Proximate control of avian sex allocation
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“Nur wenn der Winter näher kommt, legen die Blattlausweibchen ihre Eyer, und um diese Zeit ohngefähr, pflegen die Männchen zu erscheinen. Es ist also zwischen der Erscheinung der Männchen und der Legezeit ein geheimes Verhältniß. Dieses suchen wir noch, und das ist es, was uns den Grund der Begattung erklären muß.”

Charles Bonnet 1745 *Traité d’Insectologie*
(German translation by J. A. E. Goeze, 1773)
Schematic Overview

of the main factors studied in the thesis and of their
possible influences on different sex-allocation components

Male quality (e.g. attractiveness) ↔ Female quality (e.g. hormones) ↔ Food quality

↓

Parental care (male and female) ↔ Egg quality (e.g. hormones) → Primary sex ratio

↓

Before independence: Development & Survival (Pre- and posthatching) → Secondary sex ratio

↓

After independence: Reproduction & Survival → Population sex ratio
Synthesis

The existence of the two sexes, their differences and origin is one of the most challenging biological phenomena still lacking a convincing universal explanation (Charnov 1982; Maynard Smith 1982; West et al. 1999; Randerson & Hurst 2001a). 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. This thesis investigated causes of variation in avian sex allocation and potential proximate control mechanisms, with a focus on the role of maternal hormones, using the zebra finch as a model.

Background: Function and mechanism of sex allocation

Sex allocation theory

In most species we can distinguish two sexes, defined by the size of their gametes—females producing large, males producing small gametes. This is considered the result of disruptive selection for two extreme strategies of investment, either in few large gametes or in many small gametes (Parker 1972; Randerson & Hurst 2001b). The question why there usually is an equal number of males and females has first been discussed by Darwin (Darwin 1871) in the first edition of his book on the ‘Descent of Man and Selection in Relation to Sex’:

“Could the sexes be equalized through natural selection? We may feel sure, from all characters being variable, that certain pairs would produce a somewhat less excess of males over females than other pairs. The former, supposing the actual number of offspring to remain constant, would necessarily produce more females and would therefore be more productive. On the doctrine of chances, a greater number of the offspring of the more productive pairs would survive, and these offspring would inherit the tendency to procreate fewer males and more females. Thus a tendency to the equalization of the sexes would be brought about.”

Darwin was apparently not sure about this principle as he skipped this section from the 2nd edition (Darwin 1874), writing instead

“I formerly thought that when a tendency to produce the sexes in equal numbers was advantageous to the species it would follow from natural selection, but I now see that the whole problem is so intricate that it is safer to leave its solution for the future.”
It seems that Darwin’s first thoughts were right because frequency-dependent selection for equal allocation, first explicitly stated by Fisher (1930), is today considered the most important principle governing sex allocation (Frank 1990; Cockburn et al. 2002; Pen & Weissing 2002).

Local or temporal variation in conditions favourable for one or the other sex can lead to a deviation from equal allocation (Charnov 1982). This was first observed in wasp species with low natal dispersal and high local mate competition with overproduction of the sex competing less well (Hamilton 1967). Trivers and Willard (1973) proposed that “natural selection should favor parental ability to adjust the sex ratio of offspring produced according to parental ability to invest” for species in which the reproductive success of sons depends more on their condition than that of daughters. Charnov (1982) unified the different approaches in his book on Sex Allocation, stating that any factor that differentially affects the fitness returns of reproducing through male or female function is expected to affect the “allocation of resources to male versus female reproductive function”.

Who is in control?

“Parents are classically assumed to allocate investment in their young in such a way as to maximize the number surviving, while offspring are assumed to be passive vessels into which parents pour the appropriate care. Once one imagines offspring as actors in this interaction, then conflict must be assumed to lie at the heart of sexual reproduction itself – an offspring attempting from the very beginning to maximise its reproductive success would presumably want more investment than its parent is selected to give.” (Trivers 1974)

It is not clear, to what extent primary sex ratios and sex differences in offspring survival and reproductive success are controlled by parents, gametes or offspring (Reiss 1987; Eshel & Sansone 1991; Krackow 2002; Pen & Weissing 2002). Most likely all actors have some degree of control and the control depends upon the stage of development and the context. Parents need to recognise the sex of their offspring to invest differentially. During the period of parental dependence offspring cannot force parental care. When costs and benefits of differences in sex allocation are the same for parents and offspring, it does not matter who is in control. However, selection may favour sex-allocation strategies that differ between parents and offspring.

Some models show that less biased offspring sex ratios are favourable for young than for parents (Eshel & Sansone 1991; Eshel & Sansone 1994; Beukeboom et al. 2001; Komdeur & Pen 2002). An additional complexity is added,
when there are several offspring in one brood and siblings can influence sex allocation by competition (Frank 1990; Pen & Weissing 2002). It has been suggested that selection on offspring strategies is more important, because primary sex ratio adjustment is observed infrequently, while sex differences in survival are common and occur usually at the end of parental care (Clutton-Brock 1986; Clutton-Brock 1991). This is supported by findings showing sex differences in early development when parental influences are excluded by hand-rearing offspring (e.g. Martins 2004).

**Sex allocation mechanisms**

Variation in sex allocation, *i.e.*, the relative investment in reproduction through male and female function can be achieved by producing different numbers of male and female offspring. Most studies focus on such quantitative variation in avian sex allocation, *i.e.* on the numbers of sons and daughters at different stages in development. In addition to offspring numbers, offspring reproductive success, *i.e.* access to mates and fecundity, affects the fitness returns through male and female function. A highly competitive or attractive male may achieve disproportionate success even at the cost of lower survival. A female in good condition is likely to produce more or larger eggs, both resulting in a larger number of surviving offspring.

Several reviews have discussed mechanisms of primary sex ratio adjustment (Krackow 1995a; Pike & Petrie 2003) and sex-specific survival (Clutton-Brock et al. 1985; Clutton-Brock 1986). Modelling of differential investment in sons and daughters in addition to the manipulation of primary or secondary sex ratios has only just started (Lessells 1998; 2002). One of the challenges in the study of variation in sex allocation is that it is influenced by multiple factors such as the environment, parental genes and parental phenotype, by offspring genes and offspring phenotype and that selection can act in different directions on parental and offspring genes (Kirkpatrick & Lande 1989).

**Numbers: Primary sex ratios**

Variation of the primary sex ratio has been reported in many avian species (Komdeur & Pen 2002; Hasselquist & Kempenaers 2002). The details of genotypic sex-determination in birds have not been unravelled, but phenotypic sex in birds is thought to be determined by factors on their sex-chromosomes, which can morphologically be distinguished. In birds females are the heterogametic sex. Females have a Z- and a W-chromosome, and males have two Z-chromosomes. Sex is therefore determined genotypically although environmental influences can affect the phenotypic sex: disturbances during early development or diseases of the reproductive tract can lead to disturbance of
sexual differentiation (reviewed in Taber 1964). For example, extreme temperatures during embryonic development in chicken embryos result in about 10% of birds where phenotypic and genotypic sex do not match (Ferguson 1994). It is not known to what extent such effects can explain variation in offspring phenotypic sex ratios under natural circumstances but it cannot explain variation in offspring sex ratios in those studies that determined genotypic sex.

How is the primary sex ratio determined in birds?

A couple of reviews address this question (Krackow 1995a; Pike & Petrie 2003). No studies of oocyte development, meiosis, ovulation, fertilisation and early embryonic development appear to have taken offspring sex into account. This may change with the availability of molecular sex determination and methods for in vitro ovulation, fertilisation and embryonic development (Howarth 1971; Perry 1988; Olszanska et al. 1996). Sex is determined after the completion of the first meiotic division of the oocyte, when one sex chromosome is eliminated into the first polar body (Romanoff 1960). This occurs shortly before ovulation (Romanoff 1960). It is conceivable and has not been excluded that already at an earlier stage some factors determine which sex chromosome will be eliminated. In that case, the speed of growth of the oocyte, and the allocation of various substances may either determine or depend upon its prospective sex and thereby influence the primary sex ratio or investment with respect to offspring sex.

Many developing eggs become atretic (degenerate) before reaching ovulation (Gilbert et al. 1981; Gilbert et al. 1983), and atresia may in theory be related to the sex of the egg. Another possible mechanism is the resorption of eggs after meiosis and fertilisation depending on their sex. This mechanism is likely to result in laying gaps. In the absence of laying gaps it could result only in a biased sex ratio of the first egg, but not later laid eggs, which may explain why the most strong biases of the sex ratio have been observed especially in first eggs in a number of species (Emlen 1997; Komdeur & Pen 2002). These various mechanisms are not incompatible and may operate simultaneously or in different species.

Numbers: Sex-specific survival

Differences in survival of sons and daughters increases in avian species with the degree of sexual size dimorphism, with increasing food shortage (Clutton-Brock et al. 1985) and with age at fledging (Pen 2000). Sex-biased mortality occurs mostly late during the nestling period, at end of parental care, suggesting that sex-specific mortality does not require differential parental investment but can sufficiently be explained by sexual selection for large body size (Clutton-Brock 1986; Clutton-Brock 1991). In some species with larger females than males
there is no sex difference in nestling mortality, indicating that other factors besides sexual selection must play a role (Clutton-Brock 1991). Several – not mutually exclusive – hypotheses have been put forward to explain sex-specific survival in birds:

1. survival of males is lower due to stronger sexual selection in males leading to higher investment in traits influencing mating success than in traits influencing survival

2. survival of the heterogametic sex – females in birds – is lower, due to the effects of deleterious recessive alleles on the sex chromosomes

3. nestling survival depends upon sexual size dimorphism, because
   a) the larger sex requires more food and therefore has higher mortality when less food is available (Roskaft & Slagsvold 1985; Griffiths 1992; Torres & Drummond 1997; Dijkstra et al. 1998; Nager et al. 2000)
   b) the smaller sex has higher mortality when less food is available, because it has a disadvantage in sibling competition (Oddie 2000).

Reproductive success

The fitness of parents and offspring depends not only upon survival but also on the reproductive success of sons and daughters.

“This depends, not on a struggle for existence, but on a struggle between the males for possession of the females: the result is not death to the unsuccessful competitor, but few or no offspring”. (Darwin 1859)

Reproductive strategies in the sexes differ due to the fundamental differences in parental investment in gamete production (Darwin 1871; Parker 1972), which results in most species in larger variance in reproductive success in males than in females due to their lower invest in each individual offspring (Bateman 1948; Clutton-Brock 1988).

The best support for variation in success in reproduction of sons and daughters in birds comes from some size dimorphic raptors in which age at first breeding depends more on the time of the year for one sex than for the other sex and it has been observed that the sex is produced early in the season which profits more (Daan et al. 1996). In most other species, only indirect measures for reproductive success such as effects on size, body mass or condition of sons and daughters have been measured (collared flycatcher: Gustafsson et al. 1995; Merilä & Svensson 1997; pied flycatcher: Potti et al. 2002). Also, personality differences can have consequences for offspring fitness (Dingemanse et al.
2004) and such personality differences have a genetic basis but can be modified by early experience (Carere 2003). In birds, continuous and discontinuous variation in behaviour, physiology and morphology is observed in some species and is related to differences in reproductive strategies (white-throated sparrow: Lowther 1961; ruff: Lank et al. 1995; tawny owl: Roulin et al. 2003). Discontinuous variation in behaviour can also be correlated with differences in yolk hormone (Riedstra 2003; Carere 2003).

Because of the different reproductive strategies of males and females many traits, such as age at sexual maturity, attractiveness, competitiveness, parental investment or fecundity can have very different consequences for male and female reproductive success (Andersson 1994). Therefore, within- and between-sex differences in reproductive success are as important for sex allocation as effects on primary sex ratio and sex differences in survival. Surprisingly, they do not seem to be the focus of any reviews, possibly due to the difficulty in comparing how variation in the quality of sons and daughters contributes to reproductive success (Lessells 1998).

Besides direct genetic effects, the early environment and parental influences, such as egg size (Bernardo 1996), egg composition (Hill 1993), hormonal influences (vom Saal 1983), incubation (temperature)(Decuypere et al. 1985), offspring feeding and learning from parents can have long-lasting consequences for offspring morphology, physiology and behaviour and therefore for sex allocation (Crews et al. 1998; Mousseau & Fox 1998; Lindström 1999; Ketterson & Nolan 1999; Metcalfe & Monaghan 2001; West-Eberhard 2003). Offspring morphology and behaviour can vary on a continuous or discontinuous scale and can become fixed or remain plastic (Moore 1991; Crews et al. 1998; Rhen & Crews 2002).

Maternal hormones and sex allocation

In what way can maternal hormones be involved in avian sex allocation?

1. Hormonal effects on follicular maturation, ovulation and atresia may depend upon the (prospective) sex of the egg, resulting in differential growth, segregation distortion, or atresia and thereby in manipulation of the primary sex ratio

2. Hormonal effects on the production of yolk components can result in sex specific mortality or quality differences between the sexes if offspring development is differentially affected by egg composition. This may also be due to direct effects of hormones transferred into the egg yolk or albumen, which can affect embryonic and post-hatching growth, the immune system, begging and other behaviours and survival (Schwabl 1993; Schwabl
3. Hormonal effects on maternal behaviour can result in differences in the embryonic (incubation, insulation) or in post-hatching (brooding, feeding) parental care, which again can result in sex-specific mortality or quality differences between the sexes if they differ in sensitivity to the intensity of parental care.

Maternal hormones, either acting in the female during egg production and parental care, or acting in the offspring after being transferred to the egg, might affect primary sex ratios, secondary sex ratios or quality of sons and daughters (Krackow 1995a; Pike & Petrie 2003). Several lines of evidence suggest that maternal hormones play an important causal role in such a physiological mechanism. Reproductive hormones regulate follicular growth, oocyte maturation, ovulation and meiosis (Sturkie 1986). They vary in the maternal plasma and in the egg yolk across the laying sequence (Schwabl 1993; Gil et al. 1999; Eising et al. 2001) and variation of the sex ratio across the laying sequence has been reported in several species (Kilner 1998; Nager et al. 1999; Kalmbach et al. 2001). The levels of several of these hormones in the plasma or in the yolk change with the season (Schwabl 1996b; Sockman et al. 2001; Pilz et al. 2003), and seasonal variation of the sex ratio provides one of the strongest evidence for adaptive avian sex allocation (Dijkstra et al. 1990; Daan et al. 1996). Females paired to attractive males have elevated levels of testosterone in plasma and/or eggs (Gil et al. 1999; Gil 2003; this thesis: chapter 3; but see Mazuc et al. 2003) and produce male-biased offspring sex ratios (Burley 1981; Burley 1986c; Ellegren et al. 1996; Sheldon et al. 1999). Finally, the quality or amount of food received before and during egg production influences both levels of hormones in female plasma and eggs (Verboven et al. 2003) and offspring sex ratios (Kilner 1998; Bradbury & Blakey 1998; Nager et al. 1999).

Schwabl (1993) has suggested that one of the main functions of yolk androgens may be to selectively enhance the fitness of chicks from last eggs, because this is not possible by varying incubation onset. Indeed, in several avian species (canary: Schwabl 1993; kestrel: Sockman & Schwabl 2000; black-headed gull: Groothuis & Schwabl 2002; starling: Pilz et al. 2003; lesser black-backed gull: Verboven et al. 2003) yolk androgens increase with the position in the laying order and this might benefit young from later laid and therefore later hatching eggs competing with earlier hatched siblings. In other species, such as zebra finches (Gil et al. 1999) or cattle egrets (Schwabl et al. 1997), yolk androgens decrease with the laying sequence, which is thought to give first hatched young an extra advantage, facilitate brood reduction and thereby reduce costs in terms of lost parental investment in species where not all young can survive
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when food availability is low. There are also large differences in the amount of yolk androgens in repeated clutches of the same female or of different females (Gil et al. 1999; Ward et al. 2001; Groothuis & Schwabl 2002; Pilz et al. 2003). Because high yolk androgen levels in several species are associated with high breeding density and competition (house sparrow: Schwabl 1997; black-headed gull: Groothuis & Schwabl 2002; tree swallow: Whittingham & Schwabl 2002; house sparrow: Mazuc et al. 2003) it has been suggested that between clutch variation of yolk androgens may also adjust competition with unrelated conspecifics by influencing offspring aggressiveness and developmental rate.

The hypothesis that maternal hormone levels are related to offspring sex ratios has first been put forward for mammals (James 1985; James 1986) and has support mostly from correlational evidence in mammals and birds (reviewed in Krackow 1995a; James 1996; Pike & Petrie 2003). Solid experimental evidence exists neither for birds nor for mammals. The best evidence comes from studies in the late 1950’s, when the hormonal regulation of sexual differentiation was intensively investigated. A number of publications report biased offspring sex ratios after treatment of female or male birds with ovarian preparations or estrogens (Lang 1918; Schouppé 1930; Ökland 1936; Spicer 1954; Fraps et al. 1956; Pun 1958; Beilharz 1960; reviewed in Taber 1964). In some cases the presence of feminised males suggested that this was not an effect on the genetic sex but due to phenotypic sex reversal, presumably by transfer of estrogens to the egg (Riddle & Dunham 1942; Pincus & Erickson 1962; Adkins-Regan 1981).

Other studies suggested that this effect cannot be due to phenotypic reversal, because mating of feminised genotypic males should result in all-male offspring, but offspring sex ratios appeared normal (Pun 1958). In another study (Beilharz 1960) the advantage of sex-linked plumage traits was used to answer this question. Because the plumage of daughters of estrogen-treated females matched the genetically determined sex-linked plumage trait the excess of female offspring could not be explained by sex reversal of males. Unfortunately this line of research seems not to have been pursued further.

Early hormone exposure leads to within-sex differences in morphology, physiology and behaviour in a number of species (vom Saal 1983; Hews et al. 1994; Clark & Galef 1998). The effects of manipulating early hormone exposure in birds, including zebra finches, are complex, partly contradictory and not fully understood (Balthazart & Ball 1995; Agate et al. 2003). Certain differences, such as the sexual differentiation of the gonads appear to be primarily under the control of estrogen-secretion so that very early exposure to estrogens or inhibition of the enzyme aromatase, which is required for producing estradiol, can completely reverse gonadal sex (reviewed in Taber 1964; Adkins-Regan 1981; Balthazart & Adkins-Regan 2002). Other sex differences, such as in the plumage of zebra finches, appear to be entirely due to genetic sex of the indi-
vidual cells. Behavioural and neural sexual differentiation appear to be affected by gonadal hormones, cell-autonomous processes and local metabolism or secretion of steroid hormones in the brain. The picture is further complicated by the fact that the effects depend strongly upon the stage in ontogeny during which manipulations are performed, most likely because of the developmental changes in hormone receptors, hormone metabolising enzymes and endogenous hormones.

Because of the focus on the effects of estradiol, the most important hormone for gonadal sexual differentiation in birds, the effects of early exposure to testosterone have not been studied intensively and not much is known about its sex-specific effects. The discovery of maternal deposition of hormones into the egg (Schwabl 1993) and their effects (Schwabl 1996a; Eising et al. 2001; Eising & Groothuis 2003) will certainly stimulate the interest in the organising effects of early testosterone exposure in birds.

In female birds, embryonic exposure to androgens has little effect on sexual differentiation (Taber 1964). In males, estradiol, testosterone and even other androgens often have similar feminising effects, possibly due to aromatisation of testosterone to estrogens (Adkins-Regan 1985; Adkins-Regan et al. 1987). At least in zebra finches, androgen receptors are present in both male and female brains very early in embryonic development (Godsave et al. 2002) so that testosterone may act both directly and after being aromatised to estradiol.

Endogenous estrogen levels seem to be generally high in female embryos and low in male embryos, while relative levels of testosterone in male and female embryos differ depending upon the species (Woods et al. 1975; Woods & Brazil 1981; Ottinger & Bakst 1981; Abdelnabi et al. 2000). Males may be less exposed to active androgens (and estrogens) than females under normal circumstances because they have high activity of the enzyme 5-$\eta$-reductase which reduces testosterone to the inactive metabolite 5-$\eta$-dihydrotestosterone (Ottinger et al. 1984; Schumacher et al. 1989), but very little is known about actual levels and metabolism of steroid hormones during embryonic development. This makes it difficult to speculate about the mechanisms by which androgens from maternal origin exert their effects.

In quail and chicken, early exposure to estradiol or testosterone demasculinises but does not feminise male sexual behaviour (Wilson & Glick 1970; Sayag et al. 1988; Abdelnabi & Ottinger 2003) and testosterone-propionate can reduce adult levels of plasma androgens (Taylor & Glick 1983). These effects of testosterone on behaviour may directly or indirectly – mediated by estradiol – involve the vasotocinergic and serotoninergic system (Aste et al. 1998; Abdelnabi & Ottinger 2003). The vasotocinergic system is sexually dimorphic in adult birds, regulates social and affiliative behaviour, song and aggressive behaviour and can be sex-reversed by manipulations of early estrogen expo-
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Sure (Aste et al. 1998; Jurkevich 2003). Serotonin levels differ between males and females during embryonic and post-hatching development (Abdelnabi & Ottinger 2003). Embryonic treatment with estradiol or testosterone increased serotonin levels in the preoptic area in adult birds, and inhibited male courtship and mating behaviour (Abdelnabi & Ottinger 2003). Possibly, relative levels of steroid hormones during early development modify offspring sexual, agonistic and affiliative behaviour through these neurotransmitter systems.

Sex allocation in the zebra finch

Background

Mate attractiveness and food quality are the two factors generally considered as most important for sex allocation in zebra finches. Certain features of the life-history of the zebra finches support this idea, but the life-time fitness consequences of parental attractiveness and environmental conditions for sons and daughters are difficult to estimate for the zebra finch in the wild due to the high mobility and high mortality of the species (Zann 1996). Therefore predictions with respect to adaptive variation in sex allocation have to be taken with caution.

As the species readily breeds in captivity and has been used extensively in behavioural studies, it appears a promising species for research on sex allocation in birds. Experimental studies on sex allocation in zebra finches have found effects of manipulated parental attractiveness (Burley 1981; Burley 1986c), feeding regime (Kilner 1998; Bradbury & Blakey 1998; Rutkowska & Cichón 2002; Rutstein 2004), the laying (Rutkowska & Cichón 2002; Rutstein 2004) or hatching order (Clotfelter 1996; Kilner 1998) and clutch size (Arnold et al. 2003) on primary or secondary sex ratios.

Mate attractiveness

Zebra finches are socially monogamous, but genetically somewhat polygamous with rates of extra-pair paternity and intraspecific brood parasitism up to 10% (Birkhead et al. 1988; Birkhead et al. 1990; Birkhead et al. 1995; Zann 1996). Due to high mortality, repairing is frequent (Zann 1996). Both sexes have strong mate preferences (Wynn & Price 1993), males prefer more fecund females (Monaghan et al. 1996) and orange beaks (Burley & Price 1991), while females prefer males with red beaks (Burley & Coopersmith 1987), high song rates (ten Cate & Mug 1984; Houtman 1992; Collins et al. 1994) and display rates and symmetric plumage (Swaddle & Cuthill 1994a) or banding (Swaddle & Cuthill 1994b). Redness of the beak negatively affects female viability (Burley et al.
1992; Price & Burley 1994) and reproductive success (Burley & Calkins 1999). Because beak colour is heritable for both sexes, daughters of females paired to males with redder beaks will have redder beaks which negatively affects their attractiveness and survival and sons will have redder, more attractive beaks (Price & Burley 1993). Burley (1981; 1986c) found that females produce more offspring of the more attractive parental sex and suggested that the adaptive benefit is the increased attractiveness and therefore reproductive success of their offspring.

**Food quality**

Reproduction in zebra finches is opportunistic and rapid, tracing the availability of food depending upon unpredictable rainfall (Immelmann 1963) and offspring are able to reproduce at a very young age due to rapid maturation (Sossinka 1980; Zann 1996). Fecundity of daughters depends strongly upon their fledging weight (Haywood 1993), while male reproductive performance may be less affected by early conditions: several traits of adult males that are related to their reproductive performance (body size, testicular function, beak colour and song rate) are not affected by a low quality diet during the nestling period, which leads to reduced growth and immune function during the nestling period (Birkhead et al. 1999). Most studies interpret these apparently stronger effects on daughters as evidence that the fitness of daughters depends more on favourable conditions during early development than the fitness of sons (Kilner 1998; Rutkowska & Cichón 2002; Rutstein 2004) and suggest that females in good condition or in a good environment should invest more in daughters than in sons.

**Maternal hormones**

Hormones are thought to be involved in sex allocation in zebra finches, because the same factors that influence sex ratios, affect yolk androgens deposition: levels of the androgens testosterone and dihydrotestosterone in zebra finch eggs were elevated in egg laid by females paired to attractive males (Gil et al. 1999)(chapter 3), and decreased with the position in the laying order (Gil et al. 1999; Ward et al. 2001)(chapter 3). The strength of the decrease with the laying order can depend upon the attractiveness of the mate (chapter 3) and the feeding regime (Sandell 2003), which may explain why a clear decrease of yolk androgens with the laying order has not been reported in all studies (Ward et al. 2001). Further, yolk androgen levels increased in successive clutches of the same females (Gil et al. 1999) and they significantly varied between clutches of different females (Ward et al. 2001).
Another study that suggested a link between maternal hormones and offspring sex ratios (Williams 1999) reported female-biased offspring sex ratios at fledging when treating female zebra finches before breeding with estradiol. Estradiol was detectable in zebra finch eggs at very low levels (Schwabl 1993), but may have important effects on sex allocation due to its effects on sexual differentiation (Adkins-Regan 1981; Adkins-Regan et al. 1995).

Sex allocation mechanisms

Three major questions formed the basis of the thesis and will be discussed by focusing first on effects on numbers and survival of sons and daughters - primary sex ratios and sex-specific survival -, then on effects on the reproductive success of sons and daughters.

1. are there robust effects of parental traits and the feeding regime on offspring sex ratios?

2. at which stage do differences in offspring sex ratios emerge?

3. are maternal hormones involved in sex allocation?

Numbers: Primary sex ratios

We investigated the effects of parental attractiveness (chapter 2, chapter 3), the effects of the feeding regime (chapter 4) and the effect of treatment of mothers with estradiol on offspring primary sex ratios.

Offspring sex in this thesis was assessed by the amplification of sex-specific gene sequences (Griffiths et al. 1996; Ellegren & Sheldon 1997). The validity of this method was checked by comparing the genotypic sex using this method with the phenotypic sex of surviving offspring. This validation is expected to result in some error, as sexual differentiation may be disturbed due to hormonal aberrations or disease, leading usually to masculinisation of females due to differentiation of the non-functional right gonad to a testis (see Taber 1964). Out of all offspring in this thesis, for which genotypic and phenotypic sex was determined (< 300) there was one mismatch: a zebra finch with male plumage and female genotype. The genotype was repeatedly determined as female using two different types of sex-specific primers (Griffiths et al. 1996; Bradbury & Blakey 1998). Interestingly, this genetic female, but phenotypic male was fertile and breeding successfully with a normal female. It is still unclear whether this was due to a mutation in the gene sequences used for sex determination or a truly genetic female with deviating sexual differentiation.
The first studies that reported significantly biased sex ratios in relation to parental attractiveness, manipulated by coloured leg bands (Burley 1981; Burley 1986c) did not analyse primary sex ratios. Coloured Leg bands affect mate attractiveness in domesticated (Burley 1982; Burley 1985a) and wild zebra finches (Burley 1988a), although this effect could not be replicated in some studies (Ratcliffe & Boag 1987; Jennions 1998; Fawcett 2003). Two studies manipulated male attractiveness using green (unattractive), orange (neutral) and red (attractive) leg bands and found that offspring sex ratios increased with increasing attractiveness of the leg-band colour of the male (Burley 1981; Burley 1986c). The author suggested that this was due to sex-specific survival, because sex ratios were not biased in broods without mortality, but adjustment of the primary sex ratio could not be excluded.

Recent studies found no evidence (Zann & Runciman 2003), or only partial evidence (Dijkstra et al. 2000) for an effect of parental attractiveness on offspring sex ratios. These studies excluded the possibility for mutual mate choice and male-male competition by pairing birds in individual breeding cages and analysed only primary sex ratios. The exclusion of these factors and sex-specific survival might explain the failure to replicate the previous results. We therefore repeated the study in a set-up matching the original study (Burley 1986c) as closely as possible. In this experiment, described in chapter 2, females paired to males wearing attractive or unattractive leg bands, produced on average very similar secondary sex ratios to the original study, but offspring of males with a neutral band-colour produced a much higher proportion of daughters. Overall primary sex ratios in our study were 49% males for green-banded and 48% red-banded males. The sex-ratio bias of surviving young of red-banded fathers (61%) was due to sex-biased nestling survival as Burley had originally suggested (see below for further discussion of the secondary sex ratios).

In the study described in chapter 3 females were paired to their preferred or unpreferred males, but male attractiveness neither affected primary sex ratios, nor sex-specific survival (see below). Offspring primary sex ratios decreased with the position in the laying order for both females paired to attractive and unattractive males, due to a male-biased primary sex ratio of only the first egg. The function of this first-egg bias is unclear, but it supports the idea that biased sex ratios of first eggs may be easier to achieve than biased sex ratios of later laid eggs (Emlen 1997).

Two studies have reported significantly biased primary sex ratios in zebra finches in relation to the feeding regime and the laying sequence (Rutkowska & Cichón 2002; Rutstein 2004) and two studies report biased sex ratios at hatching (Kilner 1998; Bradbury & Blakey 1998) which may be due to primary sex ratio adjustment or differential embryonic survival. We investigated the effect of food quality on primary sex ratios and simultaneously increased fe-
male reproductive effort by continuously removing freshly laid eggs (chapter 4). Primary sex ratios were biased towards sons in the group receiving high quality food and biased towards daughters in the group receiving low quality food, but the effect was restricted to a subset of eggs produced (chapter 4). The direction of the bias was opposite to what was expected based upon previous research (Kilner 1998; Bradbury & Blakey 1998), but, taking into account other recent studies (Rutkowska & Cichón 2002; Rutstein 2004), the effects are not consistent: In one study sex ratios increased with the laying or hatching order under both treatments (Kilner 1998), in another study sex ratios increased with the laying order under low food quality but not high food quality (Rutkowska & Cichón 2002) and in a third study offspring sex ratios increased with the laying order when food quality was low and decreased when food quality was high (Rutstein 2004).

We found no evidence that treatment of females with estradiol leads to a bias of the primary sex ratio (chapter 5). Biased secondary sex ratios were due to sex-biased offspring survival (see below).

In conclusion, there is little evidence that parental attractiveness and maternal hormonal status influence offspring primary sex ratios. The feeding regime and the laying order had effects on offspring primary sex ratios in several recent studies and our studies also find effects of the feeding regime and the laying order on the primary sex ratios, but the direction of the bias is inconsistent between studies. This may be due to differences in the type and timing of manipulation and requires further investigation.

Numbers: Sex-specific survival

Analysis of survival in this thesis was restricted to the period of nutritional dependence. We distinguished between survival during the embryonic stage, which is influenced by egg quality and the incubation pattern and survival during the nestling stage, which can also be influenced by differential parental investment such as brooding or feeding.

Other studies investigated differences in survival of sons and daughters after independence: average life span of males and females in the wild is the same (Zann 1996), although adult sex ratios in the wild are usually slightly and consistently, but not significantly male-biased (average 52%, Zann 1996). Female zebra finches raised in enlarged broods have higher mortality after independence than males, but the actual cause of mortality is unknown (de Kogel 1997). In an aviary study, attractive leg-band colours increased survival of males, perhaps due to reduced reproductive effort of these males (Burley 1985a), but no effect of leg-band colour on survival has been observed in the wild (Zann 1994).
Embryonic survival Survival before hatching depends on parental investment in male and female eggs, and on differential vulnerability of sons and daughters to environmental conditions. We investigated sex-differences in embryonic survival of offspring in relation to the attractiveness of the father (chapter 2, chapter 3), treatment of mothers with estradiol and treatment of eggs with testosterone.

Chapter 2 provides no evidence that offspring sex ratios are correlated with paternal attractiveness, as the males wearing leg bands of intermediate attractiveness (orange) produce the most female-biased sex ratios. Our experiment lasted 3 months, while the original study took 22 months (Burley, 1986). In that study, offspring sex ratios fluctuated widely over time and the patterns were different between leg-band colour. This may explain why the sex ratios of orange-banded males in our differ from those of orange-banded males in Burley’s study and suggests that leg-band colour influences offspring sex ratio not only via their effect on paternal attractiveness but also via effects on other features, such as competitiveness, and that their effect changes over time, due to e.g. experience or season.

Elevation of yolk testosterone in eggs of attractive males may lead to male-biased secondary sex ratios by increasing survival of sons relative to survival of daughters. Our studies support observations that testosterone levels are elevated in eggs of females paired to attractive males (Gil et al. 1999)(chapter 3) and that secondary sex ratios are male-biased (Burley 1981; Burley 1986c; chapter 2). Experimental elevation of yolk testosterone indeed may influence sex-specific embryonic survival (chapter 6). Embryonic survival of sons in the control group was on average 60% that of daughters while the survival in offspring from testosterone-treated eggs did not differ between the sexes. Although these difference were statistically not significant, the average effect was large, and the possibility of a sex-specific effect of yolk testosterone on embryonic survival should be investigated with a larger sample. Females paired to attractive males deposit more yolk androgens in their eggs and, in some studies produce male-biased secondary sex ratios. If yolk testosterone indeed elevates embryonic survival of males, this could lead to a bias of the secondary sex ratio. As our studies (chapter 2, chapter 3) found no evidence that male attractiveness affects offspring sex ratios, the relation of male attractiveness, yolk testosterone and offspring sex ratios remains unclear.

In another experiment (chapter 3), females were paired either to the males they preferred or the males they rejected in a mate choice tests. We found no effect of paternal attractiveness on sex-specific offspring survival, but in both groups survival of male offspring was lower than survival of female offspring during the embryonic stage, although the difference did not reach significance ($p = 0.06$).
Chapter 5 shows that sex differences in survival can be hormonally modulated: elevation of 17-β-oestradiol in the maternal circulation increased embryonic survival of daughters at the cost of survival of sons. As primary sex ratios were not biased, this effect on survival led to the overproduction of daughters at fledging in our study (37.7% males) and, most likely also in a previous study (27.6% males) which used the same treatment but did not analyse primary sex ratios (Williams 1999). Because we manipulated hormone levels in the mother, not in the egg, it remains unclear, whether this effect is due to hormone titer in the egg, or to other egg components or to parental incubation behaviour. Sex differences in embryonic survival have been observed in other studies, showing lower embryonic survival of daughters in tree swallows (Whittingham & Dunn 2002) or of sons in zebra finches (Rutkowska & Cichón 2002).

We did not find evidence that parents adjust egg composition with respect to its sex, but we investigated only differences in egg weight (chapter 3; chapter 4; chapter 5). Studies in other bird-species found such adjustment of egg quality with respect to offspring sex: American kestrels (Weathers et al. 1997), brown song larks (Magrath et al. 2003) and spotless starlings (Cordero et al. 2001) lay smaller eggs for the sex which is ultimately larger, while white-crowned sparrows (Mead et al. 1987) and house sparrows (Cordero et al. 2000) produce smaller eggs for the sex which is ultimately smaller. It is unclear whether the differences in egg mass or size are due to a difference in the amount of yolk or albumen. This distinction is important, because the yolk is laid down before meiosis and possibly sex determination while the albumen is laid down after meiosis and fertilization and might therefore be influenced by the sex of the developing embryo. Egg composition, such as antibodies or hormones can vary with respect to offspring sex. In barn swallows, more antibodies were found in female eggs (Saino et al. 2003). In peacocks, male eggs contained more androgens than female eggs (Petrie et al. 2001; but see Eising et al. 2003). Such sex differences in yolk size and yolk composition do not imply that parents can detect offspring sex before hatching, because yolk size and composition may also determine the probability for an egg to be female or male.

It has also been suggested (Clotfelter 1996), that parents could adjust investment with respect to offspring sex without the need of recognising the sex of individual eggs if the sex ratio varies with respect to the laying order and parental investment, such as onset of incubation also varies with respect to the laying order. We observed the possibility for such an effect in chapter 3: offspring sex-ratio of only the first egg was strongly male biased and embryonic survival increases with the position in the laying when fathers were unattractive and did not vary across the laying order when fathers were attractive. This did not result in a bias of the secondary sex ratio, because only the first egg was male-biased and for this egg, survival did not differ with respect to treatment.
In conclusion, our results show that differential investment before hatching can indeed be important for avian sex allocation and the fact that manipulation of maternal plasma hormones and yolk hormones can modify the sex-specific survival patterns indicate that they may provide a flexible pattern to adjust investment with respect to offspring sex.

**Nestling survival** After hatching, sex specific survival can be affected by sex-specific parental investment and by differential vulnerability of male and female offspring to, e.g., food shortage, cold or diseases.

Our repeat of Burley’s study (chapter 2), gave some support to the idea that parental leg-band colours affect offspring secondary sex ratios, because sex ratios at independence varied from 39% (orange-banded fathers), to 50% (green-banded fathers) and 59% (red-banded fathers), although these differences were not statistically significant due to low sample sizes and therefore low power. Because primary and hatching sex ratios were unbiased for green-banded and red-banded males (see above), the deviating secondary sex ratios of red-banded males were due to sex-biased survival as Burley had originally suggested, although the effect was statistically not significant in our study. Green-banded males had the heaviest nests and red-banded males had the lowest hatching success, suggesting that parental investment differed in relation to paternal leg-band colour which could cause sex-specific survival. The results suggest that the failure of studies using individual pairs in breeding cages (chapter 3; Zann & Runciman 2003; but see Dijkstra et al. 2000) to find effects on the sex ratio, may be due to the possibility for mutual mate choice and male-male competition in an aviary setting.

In the experiment described in chapter 3 nestling survival of sons was significantly lower than that of daughters resulting in a non-significant female bias at independence in both pairs with attractive and unattractive fathers. In other studies survival of both sexes was similar (Bradbury & Blakey 1998) or lower in male than in female nestlings (Kilner 1998) when food was unrestricted and lower in female than in male nestlings when food was restricted (Kilner 1998; Bradbury & Blakey 1998). We therefore expected lower survival of daughters due to reduced paternal investment of males in the group with attractive fathers but the *ad lib.* feeding may have precluded such an effect.

Increased levels of yolk testosterone in eggs laid by females paired to attractive males may influence nestling survival by their effects on begging behaviour (Schwabl 1996a; Eising et al. 2001; chapter 6). Testosterone treatment led to a delay in early nestling mortality of both sexes (chapter 6). Furthermore, there were sex-specific effects on begging and growth (chapter 6): in the control group sons begged more than daughters and grew more rapidly. Elevation of yolk testosterone increased begging and growth only in daughters, while beg-
CHAPTER 8

ging of sons from testosterone-treated eggs was not affected and their growth even reduced.

Sex differences in begging would allow active discrimination by the parents. Balda and Balda (cited in Burley 1986c) observed that begging calls of male and female nestling zebra finches differ. Burley (1986c) suggested that offspring sex ratios were male biased due to selective rejection of female offspring, because sex ratios were biased only in broods with offspring mortality. It will be important to investigate whether parents can recognise the sex of their offspring and selectively reduce investment in offspring of a certain sex as suggested by Burley (1986c). Clotfelter (1996) found that male parents invest more in male biased broods. But there is no direct experimental proof that parental care is affected by the recognition of offspring sex in zebra finches.

If the sexes differ in vulnerability to changes in food allocation, changes in parental investment can result in sex-specific mortality even without active discrimination of sons and daughters and several studies indicate that zebra finches daughters indeed grow and survive less well when conditions during rearing are suboptimal (de Kogel 1997; Kilner 1998; Bradbury & Blakey 1998; Martins 2004). Elevated yolk-testosterone levels in eggs of females paired to attractive males may therefore benefit daughters more than sons in terms of begging and growth, because they suffer more from potentially reduced parental investment of attractive fathers. Such a compensatory function of increased yolk androgens for daughters is consistent with the observation that yolk androgens increases for female eggs with the position in laying sequence, but decrease for males (Rutstein 2004), because last hatchlings suffer most from sibling competition.

Active discrimination of the offspring sexes is possible in species, where offspring signal their sex to parents. In barn swallows (Saino et al. 2003) and red-winged black birds (Teather 1992) male and female nestlings differ in begging behaviour, presumably due to their different size at that age. In some species it has been found that parents feed male and female offspring different amounts (budgerigar Stamps et al. 1987; Stamps 1990) or quality of food (Teather & Weatherhead 1988; Magrath et al. 2004). In some species mothers and fathers differ in how much food they allocate to sons and daughters (reviewed in Lessells 1998) and/or parental investment can depend upon the offspring sex ratio (Stamps et al. 1987; Droge et al. 1991; Nishiumi et al. 1996; Clotfelter 1996).

On the other hand, even in the absence of parental influences, nestling zebra finch females grow less well than males when food is reduced (Martins 2004), so that lower nestling survival of daughters (Kilner 1998; Bradbury & Blakey 1998) is not necessarily due to parental discrimination of sons and daughters. Differences in growth between sons and daughters can be related to sex-specific mortality due to a higher mortality of the larger sex which re-
quires more resources (Teather & Weatherhead 1988; Krijgsfeld et al. 1998), or due to a higher mortality of the smaller sex, because it is less competitive (Dhondt 1970; Oddie 2000). Often these difference in growth and/or survival increase when the nestling conditions are more harsh, e.g. when brood size is enlarged, females are unexperienced or food availability is reduced (reviewed in Clutton-Brock et al. 1985; Clutton-Brock 1986).

The available data on zebra finches clearly show that the sexes can differ in survival during the nestling stage and that the direction or strength of differences in survival of sons and daughters depend upon the feeding regime experienced during breeding. Our studies do not provide evidence for effects of yolk hormones on sex differences in nestling survival, but the sex-specific effects on begging and growth suggest that sex differences in survival may emerge when sibling competition is enhanced or food availability is reduced.

Reproductive success

The last experiment of this thesis (chapter 7) aimed at investigating the long-term effects of elevated yolk testosterone on offspring attractiveness and reproductive performance. Elevation of yolk testosterone increased attractiveness of both sexes, but no differences in morphology were detected that might explain the effect on attractiveness. The treatment had sex-specific effects on reproductive behaviour: males hatching from eggs with elevated testosterone levels produced less undirected song during the first days of a breeding attempt and showed less nest-building activity, but their nests became heavier than those of control males. Female zebra finches normally do not sing and the treatment did not induce song. Nest-building behaviour of females was not affected. The specific effect on undirected male song is interesting, because the neural activity during undirected song and directed song differs (Jarvis et al. 1998; Hessler & Doupe 1999). Undirected song appears to be more dependent upon androgens and directed song more on estrogens (Pröve 1974; Arnold 1975; Harding et al. 1983; Walters et al. 1991). Undirected song induces females to stay in the nest and is correlated with rates of extra-pair copulation (Dunn & Zann 1996a; Dunn & Zann 1996b), but the actual function of undirected song is not known. The increased but less efficient nest building of control males during the first days after pairing suggests that nest-building activity may partly have a courtship function and therefore not result in actual increase in nest material accumulated in the nest.

Males from testosterone-treated eggs also started incubating later than males from control eggs, while there was no effect on females. There were no differences in reproductive performance in terms of latency to lay, clutch sizes and egg weights (chapter 7), but egg fertility, hatching and fledging success was not investigated.
What may be the mechanism mediating these effects of early testosterone exposure?

Early exposure to hormones often has specific effects on certain behaviours, while others are unaffected (Adkins-Regan et al. 1987; Adkins-Regan 1999), which may be due to specific effects on different brain circuits. In our experiment, only undirected song is reduced in males hatching from testosterone-treated eggs, which may be due to a specific influence on the song structures influencing undirected song. During undirected song in zebra finches, gene expression (immediate early gene ZENK) is higher than during directed song in the brain areas which are most important for song learning (Area X, MAN and RA) (Jarvis et al. 1998). Early exposure to dihydrotestosterone, a metabolite of testosterone, can increase the size of the high vocal centre, in which the neural pathways of the song system originate (Gurney 1982). Besides this experimental evidence, correlational evidence suggest a link between maternal androgens and neural structures: last hatching male zebra finches, which are presumably exposed to low androgens, because of the decrease with the laying order (Gil et al. 1999), start earliest to learn song and produce the best imitation of their father’s song (Tchernikovski 1999). These effects on the HVC is on the first sight not consistent with our finding that there was no effect of early testosterone treatment on the frequency of directed song, but only on undirected song. However, we did not investigate the similarity of the song of our males with their father’s song, and the other studies did not compare directed song and undirected song or their respective neural structures.

There is large variation in male mating behaviour:

“Some perform exaggerated greetings and begin the stage 1 courtship waltz..., others simply confront the female and direct a number of song phrases at her, and some mated males omit all courtship and simply jump straight on the female’s back without preliminaries” (Zann 1996).

Our results indicate that maternal androgen may modify such male reproductive behaviour. Within-brood variation in reproductive behaviour of sons and daughters may adaptively ensure that parents produce different types of offspring each of which performs best under a different circumstance. Between-clutch variation of androgens may adjust offspring behaviour to the given circumstances, but the adaptive benefits of long-term consequences of variation in maternal androgens still remain to be demonstrated.

Our study shows that natural variation in exposure to androgens can have long-term effect on offspring reproductive behaviour. The modulation of offspring phenotype by secretion of hormones into avian eggs is a fascinating and promising new research field, in which both the adaptive consequences and the
actual physiological, neural and behavioural mechanisms require attention.

**Perspectives and Conclusions**

A major challenge for studies on sex allocation in zebra finches remains the difficulty to establish the costs and benefits of producing sons and daughters under field conditions by assessing the effects on parental and offspring fitness. In the laboratory it will be especially interesting to further investigate the effects of feeding regime on primary sex ratios, because this may allow to investigate the mechanisms of primary sex ratio adjustment in more detail.

The most exciting result of this thesis are the short- and long-term effect of elevated yolk testosterone on offspring development and behaviour. Variation in yolk testosterone is thought to represent an adaptive adjustment to variation in conditions the offspring will face. Therefore it is likely that it depends upon the actual conditions whether the effects of high or low levels of testosterone are beneficial or detrimental for offspring. Such interactions between environmental quality, parental genetic quality and parental investment require more attention. The early sexual maturity of zebra finches, makes them an good laboratory model to investigate the long-term and even intergenerational consequences of maternal effects.

A particularly important area requiring further research appears to be the degree of control that parents and offspring respectively exert over avian sex allocation, which is not well studied neither from a theoretical nor from an empirical point of view (e.g. Krackow 2002, Pen 2000). The expressions ‘sex ratio manipulation’, ‘golden eggs’ (Gil 2003) or ‘mother knows best’ (Schwabl 1998) assign all control over differences in offspring quality to parents and in particular mothers which may be unjustified given the large potential for offspring influences as first suggested by Trivers (1974).

Similar studies performed at different locations with different populations of zebra finches have not produced consistent results with respect to offspring sex ratios. This may be due to varying domestication effects and it would be good to repeat studies using zebra finches from the same stock to ensure that this leads to more consistent results. Domesticated zebra finches will differ in many aspects from wild zebra finches. If stocks of zebra finches vary greatly in general traits such as mate choice or sexual dimorphism, then findings from one study cannot easily be generalised and used as assumptions in other studies and similar results are not even expected.
What can we conclude regarding the proximate control of sex allocation in zebra finches? The three main questions posed at the outset were:

1. are there robust effects of parental traits and the feeding regime on offspring sex ratios?

2. at which stage do differences in offspring sex ratios emerge?

3. are maternal hormones involved in sex allocation?

The fact that primary sex ratio adjustment has been observed in this thesis, adds to the evidence that birds are able to overcome the Mendelian constraints of genetic sex determination. Doubts remain regarding whether primary sex ratio adjustment is indeed a mechanism to adaptively adjust sex allocation because the direction of primary sex ratio biases remains unpredictable. It has repeatedly been suggested (Palmer 2000; Krackow 2002; Ewen et al. 2004) that significant findings of avian primary sex ratio adjustment may represent publication bias towards significant results and may represent the 5 or less percent of studies that are expected to yield an accidentally significant result. The effect of food quality on offspring primary sex ratios in zebra finches is one of the most frequently investigated questions in the sex-ratio literature. Three published studies (Rutkowska & Cichón 2002; Arnold et al. 2003; Rutstein 2004) and the study in chapter 4, found effects of experimental manipulation of the feeding regime on primary sex ratios, although the effect depended upon the laying order or clutch size or was only present in a subsample. If the four studies represent the studies falling outside the five-percent significance level, there should be about 80 unpublished studies that found no effect of the feeding regime on primary sex ratios in zebra finches. We are in contact with most research groups that investigate sex ratios in zebra finches and are not aware of any other studies on this topic. It seems therefore very unlikely that the evidence for primary sex ratio adjustment in relation to the feeding regime represents publication bias.

While the evidence for primary sex ratio adjustment can still be considered somewhat controversial, secondary sex ratios very frequently deviate from unity due to sex differences in survival during early development. The substantial evidence for secondary sex ratio adjustment in birds has been repeatedly reviewed (Clutton-Brock et al. 1985; Clutton-Brock 1986; Hardy 2002). In zebra finches reduced survival of daughters, when food quality or food availability is low, is well supported by experiments (Kilner 1998; Bradbury & Blakey 1998; Birkhead et al. 1999). Sex specific survival can be an adaptive mechanism to adjust the secondary sex ratio, if the costs of offspring mortality is relatively low. Such a situation is observed in chapter 4, where overall mortality was similar between treatments, and more females than males died in the control.
group, while more females than males died in the experimental group. Mate attractiveness influences secondary sex ratios, but this is restricted to a situation allowing mate choice and competition (chapter 2), and does not occur in individual breeding cages (chapter 3).

We provide evidence that the effects of early conditions depend upon the sex of the offspring. Maternal estradiol treatment reversed differences in embryonic survival. Embryonic treatment with testosterone abolished sex differences in begging and growth that were present in the control group. Natural variation of hormone secretion into the egg has important consequences for offspring development, not only during the embryonic and early nestling stage, but lasting into adulthood. These effects can be sex-specific. Variation in maternal hormones can thus play an important role in avian sex allocation, both by adjusting sex-specific survival and by affecting behaviour and morphology in a sex specific way, with potential consequences for offspring reproductive success.