Chapter 1

Introduction

$$\frac{\Delta m}{m} + \frac{\Delta f}{f} > 0$$

"Selection favours a mutant gene which alters various life history parameters if the percent gain in fitness through one sex function exceeds the percent loss through the other function."

Charnov(1982) ***The Theory of Sex Allocation***

Introduction

Sex allocation is the investment in reproduction through male and female function (Charnov 1982). Parents can either produce different **numbers** of sons and daughters (the primary sex ratio) or produce sons or daughters of different **quality**, by allocating more resources such as egg proteins, lipids or food to one sex (Charnov 1982; Stamps 1990; Lessells 1998). Allocation of resources can differentially affect survival of sons and daughters and thereby secondary sex ratios or it can influence their reproductive success by enhancing or reducing fecundity, attractiveness, *etc.* The mechanisms involved in adjusting sex allocation have remained rather elusive (Krackow 1995a; Lessells 2002; Pike & Petrie 2003).

The topic of this thesis are the mechanisms of sex allocation in birds, using the zebra finch as a model, with a focus on the potential role of the maternal endocrine state in avian sex allocation. The idea that maternal hormones play a role in avian sex allocation originated from the fact that a number of factors, such as laying sequence, mate attractiveness and time of year can affect both offspring sex ratios and maternal endocrine state, as reflected in hormones transferred to the eggs, although these factors have not been investigated simultaneously (Burley 1981; Burley 1986c; Dijkstra *et al.* 1990; Schwabl 1993; Schwabl 1996b; Kilner 1998; Eising *et al.* 2001; Whittingham & Schwabl 2002). Maternal hormones in the eggs affect offspring development and survival (Schwabl 1996a; Sockman & Schwabl 2000; Eising *et al.* 2001) and, if these effects are sex-specific, they may affect secondary offspring sex ratios or differences in reproductive success of sons and daughters.

Sex allocation theory

> *"Sex allocation theory deals with the impact of natural selection on the allocation of resources to male compared with female reproductive function"* (Charnov et al. 1981)

Because half a male genome and half a female genome are required to produce an organism (in diploid species), the sex that is rare at a given moment has the highest chance to find a partner to reproduce. Therefore, frequency dependent selection leads to equal allocation to both sexes and an even sex ratio (Fisher 1930). This is the most important principle governing sex allocation. Deviation from equal allocation may occur whenever there are predictable differences in the relative fitness (*e.g.* chances to survive and reproduce) of sons and daughters due to other influences besides the frequency of the sexes (Charnov 1982). Competition or cooperation of relatives of one sex over mates (local mate competition, Hamilton 1967) or resources (local resource competition, Clark 1978) may lead to overproduction of the sex that competes less or cooperates more. When fitness of male and female offspring is affected differentially by parental investment then parents are expected to invest most in the sex that has the steepest gain in fitness from the investment (Trivers & Willard 1973).

Sex allocation theory has been very successful in predicting qualitative and quantitative variation in sex allocation in invertebrates (Godfray & Werren 1996), but much less so in vertebrates with chromosomal sex determination (Frank 1990; Pen & Weissing 2002). It has been suggested that mechanisms of sex determination constrain the flexibility in sex ratio adjustment (Williams 1979). It is also possible that incorporation of specific features of vertebrate life histories will lead to a better understanding and predictions of vertebrate sex allocation (Frank 1990; Pen & Weissing 2000).

Offspring survival and reproduction is influenced by the offspring's own genes and, given that parents and offspring interact, the genes in its parents (Kirkpatrick & Lande 1989). When selection on traits influenced by genes in parents and offspring differs, this can result in parent-offspring conflict, because offspring are more closely related to themselves than to their parents (Trivers 1974). Sex allocation theory has mostly assigned the sole control of sex ratio adjustment to parents, while also gametes and offspring themselves are likely to exert influence (Trivers 1974; Trivers & Hare 1975; Reiss 1987). The simultaneous and potentially dissimilar selection pressures may lead to a compromise between parental and offspring strategies (Eshel & Sansone 1991; Eshel & Sansone 1994) and reduce the sex ratio bias (Beukeboom *et al.* 2001; Komdeur *et al.* 2002; Seger & Stubblefield 2002).

The interpretation of the currently available data in vertebrates is further hampered by the fact that reported examples could be subject to a publication bias towards significant findings (Csada *et al.* 1996), especially in studies with small sample sizes (Palmer 2000).

Sex allocation in birds has received increased attention in recent years (Clutton-Brock *et al.* 1985; Sheldon 1998; Komdeur & Pen 2002; Hasselquist & Kempenaers 2002). A surge of interest has been stimulated by a number of studies showing adaptive sex ratio adjustment (Dijkstra *et al.* 1990; Daan *et al.* 1996; Komdeur 1996; Komdeur *et al.* 1997) or extremely biased offspring primary sex ratios (Heinsohn *et al.* 1997) and by the possibility to sex offspring already at the stage of egg laying, with the help of molecular techniques (Griffiths *et al.* 1996; Ellegren & Sheldon 1997). Although the number of studies reporting significantly biased offspring sex ratios in birds is rapidly expanding, the results still suffer from a lack of repeatability (Radford & Blakey 2000; Ewen *et al.* 2004). This makes it difficult to design studies that specifically vary conditions and test the predicted effects on sex allocation.

The mechanisms of sex allocation in birds have remained completely in the dark (Pike & Petrie 2003). A model system with predictable and repeatable sex allocation is a prerequisite for experimental studies regarding the mechanism of sex allocation.

Mechanisms of sex allocation

Differential sex allocation can be achieved by the production of different numbers of sons and daughters through an adjustment of the primary or secondary sex ratio or by differential investment in the growth and quality of sons and daughters. There is evidence for all these forms of sex allocation in birds (Lessells *et al.* 1998; Hasselquist & Kempenaers 2002; Pike & Petrie 2003). Available knowledge on the mechanisms of primary and secondary sex ratio adjustment in birds is extremely limited (Krackow 1995a; Pike & Petrie 2003). Adjustment of the sex ratio at the egg stage could be achieved by differential chromosomal segregation, differential growth of follicles depending upon their sex, differential fertilisation, or by differential atresia after fertilisation. Differential fertilisation and atresia requires the resorption of follicles and thereby leads to laying delays and gaps in the laying sequence. Laying delays (due to decrease of fitness with the progress of season) and laying gaps (due to the disadvantage of late hatching young in competition with older siblings) may be so costly that they preclude adaptive sex ratio adjustment (Pen *et al.* 1999) or may allow only a bias of the first egg of a clutch, as has indeed been observed in a number of species (Emlen 1997). Currently evidence for the occurrence of any of these mechanisms in birds is lacking.

Sex allocation can take also the form of differential investment in sons and daughters before or after hatching. This may affect fitness by leading to differential survival, or differential reproductive success. There is ample evidence that this occurs in birds (Stamps *et al.* 1987; Stamps 1990; Droge *et al.* 1991; Clotfelter 1996; Lessells *et al.* 1998; Magrath *et al.* 2004). An extreme form of postnatal sex allocation is the killing of daughters in various human societies (*e.g.* Darwin 1874). Such differential allocation is best understood in species where the sexes differ in size and hence in energy requirements for growth till independence. Empirical observations show that the offspring of the larger sex receive more investment, but have lower fitness and that the fitness differential increases with increasing dimorphism (Maynard Smith 1982; Lessells 1998), which is consistent with models assuming unbiased primary sex ratio and sex-biased parental investment. Differential parental investment can more generally be predicted when offspring fitness depends differentially on environmental conditions for sons and daughters (Trivers & Willard 1973). The quantitative prediction for sex allocation depends strongly on the actual fitness curves, which are exceedingly difficult to determine (Lessells *et al.* 1998).

Endocrine involvement in avian sex allocation

Maternal endocrine state has been suggested to play a key role in sex allocation in mammals and birds (James 1986; Krackow 1995a; Pike & Petrie 2003). Follicular development, yolk deposition and atresia are hormonally regulated (Sturkie 1986), and several factors that influence offspring sex ratios are correlated with variation in maternal hormones, such as season, laying sequence, mate attractiveness, food quality (Krackow 1995a; Pike & Petrie 2003). Eggs with male embryos and eggs with females embryos have been found to differ in the amount of various hormones (Petrie *et al.* 2001; Müller *et al.* 2002) and manipulation of the maternal hormonal status has resulted in a biased secondary sex ratio (Williams 1999) and primary sex ratio (Veiga *et al.* 2004).

Maternal hormones are produced in different endocrine glands in the body and diffuse into the maternal circulation and into the egg and can be transferred from the maternal circulation into the egg, although apparently only a minor amount follows this route (*ca.* 0.04 – 0.1 % Arcos 1972; Adkins-Regan *et al.* 1995; Hackl *et al.* 2003). It is an old idea that maternal hormones can influence offspring sex in birds (reviewed in Taber 1964). This usually has been attributed to – temporary and pathological – phenotypic sex reversal due to exposure of estrogens. Endogenously produced estrogens in genetically female embryos induce sexual differentiation of the female phenotype as shown by elevation or reduction of estrogen exposure during embryonic development through manipulation of maternal endocrine state, injection of hormones or hormone blockers into the egg or by gonadectomy before and during sexual differentiation (Kozelka & Gallagher 1934; Riddle & Dunham 1942; Wolff & Wolff 1951; reviewed in Taber 1964; Adkins 1975; Adkins 1979; Schumacher *et al.* 1989; Aste *et al.* 1991; Balthazart *et al.* 1992; Balthazart & Ball 1995; Adkins-Regan *et al.* 1995).

This role in female sexual differentiation may be the reason why estrogen levels in avian yolk are relatively low to undetectable (Schwabl 1993; Lipar *et al.* 1999). Other steroid hormones, such as the androgens testosterone, dihydrotestosterone, androstenedione and progesterone and thyroid hormones are present in much larger amounts (Schwabl 1993; McNabb & Wilson 1997; Hackl *et al.* 2003). The idea that maternal deposition of hormones into the egg is not pathological but can subserve an adaptive function was first suggested by the finding that yolk testosterone levels in canary eggs correlated with social rank of offspring at 7 – 20 weeks of age and increased offspring begging and growth (Schwabl 1993; Schwabl 1996a). There are very few experimental studies on the effects of natural variation in yolk testosterone levels on offspring morphology, physiology and behaviour and their potential role in sex allocation by exerting sex-specific effects on traits influencing offspring survival and reproduction.

Sex allocation in the zebra finch Taeniopygia guttata

The zebra finch is an attractive model organism to study avian sex allocation as it varies sex ratios with respect to a number of factors, such as artificial parental ornaments, food quality and laying order (Burley 1982; Burley 1986c; Kilner 1998; Bradbury & Blakey 1998; Rutstein 2004). Also, the survival and quality of sons and daughters is differentially affected by environmental conditions (de Kogel 1997; Kilner 1998; Birkhead *et al.* 1999; Martins 2004). Posthatching parental investment may also depend upon offspring sex (Burley 1986c; Clotfelter 1996). Zebra finches are particularly suited for experimental research regarding mechanisms of sex allocation as they breed easily in captivity and have been widely used in research on avian behaviour, physiology and ontogeny, especially in the context of sexual differentiation (Morris 1954; Immelmann 1962a; Immelmann 1962b; Immelmann 1963; Gurney 1982; Kruijt *et al.* 1983; Kruijt & Meeuwissen 1993; Adkins-Regan *et al.* 1996; see Zann 1996; Arnold 1997a; Arnold 1997b; Adkins-Regan 1999; Adkins-Regan 2002). As zebra finches adjust offspring primary sex ratio, secondary sex ratio and the quality of sons and daughters, they may allow to study simultaneously the respective roles of these different ways of adjusting reproduction through male and female function.

A possible role for hormones in zebra finch sex allocation is suggested by the fact that mate attractiveness and laying order influence sex allocation in the zebra finch (Burley 1981; Burley 1986c; Kilner 1998) and also maternal hormone deposition in the eggs (Gil *et al.* 1999; Ward *et al.* 2001), and that treatment with the hormone estradiol led to female-biased offspring sex ratios (Williams 1999).

Outline of the thesis

This thesis has two main goals. Firstly, it repeats previous research to establish the robustness of reported effects of various factors on sex allocation. Secondly, it explores the possible role the maternal endocrine state plays in sex allocation by investigating hormonal effects on primary or secondary sex ratio and the quality of sons and daughters.

In the first three chapters, external factors are studied that in previous studies affected offspring sex ratio and maternal hormone deposition in eggs. As in these previous studies only secondary sex ratios (at hatching and at independence) were studied, we analysed also primary sex ratios (at laying) by molecular sex determination using embryonic tissue.

In **chapter 2**, we study the effect of mate attractiveness or dominance, manipulated by artificial ornaments. In an intriguing series of studies, Nancy Burley (Burley 1981; Burley *et al.* 1982; Burley 1985b; Burley 1986b; Burley 1986c; Burley 1988b) reported that red leg bands render male zebra finches more dominant and more attractive to females than green leg bands, and at the same time cause their female partners to produce more surviving male offspring. This finding, while originally meeting some scepticism (Immelmann *et al.* 1982; Thissen & Martin 1982), has loomed large over the field, and we decided to repeat the study on a large scale in an international multilaboratory setting, together with the Universities of Bielefeld and Melbourne. In addition to the secondary sex ratio, we study the sex ratio at the egg stage and at hatching and several other parameters of reproduction in this large multi-aviary experiment.

In **chapter 3**, the effect of male attractiveness on offspring primary sex ratios and sex-specific survival is investigated, by allowing female zebra finches to exhibit their preference to associate with a certain male in two-way choice tests and assigning them to breed with their preferred or their unpreferred male.

In **chapter 4**, we concentrate on a different external factor, the feeding regime before and during breeding, which frequently has been shown to affect both primary sex ratios and sex-specific survival. This effect may be mediated by the food directly or via an effect on female condition and reproductive effort. We manipulate food quality and reproductive effort simultaneously, by continuous egg-removal, which induces females to lay extended clutches, while giving food of low and of high quality.

In **chapter 5**, we manipulate the maternal hormonal state by injections of 17-β-estradiol, which has been found to influence secondary sex ratios in zebra finches (Williams 1999) and analyse whether offspring primary or secondary sex ratio is adjusted.

Females deposit hormones in the egg and **chapter 6** focuses on the short-term consequences of increased yolk androgen levels on begging, growth and survival of sons and daughters.

Yolk hormones may not only affect offspring during the nestling stage but have also long-term consequences on fitness of sons and daughters. In **chapter 7**, we study therefore the long-term effects on offspring attractiveness and breeding performance.

Chapter 8 reviews the current knowledge and insights regarding the function and mechanism of avian sex allocation. It summarises the evidence for sex allocation in zebra finches in the light of the results of this thesis and discusses the potential role of maternal endocrine state in avian sex allocation.