embryo viability. However, it could also explain sex-ratio bias in the last egg, especially in those species where the last egg is clearly smaller (Genovart et al., 2003; Fletcher and Hamer, 2004).

Alternatively, when the overlap among the slopes of RYD curves is large (i.e. during late growth phase, Figure 1A; Badyaev et al., 2005), sex-specific ovulation could be allowed, which would avoid the costs of re-absorption and gaps. In this sense, the fact that inhibit mostly acts on neighbouring follicles (Yang et al., 2001; Johnson et al., 2005) might allow distant ones to quickly ovulate (see “clustered follicles” in Badyaev et al., 2006b).

Finally, selective abortion could also allow sex ratio manipulation at this stage. This mechanism would be based on the rejection of ovulated ova out of the infundibulum and into the abdominal cavity (i.e. “internal ovulation”; Johnson, 2000). This leads to the production of phantom post-ovulatory follicles, also known as “un-reconciled follicles” (Robinson et al., 2003). These are detected when the number of eggs laid does not correspond to the number of post-ovulatory follicles in the ovary. In some cases, ova could be reabsorbed, but in others, it could cause peritonitis, which can be fatal (Johnson, 2000). However, in spite of the apparent risk of peritonitis, the presence of un-reconciled follicles seems to be high in poultry species with records of about 3.4 per domestic hen (Robinson et al., 1998) and about 1.6 per female turkey (Melnychuk et al., 1999), both determined on the day of the first oviposition. In fact, the phenomenon is more frequent when the infundibulum is not ready to “catch” the follicles, typically at the beginning of ovulation sequence (Renema et al., 1995; Robinson et al., 2003). Therefore, this mechanism could also explain those examples of sex ratio bias at the first egg (see above) and only ovarian examination would allow us to discard this possibility.

2.4 Sex-specific fertilisation

The ovum remains in the infundibulum during a short time period (15–30 min; Johnson, 2000) during which it is fertilised. Spermatozoa are stored and regularly released from sperm-storage tubules (SST) located in the utero-vaginal junction (Birkhead and Møller, 1992). In these tubules, stored spermatozoa can remain viable from 6 to 45 days, depending on the species (Birkhead and Møller, 1992). Sperm are released from the SST following oviposition of each egg (Zavaleta and Ogasawara, 1987; Bakst, 1994), but the exact mechanism and timing involved in this release is barely known although there are suggestions of estrogenic regulation (Yoshimura et al., 2000) or neuronal-control (Freedman et al., 2001). Therefore, if spermatozoa are selectively released from SST, females could regulate fertilisation probabilities of male and female oocytes. Pike and Petrie (2003) discarded such a possibility because sperm are released from SST before the time of ovulation and sex determination (Birkhead, 1995). Nevertheless, in some species the amount of sperm in SST drops dramatically after a single insemination event.
(Birkhead and Fletcher, 1995), and repeated copulation may be required to ensure successful fertilisation (Török et al., 2003). In such cases, sperm from the latest copulation could directly fertilise the last ovulated follicle, allowing females to avoid copulation when the oocyte is of non-desired sex.

Alternatively, the female could limit the motility of any released sperm by altering oviducal fluid and/or the composition of the fluid composition located between the ovary and the infundibulum. Thus, changes in calcium content, pH and viscosity of these fluids, as well as changes in body temperature could selectively limit sperm fecundity when the ovum is not from the expected sex (Ashizawa et al., 2000).

Pike and Petrie (2003) suggested several additional mechanisms: (1) female changing the membrane ova composition, thus preventing spermatozoids to penetrate it; (2) maternal inhibition of zygote development after sperm penetration into the oocyte; and (3) spermatozoids being able to detect the sex of ova and selectively fertilise them (parental control in this case). In any case, it must be considered that, in addition to possible laying gaps, an unfertile egg can constitute an important cost in terms of energy because it would be difficult to reabsorb and it could be laid and incubated. Therefore, an imbalance between costs and benefits could have prevented the evolution of any mechanism based on sex-specific fertilisation.

### 2.5 Sex-specific embryo mortality

Females could manipulate sex-ratio by hatching time by differential embryo mortality. Although it could be considered as secondary sex ratio, the potential mechanisms would be acting throughout follicle and egg formation. Pike and Petrie (2003) did not find studies showing natural differences in embryo mortality between sexes but several examples have recently appeared. Thus, higher mortality rates of male embryos from three different passerine species have been described (Cichón et al., 2005). Similarly, in the megapode brush turkey (Alectura lathami), environmental temperature influences sex-specific embryo mortality in a quadratic fashion: more females died at 31°C, more males died at 36°C, and the ratio almost 1:1 at 34°C (Goeth and Booth, 2005).

The absolute cost of this sex-ratio manipulation should be large because materials invested in the eggs will be lost. However, the relative cost would depend on the number of eggs laid. Thus, it could be expected that this mechanism would evolve in birds with large clutch sizes. The cited species could agree with this requirement since their clutches ranges about 6–15 eggs in the case of passerines and 18–20 eggs in the case of brush turkeys.

How females could manipulate sex-specific embryo survival? In the case of brush turkey it would be obvious that females directly manipulate temperature at the nest site. Additionally, female could also perhaps alter egg composition: (1) depending on the sex of each embryo or, (2) irrespective of egg sex.

#### 2.5.1 Sex-specific manipulation of egg composition

A sex-specific maternal manipulation of egg composition could lead to differential survival rates of male and female embryos. In yolk, females can differentially allocate substances such lipids, antioxidants, immunoglobulins and hormones (Royle et al., 1999, 2003; Blount et al., 2002; Gasparini et al., 2002) on a sex-specific basis. Since the bulk of the yolk is produced before meiosis, those considerations referred to sex-specific developing rate of follicles (Section 1.1.1) can also apply here. There are also those cases where sex-specific allocation of hormones at the yolk has been reported (Petrie et al., 2001; Müller et al., 2002; Rutstein et al., 2005a; but see Eising et al., 2003 and Pilz et al., 2005). After ovulation, female would be only able to manipulate composition and/or quantity of albumen and shell (Mead et al., 1987; Johnson, 2000), although a selective re-absorption of part of the yolk cannot be excluded. In this sense, recent findings show that the relative contribution of yolk and albumen amounts depends to a certain degree on the sex of the preceding oocyte (Badyaev et al., 2006b).

In any case, the sex-specific manipulation of embryo survival probabilities would depend on the capacity of the female to recognise the sex of each gamete from the moment of sex determination (meiosis I). However, females would face again the theoretical limitations inherent to the parent-gamete conflict. Moreover, there is no evidence as yet of maternal recognition of embryo sex in any bird species. In mammals, it has been proposed that some differences between male (Y) and female (X) spermatozoa, for instance electrical surface charge, size, mass/density, cell membrane receptors, and some substances (Cohen, 1975; Pike and Petrie, 2003), could be used by females to actively manipulate fertilisation, inducing sex-ratio bias. Similarly, the study of sex-specific differences in avian female
gametes could provide some clues on the mechanism of maternal recognition.

2.5.2 Non-sex-specific manipulation of egg composition

Females could manipulate egg composition by investing equally in all the eggs, with independence from each sex. Any substance added to egg would interact with male and female embryo characteristics, resulting in differential mortality. Recent experiments suggest that increased levels of maternal hormones differentially affect embryo survival. Injection of laying females with corticosterone (Love et al., 2005; see also Hayward and Wingfield, 2004) and 17-beta-estradiol (von Engelhardt et al., 2004) induced increased mortality of male but not female embryos. Similarly, Rutstein et al. (2005b) found that female zebra finches paired with males with experimentally increased attractiveness produced eggs with higher mortality of male embryos, perhaps as consequence of increased allocation of maternal testosterone to the yolk (Gil et al., 1999). In fact, female zebra finches injected with testosterone produced male eggs with lower hatching success than female eggs (Rutkowska and Cichon, 2006). By contrast, injecting yellow legged-gull (Larus cachinnans) eggs with testosterone resulted in a reduction of female embryonic survival (Rubolini et al., 2006). In any case, we do not know if it was the result of similar amounts of hormones at the yolk interacting with sex-linked differences of embryos or treatments altering the amount of yolk hormones in a sex-specific manner, e.g. due to sex-specific growth rates of follicles (Badyaev et al., 2005).

Non-dosage compensation in birds would imply that males (ZZ) could present higher concentrations of some products from Z-chromosome genes. The strongest evidence is related to the aconitase enzyme (Baverstock et al., 1982). Krackow (1999) suggested that these products could interact with maternally derived egg constituents, enhancing or reducing embryo survival depending on its sex. This would allow the evolution of some mechanism of sex-ratio manipulation based on sex-related differences in these chromosomal gene products. However, there is some evidence to suggest that birds could have in fact some mechanism of dosage compensation (McQueen et al., 2001).

Nonetheless, egg composition could interact with other more evident sex-specific traits of embryos. Several recent studies suggest that the duration of embryo development can differ between sexes. In Eurasian kestrels, female embryos showed shorter embryonic periods, hatching sooner than males (Blanco et al., 2003a,b). In guillemots (Cepphus grylle), it is the male embryo who shows a shorter developing period (Cook and Monaghan, 2004, but see also a negative result in Salomons et al., 2006). These differences in developmental rates could imply different levels of dependence on egg resources. In such case, an overall reduction in yolk lipids or proteins (albumen) could mean a reduced survival of that sex more dependent on these substances. In this sense, Pérez et al. (2006) have experimentally demonstrated that survival of male yellow-legged gull embryos is more dependent on food availability than for females.

In addition, male and female embryos could be sexually dimorphic in size, which could also interact with maternal control. At least nine different avian species present sexual dimorphism in egg size (Howe, 1977; Fiala, 1981; Ankney, 1982; Mead et al., 1987; Anderson et al., 1997; Cordero et al., 2000, 2001; Petrie et al., 2001; Magrath et al., 2003; see also Blanco et al., 2003a, 2003b for differences only at the first egg). Nevertheless, these differences could be not due to variability in embryo size per se, but to differences in albumen content (Mead et al., 1987; Finkler et al., 1998; Badyaev et al., 2006b). Albumen is allocated to yolk in the oviduct, when sex is already determined. Thus, females could actively distribute more or less albumen depending on the sex (a sex-specific manipulation; see previous Section). Unfortunately, the cited studies did not analyse egg content. Pike and Petrie (2003) also suggested that egg-size dimorphism could be used as a cue in sex-selective incubation, which could lead to sex-specific mortality. However, as in the case of sex-related survival of chicks, such mechanism would exclusively work on secondary sex ratio, being not the aim of this review.

3. PATTERNS OF PRIMARY SEX RATIO ALLOCATION: NEW FINDINGS

Twenty-eight studies showing significant correlations between natural primary sex-ratio bias and any source of variation are presented in Table 1. In addition, Table 2 shows eleven experimental works providing important clues on the potential mechanism involved.
Table 1: Recent correlational studies (mid-2002 to the present) showing primary sex ratio manipulation in avian species. Studies were embryos were not sexed are explicitly indicated.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex ratio bias related to:</th>
<th>Limitations on primary sex ratio assessment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelecaniformes</td>
<td><em>Female body condition</em></td>
<td>Embryo sexing was not needed since all eggs hatched</td>
<td>Velando (2002)</td>
</tr>
<tr>
<td>Sula nebouxii*</td>
<td><em>Good maternal condition, low corticosterone and high testosterone plasma levels</em></td>
<td></td>
<td>Pike and Petrie (2005)</td>
</tr>
<tr>
<td>Galliformes</td>
<td><em>Laying date</em></td>
<td></td>
<td>Andersson et al. (2003)</td>
</tr>
<tr>
<td>Charadriiformes</td>
<td><em>Laying date, laying order interacting with laying date</em></td>
<td>Embryos were not sexed. Complete versus incomplete-brood comparisons</td>
<td>Krebs et al. (2002)</td>
</tr>
<tr>
<td>Actitis hypoleucus*</td>
<td><em>Laying order</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larus cachimans*</td>
<td><em>Laying order</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larus ridibundus*</td>
<td><em>Laying order</em> and egg size interaction*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Philomachus pugnax*</td>
<td><em>Female body condition</em>, mostly arising in years when females are in worst condition</td>
<td>Complete versus incomplete-brood comparisons</td>
<td>Thuman et al. (2003)</td>
</tr>
<tr>
<td>Sterna hirundo*</td>
<td><em>Laying order (last-egg)</em></td>
<td>Some fertile eggs could have been discarded</td>
<td>Fletcher and Hamer (2004)</td>
</tr>
<tr>
<td>Pittaciiformes</td>
<td><em>Laying order; statistical reanalysis of first evidences and new data</em></td>
<td>Information not available</td>
<td>Neuhäuser (2003)</td>
</tr>
<tr>
<td>Eclectus rotatus*</td>
<td><em>Laying date; statistical reanalysis of first evidences and new data</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platycercus elegans*</td>
<td><em>Laying date</em></td>
<td></td>
<td>Krebs et al. (2002)</td>
</tr>
<tr>
<td>Strigops habroptilus*</td>
<td><em>No bias in second egg; statistical reanalysis of first evidences</em></td>
<td>Information not available</td>
<td>Neuhäuser (2003)**</td>
</tr>
<tr>
<td>Strigiformes</td>
<td><em>Population bias related to food availability</em></td>
<td></td>
<td>Brommer et al. (2003)</td>
</tr>
<tr>
<td>Strix uralensis*</td>
<td><em>Pair bond duration, which was related to chick feeding rates of mates</em></td>
<td>Embryos were not sexed. Complete versus incomplete brood comparisons</td>
<td>Green (2002)</td>
</tr>
<tr>
<td>Passeriformes</td>
<td><em>Pair bond duration, which was related to chick feeding rates of mates</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acanthiza pusilla*</td>
<td><em>Incubation start with respect to laying order, interacting with population (probably effect of environmental temperature)</em></td>
<td>Scarce information about the proportion of unsexed eggs</td>
<td>Badyaev et al. (2003a, 2003b)</td>
</tr>
<tr>
<td>Carpodacus mexicanus*</td>
<td><em>Resource availability and female condition</em></td>
<td>Complete versus incomplete brood comparisons</td>
<td>See also Young and Badyaev (2004), Badyaev et al. (2005, 2006a, 2006b)</td>
</tr>
<tr>
<td>Certhia familiaris*</td>
<td><em>Resource availability and female condition</em></td>
<td></td>
<td>Suorsa et al. (2003)</td>
</tr>
</tbody>
</table>
Table 1 Recent correlational studies (mid-2002 to the present) showing primary sex ratio manipulation in avian species. Studies were embryos were not sexed are explicitly indicated (continued)

<table>
<thead>
<tr>
<th>Species</th>
<th>Trait Description</th>
<th>Methodology</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Manorina melanophrys</em></td>
<td>Food availability between population</td>
<td>Complete versus incomplete-brood comparisons</td>
<td>Ewen et al. (2003)</td>
</tr>
<tr>
<td></td>
<td>More helpful sex</td>
<td>Complete versus incomplete-brood comparisons</td>
<td>Clarke et al. (2002)</td>
</tr>
<tr>
<td><em>Megalurus gramineus</em></td>
<td>Laying date</td>
<td>Some eggs sexed by no information about the sex of them</td>
<td>McIntosh et al. (2003)</td>
</tr>
<tr>
<td><em>Parus caeruleus</em></td>
<td>Male attractiveness (colour), consistent between years</td>
<td>Some fertile eggs could have been discarded</td>
<td>Griffith et al. (2003)</td>
</tr>
<tr>
<td></td>
<td>Male attractiveness (song)</td>
<td></td>
<td>Dreiss et al. (2006)</td>
</tr>
<tr>
<td><em>Parus major</em></td>
<td>Male tarsus length</td>
<td>Embryos were not sexed. Conserved assignation of un-hatched eggs would not change the bias</td>
<td>Yamaguchi et al., 2004</td>
</tr>
<tr>
<td></td>
<td>Male body condition and pair bond. Within-individual annual changes analysed</td>
<td>Although embryos were sexed, incomplete clutches were included. However, the proportion of eggs hatching did not depend on sex-ratio in the clutch</td>
<td>Oddie and Reim, 2002</td>
</tr>
<tr>
<td><em>Philetairus socius</em></td>
<td>Helpful sex but in pairs with helpers (unpredicted result)</td>
<td>Embryos were not sexed. Reanalyses including unsexed chicks as male or female</td>
<td>Doutrelant et al. (2004)</td>
</tr>
<tr>
<td><em>Petrochelidon ariel</em></td>
<td>Female tarsus length, but contrarily to predictions</td>
<td>Embryos were not sexed. Complete versus incomplete-brood comparisons, but not clearly stated</td>
<td>Magrath et al. (2002)</td>
</tr>
</tbody>
</table>

* New species from Pike and Petrie (2003)' review.
** Negative results not included in Table 3.
Table 2 Recent experimental studies (mid-2002 to the present) showing primary sex ratio manipulation in different species

<table>
<thead>
<tr>
<th>Species</th>
<th>Manipulated Trait</th>
<th>Main effect</th>
<th>Limitations on primary sex ratio assessment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Colubiformes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Columba livia</em></td>
<td>Female body condition by removing eggs</td>
<td>Female bias in first and second eggs</td>
<td>Restricted to final clutch after a 4-month laying period</td>
<td>Pike (2005)</td>
</tr>
<tr>
<td><strong>Galliformes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Coturnix coturnix japonica</em></td>
<td>Circulating levels of testosterone, 17-b-estradiol and corticosterone in females</td>
<td>Only high corticosterone levels induced female biased sex ratio</td>
<td>Infertility rates not analysed per treatment</td>
<td>Pike and Petrie (2006)</td>
</tr>
<tr>
<td>*<em>Gallus gallus domesticus</em></td>
<td>Circulating levels of progesterone</td>
<td>High progesterone induced female bias</td>
<td>Seven eggs with no apparent embryo development were not sexed</td>
<td>Correa et al. (2005)</td>
</tr>
<tr>
<td>*<em>Pavo cristatus</em></td>
<td>Male attractiveness</td>
<td>More corticosterone in egg yolk and female-biased sex-ratio when paired with less attractive males</td>
<td></td>
<td>Pike and Petrie (2005)</td>
</tr>
<tr>
<td><strong>Passeriformes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Sturnus unicolor</em></td>
<td>Male courtship display (green plants at the nest)</td>
<td>More plants removed previous primary sex ratio bias</td>
<td>Complete versus incomplete-brood comparisons</td>
<td>Polo et al. (2004)</td>
</tr>
<tr>
<td></td>
<td>Circulating levels of testosterone in females</td>
<td>Male bias during three years, though only first was treated. Change in social status of females</td>
<td>Embryos not sexed. Unhatched eggs at last position. Justified by no laying sequence effect in previous article</td>
<td>Veiga et al. (2004)</td>
</tr>
<tr>
<td><strong>Taeniopygia guttata</strong></td>
<td>Diet quality of females</td>
<td>Diet quality induces sex-ratio skews, but depending on clutch size</td>
<td></td>
<td>Arnold et al. (2003b)</td>
</tr>
<tr>
<td></td>
<td>Diet quality of females</td>
<td>Diet quality induced overall sex-ratio skews and laying order x diet quality interaction</td>
<td></td>
<td>Rutstein et al. (2004)</td>
</tr>
<tr>
<td></td>
<td>Male attractiveness by colour rings</td>
<td>Sex ratio bias due to embryo mortality, rejecting previous evidences on primary sex ratio effects (Burley 1986)</td>
<td></td>
<td>Rutstein et al. (2005b)**</td>
</tr>
<tr>
<td><strong>Taeniopygia guttata</strong></td>
<td>Circulating levels of testosterone in females</td>
<td>More males in eggs from injection time</td>
<td></td>
<td>Rutkowska and Cichon (2006)</td>
</tr>
<tr>
<td><strong>Trachycineta bicolor</strong></td>
<td>Female body condition by clipping feathers</td>
<td>Body condition predicted sex ratio bias, but also foraging skills</td>
<td></td>
<td>Whittingham et al. (2005)</td>
</tr>
</tbody>
</table>

* New species from Pike and Petrie (2003)'s review.
** Negative results not included in Table 3.
All these articles were published since mid-2002 (end of Pike and Petrie, 2003’s review, which only described seven studies). All of these studies reported used molecular sexing techniques. Articles where embryos from unhatched eggs were not sexed are specified in the “limitations on primary sex ratio assessment” column. Among avian orders represented, only Galliformes is a new addition to that of the previous review (Pike and Petrie, 2003). These articles represent 27 species with 18 of them being new ones for the study of primary sex ratio manipulation (Tables 1 and 2). Table 3 summarises the number of avian species presenting the influence of a specific parameter on primary sex ratio with the aim of providing some indication on what sources of variation are often related to primary sex ratio in birds.

### Table 3 Number of avian species showing primary sex ratio bias related to different parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pike and Petrie (2003)</th>
<th>Current review</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laying order</td>
<td>11</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>Laying date</td>
<td>5</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Female body condition</td>
<td>2</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Food quantity/quality</td>
<td>5</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Male attractiveness</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Helpers presence at the nest</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Clutch size</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Circulating testosterone levels of female</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Circulating corticosterone levels of female</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Incubation start or hatching asynchrony</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Circulating progesterone levels of female</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

* Body size, condition, foraging/nesting ability, harem size and pair bond duration.

4. **IS PRIMARY SEX RATIO MANIPULATION A WIDESPREAD TRAIT IN BIRDS?**

To this point emphasis has been on analysis of the potential proximate mechanisms of primary sex ratio manipulation in birds, as well as a summary of significant relationships between sex ratio and reproductive/life-history traits. Although it seems evident that avian sex ratio manipulation exists, some authors still maintain that it could have not evolved in birds, or at least, would not be widespread in avian taxa (Clutton-Brock, 1986; Krackow, 1999; Palmer, 2000). This opinion is mostly founded on the three main problems that any potential mechanism must solve: the parent-gamete conflict, the meiotic constraints and the maternal recognition of embryo sex.

Three studies to date have tried to test the hypothesis that primary sex ratio manipulation is a widespread trait in birds by means of meta-analyses (Palmer, 2000; West and Sheldon, 2002; Ewen et al., 2004). Palmer (2000) concluded that the selective reporting of significant results (i.e. publication bias) could explain the high number of articles showing sex-ratio manipulation in birds. By means of funnel graphs, he showed that the number of studies reporting significant results decreases when the sample size of each study increases. However, West and Sheldon (2002) replied that Palmer’s study only analysed sex ratios at the population level, which would prevent to detect variability at the individual scale. For instance, some females manipulate sex ratio to produce male bias, whereas other females do the opposite, neutralising the effect in the whole population.
In addition to performing analyses at the individual level, West and Sheldon (2002) restricted their approach to those particular cases in which there was a clear theoretical prediction as to the direction of an effect (i.e. mate quality and cooperative breeding). In contrast to Palmer (2000), their meta-analyses did not reflect any publication bias. Instead, their results suggest that mechanisms of sex determination do not constrain the evolution of sex ratio adjustment.

Ewen et al. (2004) did not restrict meta-analysis to these two specific parameters and were the first to limit the approach to those articles exclusively reporting primary sex ratio. In agreement with Palmer (2000), their results revealed that those studies published until 2002 (also two works from 2003) do not exhibit any variability beyond that which could be expected by chance or sampling error, although some exceptions were considered. However, they assumed that analyses on complete broods should equal to primary sex ratio. Instead, this condition could remove initial primary bias when the favoured sex is also that with higher mortality, or alternatively, provide bias to the sex with higher survivorship (Fiala 1980; van den Burg et al., 2002; Dyrcz et al., 2004). Recent data could be used in future meta-analytical-approaches but we must take into account several research lines can be proposed and these are briefly described below.

5. FUTURE DIRECTIONS

As has been shown recent correlational and experimental evidence has opened new perspectives in this field. Taking these into account several research lines can be proposed and these are briefly described below.

5.1 Hormones and segregation distortion: an integrative approach

Research should be firstly directed to those mechanisms operating during meiosis I because a priori they would be less expensive in terms of energy and/or time than those present in subsequent stages (e.g. Oddie, 1998; Komdeur and Pen, 2002). Krackow (1995) was the first to suggest that hormones (progesterone) could play an important role in sex determination during meiosis. Recent experiments have shown that, in addition to progesterone (Correa et al., 2005), testosterone and corticosterone can also induce primary sex ratio bias (Table 2). However, we must consider that all these hormones are closely related through the aromatisation processes (Norris, 1997). Thus, injections or implants producing high levels of one hormone could induce high levels of the other hormones as result of metabolism. Moreover, the levels of other hormones could also change as consequence of feed-back responses (Norris, 1997). Thus, circulating levels of corticosterone have been negatively related to prolactin secretion (Crisculo et al., 2006). Such possibilities should be tested by simultaneously using receptor blockers or synthesis-inhibitors (Pike and Petrie, 2006) as well as by measuring the levels of related hormones (Correa et al., 2005). However, the complete control of all these substances is difficult. For instance, although Pike and Petrie (2006) made an important effort in this sense, they could not discard that the effect of corticosterone on sex ratio was not indirectly due to changes in progesterone values. In any case, an integrative approach is required, taking into account the role of different hormones simultaneously.

In addition, we need to understand how these hormones could influence meiotic distortion at the follicle level. Badyaev et al. (2006b) found that follicles that will produce male or females are separated (clustered) in the ovary of female house finches. Such spatial segregation could allow females to differentially provide hormones among specific follicle groups. Moreover, we should also establish if skewed sex-ratios are caused by circulating hormone levels alone or in combination with hormones accumulated in egg yolk. Finally, we would need to determine the presence or absence of different hormone receptors at the oocyte surface and determine if they are differentially expressed in oocytes that will lead to male or female embryos (i.e. maternal recognition problem).

5.2 In vitro experimental approach

In addition to manipulation of circulating levels of hormones in vivo, in vitro experiments on first meiosis stages could be crucial, allowing testing some of suggested questions. In fact, in vivo manipulations of hormone levels can not completely exclude the possibility of differential embryo mortality. Thus, all experiments cited in Table 3 did not sex apparently unfertile eggs. However, many of these eggs could have contained embryos that had died at the first stages...
of development. Sexing undeveloped embryos is very difficult, leading to errors due to contamination from maternal tissues (see Arnold et al., 2003a). In vitro experiences could overcome this limitation. To induce meiosis into a milieu with different levels of hormones could recreate the ovarian environment, providing definitive evidences for segregation distortion mechanisms.

5.3 Hormones and segregation distortion: patterns of primary sex ratio allocation

Hormones could be involved in at least two of the most frequently reported parameters related to primary sex ratio allocation: laying order and maternal body condition (Table 3). In the first case, the interaction between steroids and prolactin (PRL) could explain some of these findings (i.e. Badyaev et al., 2003a,b). The start and continuity of incubatory behaviour is controlled by PRL, which also influences steroid production by the ovary (Johnson, 2000). When the incubation start is advanced to the beginning of the laying sequence due to ambient temperature (Deeming, 2002), an overlap between high PRL secretion and oogenesis occurs. High PRL levels needed for egg incubation could thus constrain the sex-specific steroid requirements of growing follicles (Sockman and Schwabl, 1999; Sockman et al., 2000; Deeming, 2002). Badyaev et al. (2003a, 2003b) have shown that female house finches produced sex-ratio bias when they start incubation with the first egg, but not when incubation is delayed until the last egg. The interaction between PRL and steroid levels could thus influence segregation distortion during meiosis, producing sex-ratio bias with laying order.

On the other hand, hormones could also mediate in the relationship between primary sex ratio and maternal condition. The effect of corticosterone on primary sex ratio is suggestive of adaptive manipulation related to the level of stress in females. High corticosterone levels (high stress) would induce a primary sex ratio skewed to the cheapest sex (Pike and Petrie, 2006), being also associated to low body condition of females (e.g. Schoech et al., 1997; Kitaysky et al., 1999; Love et al., 2005). Similarly, Rutstein et al. (2005a) found that sex-specific allocation of maternal testosterone to egg yolk, which has been related to primary sex-ratio allocation (Veiga et al., 2004; Pike and Petrie, 2005b), depends on female condition at laying (see Gilbert et al., 2005).

Other previously overlooked hormones might also establish an endocrine link between female body condition and sex ratio bias. Among them, leptin (Zhang et al., 1994) could be a good candidate. Leptin is produced in adipocytes, and their circulating values are positively correlated to the fatness degree in mammals (Brann et al., 2002). It is also involved in the regulation of energy stores, food intake and satiety (Bado et al., 1998; Brann et al., 2002). Leptin plays a role as activator of hypothalamic-pituitary-gonadal axis, inducing FSH-LH release and modulating steroidogenesis (McCann et al., 2001; Paczoska-Eliasiewicz et al., 2003). Thus, high leptin levels induce the production of gonadal steroids when a certain threshold of fatness is attained, acting as a permissive factor for reproduction (Brann et al., 2002). Leptin levels in ovarian tissue increases during follicular maturation and ovulation in rats (Ryan et al., 2003), and leptin receptors have also been described in avian ovary (Ohkubo et al., 2000; Paczoska-Eliasiewicz et al., 2003). Moreover, leptin attenuates follicular atresia in chickens (Paczoska-Eliasiewicz et al., 2003). Therefore, leptin could have evolved as a permissive factor that informs organism about the state of energy reserves before skewing sex ratio in a direction or in another.

5.4 Integrating segregation distortion and selective embryo mortality

As described in Section 2.5, high levels of maternal hormones could also lead to sex-specific embryo mortality. Thus, if segregation distortion is mediated by the amount of maternal hormones at the yolk, such mechanism could also imply a cost when an excess of hormone produces embryo mortality, even neutralising primary sex ratio manipulation. Rutkowska and Cichon (2006) found that female zebra finches injected with testosterone produced a male-biased sex ratio at laying, but male-biased mortality at hatching. Therefore, females should carefully regulate the amount of hormones allocated to the egg yolk. Alternatively, hormone-mediated mechanisms acting both on segregation distortion and embryo development could interact, leading to optimal sex ratio allocation at the hatching time. Such possibilities should be explored in the future.
5.5 Fitness consequences

In addition to the knowledge of the exact mechanisms of sex ratio manipulation, and their proximate costs, we need to understand their ultimate consequences in terms of individual fitness, i.e. as the contribution an individual makes to the gene pool (e.g. number of fertile descendants). How can an unbalanced sex ratio negatively or positively affect to the fitness of both parents and offspring of both sexes (Hasselquist and Kempenaers, 2002; Komdeur and Pen, 2002)? Such approach has been rarely addressed, although it is imperative to demonstrate that this trait is adaptive and can be spread in the population.

Hasselquist and Kempenaers (2002) identified the factors that potentially could have sex-specific effects on offspring fitness, by directly (e.g. condition, age) or indirectly (e.g. environmental, social and offspring) affecting parental care. Moreover, they made predictions about the occurrence and expected direction of sex ratio manipulation under different scenarios of parental care which could be present in different species.

Hence, in addition to experiments performed to determine the physiological mechanism involved, other studies should manipulate the cited factors (Hasselquist and Kempenaers, 2002), analysing the impact on parental and offspring fitness. Moreover, it is important to investigate whether one sex is more costly to produce than the other due to perhaps higher growth, begging rates or metabolic rates (Hasselquist and Kempenaers, 2002). Both Hasselquist and Kempenaers (2002) and Komdeur and Pen (2002) claimed that only a few studies have investigated whether sex ratio manipulation does lead to increased fitness (Appleby et al., 1997; Komdeur, 1998). These studies must be focused on those species where primary sex ratio manipulation has been previously documented. Several articles addressing this subject have recently appeared (see also Badyaev et al., 2005). Thus, Green (2002) and Müller et al. (2005a) manipulated brood sex ratio and determined the cost of different sex ratio bias for parents and nestlings, respectively. Meanwhile, Velando (2002) manipulated maternal effort in order to assess the impact on offspring of both sexes (also Velando and Alonso-Alvarez, 2004). Similarly, Fernandes Martins (2004) and Gorman and Nager (2004) manipulated food availability/quality to determine its effect on male and female chicks.

5.6 Primary sex-ratio bias: strategy or constraint?

A question remains. Is primary sex ratio bias a passive consequence of maternal reproductive constraints or is it a manifestation of maternal ability to adaptively modify the sex of the progeny?

As previously commented, the interaction between PRL and steroid levels could constrain sex determination, biasing sex ratio with laying order (Badyaev et al., 2003a, 2003b). Alternatively, females could face this constraint by adjusting the sequence in which sexes are produced to her hormonal state, thus allocating steroids and other resources to eggs in a sex-specific manner. In house finches, females bias sex ratio when they start incubation with the first egg, but not when is delayed until the last egg (Badyaev et al., 2003a, 2003b). However, the direction of the sex ratio manipulation in females with early onset of incubation differed between two populations with different environmental conditions (Montana and Alabama, USA), and such sex ratio adjust was adaptive in terms of growth and survival of chicks (Badyaev et al., 2002). This example suggests that, at least in this species, the pattern of sex ratio allocation was the result of an adaptive strategy more than the consequence of constraints (see also Badyaev et al., 2005).

The manipulation of sex ratio based on female body condition or food availability could be also the result of energetic constraints suffered by poorly nourished females when attempting to produce more offspring of the expensive sex. Such energetic constraints could limit the amounts of lipids allocated to the yolk, which could influence the provision of other substances potentially involved in sex determination during meiosis (e.g. hormones). However, this would also constitute a good strategy in order to predict food availability at the moment of chick development (West and Sheldon, 2002). To distinguish between strategy and constraint remains as a challenge for the future.

ACKNOWLEDGEMENTS

I must first thank the editor of Avian and Poultry Biology Reviews (C. Deeming) for inviting me to write this review for the journal. I am also grateful to Javier Víñuela for his comments and suggestions on a first draft of this manuscript. The author was supported by the Ramón y Cajal fellowship (Spanish Ministerio de Educación y Ciencia).


Endow, S.A. (1999) Microtubule motors in spindle and chromo-


