Causes of between-individual differences in mating preferences

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Contents

General introduction

Sexual selection, mate choice and mating preferences

Sexual selection, the intra-specific selection for successful reproduction, is a mechanism that helps explaining the evolution of exaggerated characters that appear counterintuitive, when seen from the natural selection perspective alone (Darwin 1859). Darwin himself proposed sexual selection as an additional force of evolution and dedicated an entire book to it (Darwin 1871). Although conceptually important, there is no strict distinction between natural and sexual selection, since both are, ultimately, about the representation of an individual's own alleles in future generations (Andersson 1994). In this sense, 'survival of the fittest' (Darwin 1859) always pertains to the ability of an individual to leave copies of it's own alleles.

Sexual selection can be conceptually separated into intra-sexual competition and mate choice (Andersson 1994). Intra-sexual competition describes the competition for access to individuals of the other sex and can take different forms (e.g. contest, scramble, endurance rivalry or coercion, Andersson & Iwasa 1996). Competition is usually most intense in the sex that shows greater variance in reproductive success (Bateman's principle, Bateman 1948), which is usually also the sex that invests less in each individual offspring (Trivers 1972). Mate choice, the inter-sexual component of sexual selection, describes the selection of phenotypes imposed by one sex (usually the one with lower variance in reproductive success) on the other (Andersson 1994). The variance in reproductive success is generally higher in systems with multiple matings and hence lower in strictly monogamous species. However, even in socially monogamous species there is often a fair amount of extra-pair paternity, which tends to increase the between-individual variance in reproductive success (Reynolds 1996; Petrie & Kempenaers 1998).

Terminology

In a very broad sense, mate choice or mating biases can be defined as 'a process leading to the tendency of members of one sex to mate non-randomly with respect to one or more varying traits in members of the other sex' (Kokko *et al.* 2003). This definition does not require any active choice and includes 'passive acceptance of the first conspecifics encountered' (Kokko *et al.* 2003). Such a definition, however, encompasses all of sexual selection and is hence too broad to help understanding the mechanisms that lead to non-random mating. A narrower definition of mate choice requires the existence of one or more traits in the choosing sex that lead to non-random mating (Heisler *et al.* 1987; Maynard Smith 1987). This sets the focus on characteristics of the choosing sex, while still covering active forms of choice as well as resistance. Mate choice can be seen as the outcome of matings that arise from mating preferences, i.e. the traits that induce non-random mating (Jennions & Petrie 1997). Throughout this thesis, I have followed this definition and have focused on preferences as a characteristic of the choosing sex.

It is further useful to divide mating preferences into preference functions (i.e. the ranking order of stimuli) and choosiness (i.e. the investment into mating with the preferred stimulus) (Jennions & Petrie 1997; Widemo & Sæther 1999). Widemo & Sæther (1999) have additionally separated the sampling strategy (the 'how to choose') from the 'how much to invest' (choosiness) and 'what to choose' (preference functions). Throughout my thesis, the distinction between preference functions and other aspects influencing mate choice is most important.

Costs and benefits of mate choice

Costs of mate choice

Mate choice necessarily has some costs associated with it. Discrimination against some phenotypes involves the resistance to mating when harassed by members of the other sex (Andersson & Iwasa 1996; Kokko *et al.* 2003). More active forms of mate choice entail searching for mates, which involves investment in terms of time and energy (Alatalo *et al.* 1988; Vitousek *et al.* 2007; Booksmythe *et al.* 2008). Costs of mate choice can be low, if distances to travel between potential mates are short, traits are easy to evaluate and when time and energy constraints are low (Byers *et al.* 2005, 2006; Vitousek *et al.* 2007). Under other circumstances costs might be large and this tends to decrease choosiness (Milinski & Bakker 1992; Backwell & Passmore 1996; Booksmythe *et al.* 2008). The costs associated with mate choice are mainly determined by the ecology of a species or population. It is difficult to quantify the costs of mate choice, because it is often difficult to unambiguously assign some behaviour to the mate choice context. This seems to be

the main reason why we are largely lacking estimates of mate choice costs, although such estimates would be highly valuable (Jennions & Petrie 1997; Kokko *et al.* 2003).

Marginal costs might differ between individuals and this might lead to condition-dependent mate choice. This should mainly affect the choosiness and hence the range of accepted stimuli rather than the preference function itself. However, there are two reasons why it might also affect preference functions: First, if there is strong assortative mating for quality (either because of mutual mate choice or competition within the choosing sex), individuals might benefit from adjusting their preference to stimuli that are achievable (Johnstone 1997). Such anticipated competition would induce condition-dependent variation in preference functions (Burley & Foster 2006). Second, if attractiveness is multidimensional because there are different benefits to be optimised and there is condition-dependent variation in needs (e.g. direct versus indirect benefits), this would also affect how individuals rank opposite-sex stimuli, because they might shift the importance of attractiveness axes. The effect can be strong if these axes are relatively independent, while a correlation between them would diminish this effect.

Benefits of mate choice

For investment into mate choice to be an overall successful strategy, benefits of choice have to outweigh the costs. These benefits can be broadly classified into direct and indirect benefits (Kirkpatrick & Ryan 1991; Kokko *et al.* 2003). Direct benefits help reducing the investment into the current breeding event and hence to increase an individual's prospects for future reproduction. The most obvious forms of direct benefits are nuptial gifts and help in parental care (Badyaev & Hill 2002; Nakagawa *et al.* 2007). But also a high quality territory or protection from harassment by other members of the opposite sex can be considered direct benefits.

Indirect benefits are somewhat less obvious and harder to understand. Nevertheless, they have been an important research focus in the field of sexual selection (Møller & Alatalo 1999). Two main ideas have been put forward by Fisher (1930):

Good-gene indicator hypothesis: Choosing individuals should prefer potential mates that carry alleles for high viability, since this promises increased offspring viability and hence higher (long-term) fitness. Empirically, these effects are small, but significant (Møller & Alatalo 1999). Since viability depends on a large number of loci, good-gene indicators are expected to reflect genome-wide quality (Andersson & Iwasa 1996). Some mechanism has to ensure honesty of the signal, since the invasion of 'cheating' mutations (that produce a strong signal, but are otherwise not associated with genetic quality) will diminish the indicator value of a trait. Honesty is most easily ensure by condition-dependent trait expression (e.g. by indicating resistance to parasites, Hamilton & Zuk 1982) or by constituting a handicap to the carrier (Zahavi 1975).

Sexy-son hypothesis: The sexy-son hypothesis puts the focus more on good-genes for attractive sons and less on viability. The main conceptual difference between good-genes for viability and good-genes for attractive sons, is that the trait does not have to signal genome-wide quality, since the indirect benefits are realised via the mating advantage of the (male) offspring (Andersson & Iwasa 1996). Hence, if there is a population-wide preference for a particular trait, novel mutations that increase the strength of the signal will be selected for independent of their influence on viability. If preference and trait are genetically correlated (e.g. via linkage disequilibrium due to assortative mating), this can lead to runaway selection, where the preferences for and the expression of the trait co-evolve and are driven to extremes (Fisher 1930; Lande 1981). Since sexy-son traits signal quality by promising high fitness via high mating success in male offspring, it has been suggested that the good-gene indicator and the sexy son-hypothesis are not as different as often suggested and might be combined to a 'Fisher-Zahavi hypothesis' of indirect benefits (Eshel *et al.* 2000; Kokko *et al.* 2002, 2003).

The good-gene indicator and the sexy-son hypothesis predict a unifying overall best solution to mate choice. The sexy-son hypothesis requires a heritability of preferences and if preferences are heritable, both hypotheses predict a genetic correlation between preferences for and expression of a trait.

Other causes of mating preferences

Preferences might have been selected for in contexts other than mating and hence be due to sensory bias (Ryan 1998; Fuller *et al.* 2005). For example, a preference for orange spots in guppies and sticklebacks seems to have been shaped by a preference for colourful food items (Rodd *et al.* 2002; Smith *et al.* 2004). Mating preference might also originate from apparently non-adaptive sensory biases (Burley *et al.* 1982; Ryan *et al.* 1990; Ryan & Rand 1990; Basolo 1995). Holland & Rice (1998) have proposed an antagonistic chase-away model of sexual selection, in which members of the chosen sex exaggerate traits for purposes of sensory exploitation, while the choosing sex develops a resistance to these stimuli (since they may convey costs, but no benefits) and this can lead to an acceleration of the process very much like in a Fisherian runaway scenario.

Between-individual differences in mating preferences

Most of the ideas described above suggest that all individuals should aim at mating with the same superior individual(s). Empirical studies, however, have shown that this is often not the case (Bakker & Pomiankowski 1995; Jennions & Petrie 1997; Widemo & Sæther 1999; Brooks & Endler 2001; Brooks 2002; Forstmeier & Birkhead 2004). This is at first glance a puzzling situation. However, there are potential adaptive explanations

for between-individual differences in preferences (Figure 1). Furthermore, it is useful to study the proximate causes in order to understand the origins of this variation (Figure 2).

Adaptive explanations

If there is non-additive genetic variation in fitness-relevant traits, the interaction between the parental genomes matters for offspring fitness (Zeh & Zeh 1996; Neff & Pitcher 2005). Special cases of genetic compatibility that are well-documented in natural systems are heterozygote advantage (Kempenaers 2007), inbreeding depression (Charlesworth & Charlesworth 1987; Pusey & Wolf 1996) and outbreeding depression (LeBas 2002; Price & Bouvier 2002; Peer & Taborskyi 2005). It is unclear, however, how important mate choice for compatible alleles is independent of an outbreeding-inbreeding axis (i.e. different degrees of relatedness, Tregenza & Wedell 2000). Best evidence comes from MHC-disassortative preferences (Wedekind *et al.* 1995; Penn 2002) and from studies showing that extra-pair young are more dissimilar than within-pair young (Johnsen *et al.* 2000; Blomqvist *et al.* 2002; Foerster *et al.* 2003).

The most likely situation in which mate choice for compatibility might be relevant between unrelated individuals within single populations is when the recombination in chromosomal regions relevant for compatibility is suppressed (Tregenza & Wedell 2000). Additionally, the polymorphism has to be phenotypically expressed, so that it can be detected by the chooser. In mice, for example, females heterozygous for a *t* allele avoid smells of males also carrying a t allele (which is genetically incompatible), while wild-type homozygotes do not (Williams & Lenington 1993). Zeh & Zeh (1996) suggest that by mating multiply, females adopt a bet-hedging strategy against the 'cumulative toll of genetic incompatibility'. Chapters 1, 2 and 3 of this thesis address mating preferences in relation to genetic compatibilities.

Behavioural compatibility seems particularly advantageous in breeding systems with bi-parental care. Consistent behavioural differences between individuals came into focus of behavioural ecology only recently (DeWitt *et al.* 1998; Sih *et al.* 2004a,b; Réale *et al.* 2007). It seems possible that partners of a pair have advantages when behaving similarly, since that might reduce conflict in the pair bond (van Oers *et al.* 2008). On the other hand, disassortatively mated pairs might have an advantage, since the pair might have a larger behavioural repertoire that ensures good foraging success in unpredictable environments (Both *et al.* 2005). Only few studies have analysed behavioural compatibility in pair bonds (Spoon *et al.* 2006, 2007). Chapter 4 addresses questions of behavioural compatibility for exploratory behaviour and activity.

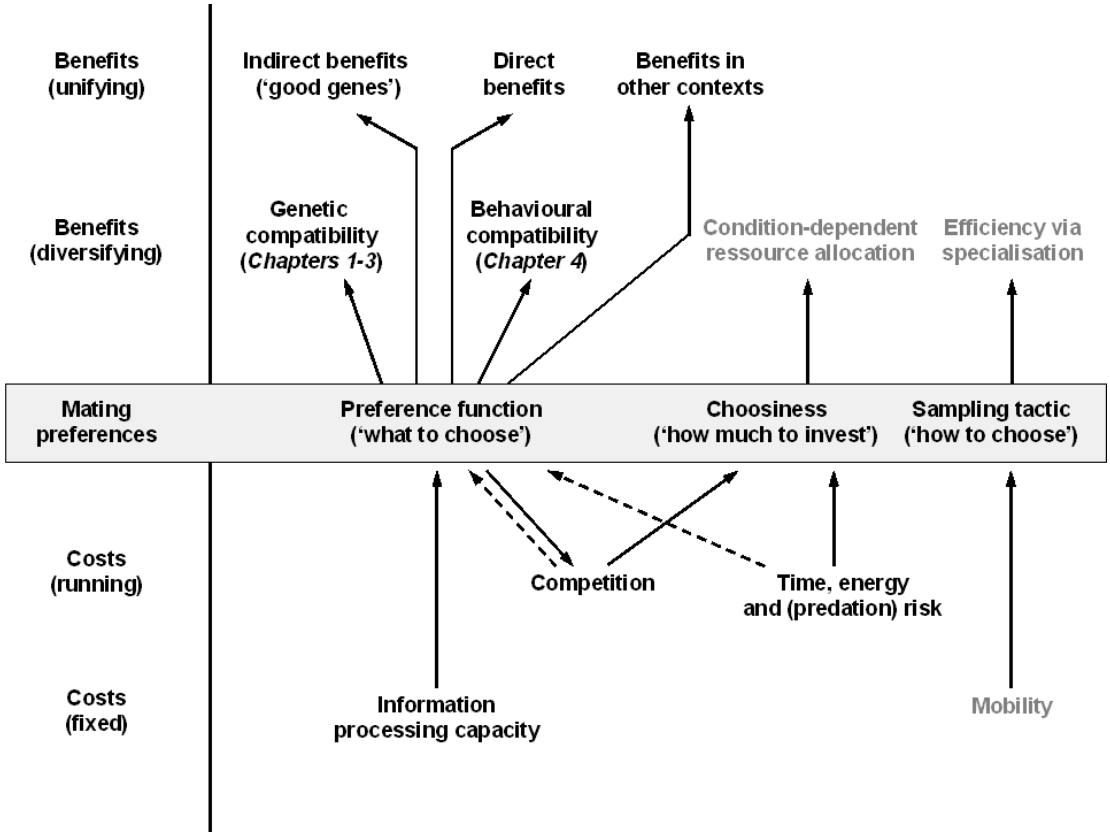

Figure 1: *Costs and benefits of mate choice. The figure illustrates the most important ultimate causes that might produce unifying and diversifying variation in mating preferences.*

Proximate mechanisms for the formation of mating preferences

Between-individual differences in mating preferences can arise from the proximate mechanisms that form preference templates. In my thesis, I address a number of those mechanisms experimentally (Chapters 1, 2 and 3).

Additive genetic determination of preferences is particularly interesting, because it is one of the critical assumptions of Fisherian runaway selection (Fisher 1930). There is very good evidence for innate preferences for discrete variation (between populations or morphs) (Bakker & Pomiankowski 1995). There is also good evidence for heritable variation in choosiness (Bakker 1993; Bakker & Pomiankowski 1995). However, estimates of heritability of preference functions are very scarce and only three out of seven studies report significant results (Collins & Cardé 1989; Gray & Cade 1999; Jang & Greenfield 2000; Brooks & Endler 2001; Iyengar *et al.* 2002; Hall *et al.* 2004; Simmons 2004). This indicates that within-population heritability of preference functions is relatively low and difficult to measure. I therefore approach this issue by estimating the heritability from a

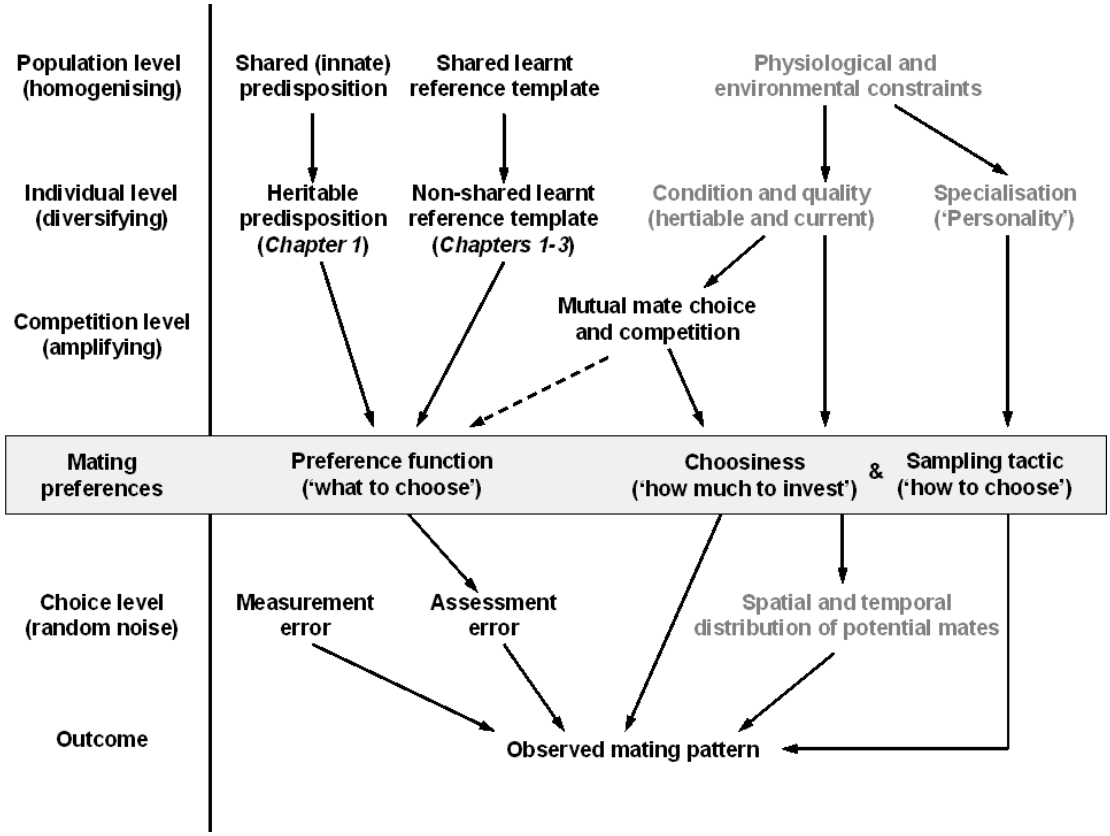

Figure 2: *Proximate causes of observed mate choice. The figure conceptually separates the most important routes that produce unifying, diversifying, amplifying and random noise in observed mate choice outcomes.*

large sample of 44 pairs of sisters tested eight times each for their preference (i.e. a total of 704 trials, Chapter 1).

Sexual imprinting is a mechanism that, if it works perfectly and if egg dumping is negligible, would ensure a vertical transmission just like heritable variation in preferences. Sexual imprinting describes the formation of mating preferences during early development usually by using the own parents as models for preference formation (Bolhuis 1991). Most of the sexual imprinting literature has focused on positive sexual imprinting on heterospecific fosters, morphs and novel-traits (see Chapter 2 for references and discussion). This would serve species recognition and would help avoiding hybridisation and outbreeding depression. However, parents could also be used as negative templates to ensure inbreeding avoidance, a mechanism that has been discussed as 'family phenotype matching' or 'indirect familiarisation' in the kin recognition literature (Nakagawa & Waas 2004). I have applied two rigorous tests for imprinting-based preferences: Once by testing for a similarity in preferences between foster sisters (which

includes negative and positive sexual imprinting, Chapter 1) and once by testing female preferences for sons of the foster parents, which separates positive and negative sexual imprinting (Chapter 2). In both tests I ensured independence from genetically determined preferences by cross-fostering eggs between clutches. Besides one study on sexual imprinting in humans (Bereczkei *et al.* 2004), these are the first experimental tests for sexual imprinting on continuous variation in any animal species.

Individuals will commit measurement errors, when mapping phenotypic traits on an attractiveness or quality axis. Such mapping is particularly difficult, if traits vary in multiple dimensions (Candolin 2003; Fawcett & Johnstone 2003). However, multiple cues might sometimes facilitate choice if they are involved in a hierarchical mate choice process, hence rendering choices more accurate (Backwell & Passmore 1996; Robson *et al.* 2005). Nevertheless, individuals are likely to conduct inaccurate choices that might produce patterns of disagreement between females, if stimulus individuals differ little in their attractiveness. But unless there are between-individual differences in the ability to discriminate between stimuli, the assessment error is expected to produce only random noise.

Studying mating preferences in zebra finches

The ecology of (domestic) zebra finches

Throughout my study, I used the zebra finch as a model organism. The zebra finch is a small passerine bird that is highly gregarious and populates the dry biomes of central Australia (Zann 1996). It forms large flocks during the non-breeding season and is a colonial breeder. Colony sizes ranged between seven and 47 pairs in a sample of ten colonies (Zann 1996). Zebra finches are largely granivorous, and since rainfall and hence seed production is usually unpredictable in its native habitat, zebra finches are very fast and flexible in changing from non-breeding to breeding whenever conditions are suitable (Zann 1996). The zebra finch is very unusual among small, relatively short-lived birds in forming stable pair-bonds across breeding attempts and even across breeding seasons (Black 1996; Zann 1996).

Like many other species of passerines, the zebra finch shows a pronounced sexual dimorphism in plumage colouration and voice (Figure 3). Males have many colourful ornaments: rusty-orange cheek patches, chestnut flanks with white spots, a finely black-and-white barred breast, a bold black breast band and a bright red beak. Females, in contrast, are predominately grey with an orange beak. Both sexes share a marked head pattern with white and black stripes and conspicuous black-and-white uppertail coverts. Only males produce a short, rhythmic song that varies greatly between

individuals, but is highly constant throughout an individual's adult life. The song is learnt from tutors (often the father, but also from other males, Williams *et al.* 1993; Zann 1996) during adolescence, with a sensitive period chiefly between 35 and 65 days of age (Immelmann 1969; Eales 1985; Zann 1996). Calls, most importantly the most common distance calls, also differ between sexes (Zann 1996; Forstmeier *et al.* in press).

Zebra finches have been kept in captivity for more than 200 years (Zann 1996). Up to the 1920ies there was a regular import of wild-caught birds from Australia to Europe and America, but in the 1960ies legal export of wild-caught birds from Australia was stopped officially (Zann 1996). Since then, captive populations have been bred without new imports. Captive zebra finches are usually larger than wild-caught birds and some populations have been selected for unusual colour morphs. A population-genetic study shows that genetic variation in captivity is lower than in the wild, but gene flow between captive populations has ensured that a large genetic variation has been maintained (Forstmeier *et al.* 2007b).

Although, at first glance, it seems inappropriate to study evolution in captive birds, some questions that require careful manipulations and control are much easier conducted in the lab. Importantly, evolution does not stop in captivity as long as there is heritable variation in traits and variation in reproductive success. Heritable variation is always present, unless in a population of clones (when neglecting mutations). Variance in reproductive success, is very difficult to equalise even when special breeding designs are applied (which is sometime done to maintain genetic variation in endangered species, Frankham *et al.* 2004). It was certainly always present when breeding captive zebra finches. However, it is important to consider the selection pressures that have acted in the process of domestication, to understand the results obtained from captive populations. It is usually easier to imagine past selection pressures in the wild, by assuming that the environment has not changed systematically. This is more difficult in captivity, since selection pressures have clearly changed during domestication (e.g. absence of predators) and it is often unclear, how subjects have been bred in the past. It seems possible that breeders have been selecting for fast reproduction and thus against choosy females (see General discussion).

Although very different in a multitude of aspects, there are some interesting similarities between captive zebra finches and humans. First, the environment is largely determined by conspecifics, with little unpredictability in the abiotic and inter-specific environment. Second, the mating system is remarkably similar. Both species form relatively stable pairs bonds, often with life-long social monogamy, but both species also exhibit some unforced divorces. Both species show low, but significant levels of extra-pair paternity and hence, despite biparental care for the offspring, both species show higher variance in reproductive success of males compared to females. Although one

should not over-interpret those similarities, it offers the possiblility to compare between zebra finhces and our own species.

Mating preferences of female zebra finches

Mating preferences of female zebra finches have been studied for quite some time. The focus of attention has been on beak colour and song rate. However, there is no universal trait that makes males attractive to females.

Beak colour varies between orange and red and this has been shown to covary with circulating carotenoids concentrations (Blount *et al.* 2003b; McGraw & Ardia 2003; McGraw *et al.* 2003). Carotenoids have to be acquired with the diet, they have antioxidant properties and are potentially important for immunocompetence (Saino *et al.* 1999; Fitze *et al.* 2007). Indeed, in zebra finches, males with redder beaks have been found to be of better (genetic) quality and to have higher reproductive success (Price & Burley 1994). A number of studies have confirmed a preference of females for males with redder beaks (Burley & Coopersmith 1987; Houtman 1990; Blount *et al.* 2003b). Others, however, did not find overall preferences for redder beaks in experimental (Collins *et al.* 1994) or correlational studies (Burley *et al.* 1994; Collins *et al.* 1994; Forstmeier & Birkhead 2004; Roberts *et al.* 2007) and one experimental study even found a preference for orange beaks (Sullivan 1994). Collins & ten Cate (1996) have reviewed the ambiguous evidence for beak-colour preferences. They suggest that beak colour might be less important than song rate (see below), that the preference depends on female mating experience or that beak colour acts via male-male competition. The last hypothesis has been tested, but was not verified, since beak colour did not correlate significantly with aggression in competition trials (Etman *et al.* 2001; Bolund *et al.* 2007).

Male song rate is another important predictor of male attractiveness (Ratcliffe & Boag 1987; Houtman 1990, 1992; Collins *et al.* 1994; Collins 1995; Balzer & Williams 1998; Forstmeier & Birkhead 2004) and has been suggested to be a confounding mechanism when preferences for other traits are measured (Collins & ten Cate 1996). However, song rate is often measured during mate-choice trails and since song is to a large degree a feedback to female approach (Rutstein *et al.* 2007), high song rates of the preferred males are partly the outcome rather than the cause of the preference (Collins & ten Cate 1996). But even when measured independently of the mate choice trails, song rate has an influence on mating preferences (Forstmeier & Birkhead 2004). However, while females generally tend to spend more time with the high song rate males in the choice chamber, they do not seem to prefer them for extra-pair copulations (Forstmeier 2007).

Some studies have tested if song structure itself matters for mate choice. Females preferred high between-strophe variation over standard strophe repetition in a Skinner box (Collins 1999) and males that had sham surgeries were preferred over males that

had their song distorted by surgery (Tomaszycki & Adkins-Regan 2005). Holveck & Riebel (2007) found that in phonotaxis and operant conditioning tests (where females could hear male song, but could not see males) females preferred high 'relative performance' and 'sound density' (principal components one and two from a analysis of six song parameters). Similarly, zebra finch females preferred longer songs with more elements (Clayton & Prove 1989; Neubauer 1999). There was no indication that females preferred a particular motorically-demanding song motif in a study using manipulated song (Leadbeater *et al.* 2005). While every study reports some significant effect, there is no general consensus about which aspects of song structure might cause mating preferences (Forstmeier *et al.* in press). The large between-individual variation (and the high within-individual consistency) of male song indicate that song might be used as a signal of identity rather than as a signal of quality (Dale 2006).

The effect of other male ornaments on preferences has received much less attention. In a choice experiment with males selected for high and low corticosterone, female preferred low-corticosterone males and males with brighter leg colour and cheek patch colour (Roberts *et al.* 2007). Body size and body mass did not seem to have an effect in correlational studies (Balzer & Williams 1998; Forstmeier & Birkhead 2004; Roberts *et al.* 2007). There are indications that females prefer symmetrical leg band combinations and symmetrical breast ornamentation (Swaddle & Cuthill 1994a,b, but see Jennions 1998).

Interestingly, female zebra finches have been shown to prefer males wearing red leg bands over unbanded or orange-banded birds while green-banded birds were avoided (Burley *et al.* 1982). This finding has been frequently used as an easy way to manipulate male attractiveness (Burley 1986b, 1988; Gil *et al.* 1999; Rutstein *et al.* 2004; Gorman *et al.* 2005; Gilbert *et al.* 2006; Williamson *et al.* 2006). It has been repeated mainly in the Burley population (Burley 1986a; Burley *et al.* 1994, 1996; Hunt *et al.* 1997). However, in other populations (including our) there are apparently no preferences for red-banded males (e.g. Jennions 1998, own unpublished data). Although certainly interesting, these hidden preferences for red rings do not help much in understanding the causes of between-individual differences in mating preferences. Hence, I did not pursue the colour ring manipulation in my thesis.

Some studies have manipulated early condition of nestlings and have found a preference for males from good early-rearing conditions and this has been suggested to act via beak colour (de Kogel & Prijs 1996), cheek patch sizes (Naguib & Nemitz 2007) or song complexity (Spencer *et al.* 2005). Others have failed to find effects of early-rearing (Blount *et al.* 2003a; Naguib *et al.* 2008; Bolund *et al.* in prep.). Collectively, these findings indicate that females may prefer males in good condition (long-lasting effects).

From the above discussion of what makes male zebra finches attractive to females (see Figure 3 for a summary) it becomes clear, that the topic is not fully understood. Also the low between-female repeatability of mating preferences (Forstmeier & Birk-

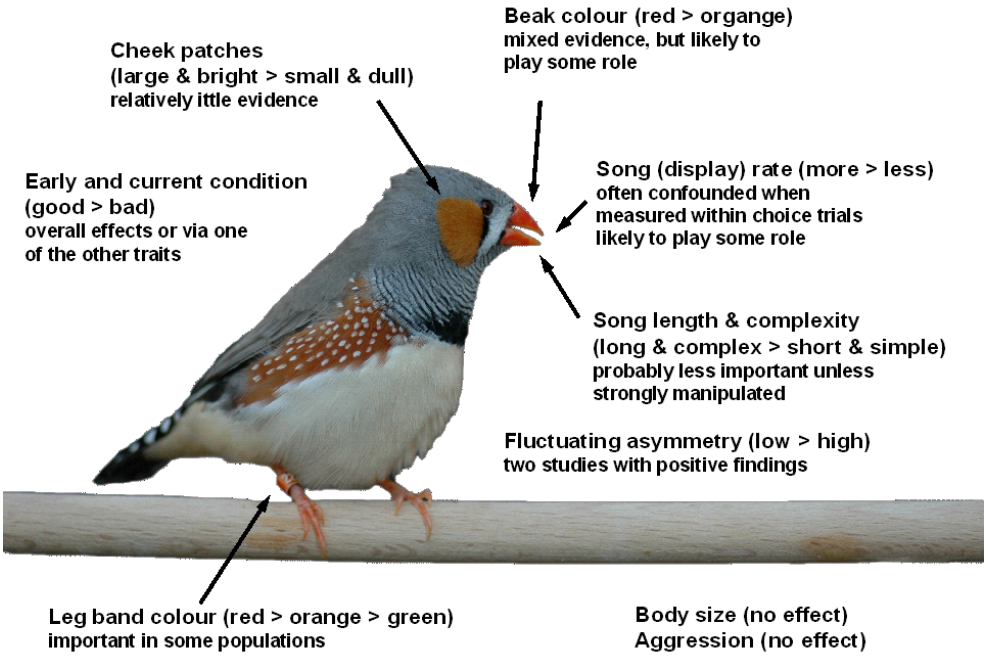

Figure 3: *Summary of traits that make male zebra finches attractive to females. For details see text.*

head 2004, Chapter 2) indicates that there is no single trait that makes zebra finches attractive. This is the starting point of my thesis. It seems likely that attractiveness is multidimensional and females might use different axes to assess male attractiveness.

Choice chamber setup to test for mating preferences

When measuring mating preferences in the sense of preference functions, one has to measure how individuals discriminate between stimuli (Widemo & Sæther 1999). In principle, stimuli can be assessed sequentially or simultaneously. There are some drawbacks when measuring preferences sequentially. Most importantly, there might be order effects that are difficult to control for (e.g. Milinski & Bakker 1992; Downhower & Lank 1994; Collins 1995). Since sample sizes are usually relatively low when measuring mating preferences in vertebrates, this is not desirable. It is more efficient to measure preferences in simultaneous choice trials, where focal (choosing) subjects are forced to discriminate between stimuli. However, simultaneous choice tests have also been criticised for introducing aspects of mate sampling (Wagner 1998). When measuring mating preferences it is important to clearly separate mating preferences (between sexes) from competition within sexes. Hence, stimulus individuals from the chosen sex have to be

physically separated. Unless one is interested in social factors influencing mating preferences, mate choice is best assessed when the choosing individual is alone.

A frequently used approach to achieve both simultaneous presentation and spatial separation and to accommodate large numbers of tests to achieve reasonable sample sizes, are choice-chamber setups. In these, stimulus individuals from the chosen sex are kept in medium-sized stimulus cages or compartments, while the choosing individuals is kept in a larger arena that allows them to visit stimulus individuals while keeping the stimulus individuals separated. Such choice-chambers have been used for birds (e.g. Bateson 1980; Burley 1981; Bateson 1982; Burley *et al.* 1982; Collins 1994; Forstmeier & Birkhead 2004; Rutstein *et al.* 2007) and in fish (e.g. Brooks & Endler 2001; Aeschlimann *et al.* 2003; Simcox *et al.* 2005). I have used a four-way choice apparatus, where four males were presented simultaneously (Figure 2.1 on page 33) and a two-way choice apparatus (Figure 2.2 on page 34).

Preferences in a choice-chamber are usually measured as the time spent close to stimulus individuals (Burley *et al.* 1982; Burley 1986c; Wynn & Price 1993; de Kogel & Prijs 1996; Jennions 1998; Forstmeier & Birkhead 2004; Rutstein *et al.* 2007). This preference has been shown to correlate positively with solicitation to copulations in mate choice trials (Witte 2006) and extra-pair copulations in trials with paired females (Houtman 1992; Forstmeier 2007). Individuals might differ in whether they are sexually or socially motivated during mate choice trials. This might influence their preference measurements, when different stimuli are preferred in the two contexts. This is likely to introduce some measurement error to measurements of preferences in the choice-chamber, that I have addressed by using large sample sizes in all experiments (Chapters 1, 2 and 3). Mating preferences when measured as time spent with males show relatively low, but significant repeatability when measured several weeks apart ($R = 0.29$, Forstmeier & Birkhead 2004, $R = 0.26$, Chapter 2).

The candidate trait approach

Frequently, researchers have manipulated specific traits in the study of mating preferences (Burley 1986b; Burley & Coopersmith 1987; Burley 1988; Clayton & Prove 1989; Collins *et al.* 1994; Swaddle & Cuthill 1994a; Collins 1999; Gil *et al.* 1999; Rutstein *et al.* 2004; Gorman *et al.* 2005; Gilbert *et al.* 2006; Williamson *et al.* 2006). This is certainly very valuable for understanding whether specific traits influence attractiveness. However, there are several problems with such a candidate trait approach when studying between-individual differences in mating preferences.

First and most importantly, it changes attractiveness in only one dimension. This is problematic, since in most species (as in the zebra finch), individuals differ in multiple dimensions (e.g. multiple ornaments, song characteristics, display behaviour) and fe-

males might differ in how they value the single trait under investigation. This might create differences between individuals, but it might also unify preferences, if the differences on one axis are maximised. Hence, it is not always clear how preferences for individual traits relate to mating preferences within more or less diverse populations.

Second, a manipulation of a single dimension often (though not necessarily) involves creating distinct classes. This might shift the context from mating preferences to species-recognition or other contexts of categorisation. For example, ten Cate *et al.* (2006) manipulated beak colour of foster parents in white-morph zebra finches and found that males prefer the beak colour of the foster mother. However, since white-morph zebra finches do not show sex differences in the plumage, this is most likely related to the classification of individuals (in this case to the two sexes).

Thirdly, manipulation of individual traits relies on these traits being important in mate choice. This makes it difficult to interpret negative results.

Multidimensional overall similarity

For most of my PhD work, I have used overall (multidimensional) similarity based on close relatedness. Since most traits (morphological as well as behavioural) show substantial heritability (e.g. Forstmeier *et al.* 2004), close relatives can be expected to be more similar than unrelated birds from the same population. The degree of similarity depends on the heritability of the traits. Although using relatives always allows for some component of noise (the fact that in the case of siblings they share only 50% of their alleles plus environmental noise), it puts similarity into a very natural setting.

In order to study mating preferences based on self-referent phenotype matching (Chapter 3), I have tested female preferences for unfamiliar brothers. A cross-fostering regime has ensured that females had never had contact with biological kin. Hence they could have used only their own phenotypes to identify unfamiliar brothers. By separating early experience from genetic similarity, it was possible to test for assortative versus disassortative preferences, both of which might have been predicted from current theory.

In order to study the effects of sexual imprinting, I took two approaches. In one experiment, I tested female preferences for the son of the foster parents (Chapter 2). Again, the son of the foster parents is similar to the foster parents for all heritable traits. Hence, if sexual imprinting works, I would expect females to prefer him over unrelated and unfamiliar individuals. In a second experiment, I tested zebra finch foster sisters that were unrelated, but had grown up with the same foster parents for their similarity in ranking unfamiliar males (Chapter 1). In this situation males were random samples from the population, while females were similar with respect to their experiences during the nestling phase independent of their genetic backgrounds.

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Chapter 1

Heritability of and early-environmental effects on variation in mating preferences

Abstract

Many species show substantial between-individual variation in mating preferences, but studying the causes of such variation remains a challenge. For example, the relative importance of heritable variation versus shared early-environmental effects (like sexual imprinting) on mating preferences has never been quantified in a population of animals. Here, we estimate the heritability of and early-rearing effects on mate choice decisions in zebra finches based on the similarity of choices between pairs of genetic sisters raised apart and pairs of unrelated foster sisters. We found a low heritability of preference functions and no evidence for early-rearing effects. A literature review confirms that heritable variation is low in most species and that there is no good evidence for sexual imprinting on continuous variation in any animal (except humans). This highlights that within populations, heritable variation and sexual imprinting play a limited role in explaining between-individual variation in preferences. While effects on preference functions were weak, we found strong individual consistency in choice behaviour and part of this variation was heritable. It seems likely that variation in choice behaviour (choosiness, responsiveness, sampling behaviour) would produce patterns of non-random mating and this might be the most important source of between-individual differences in mating patterns.

Understanding the origin of mating preferences is a critical issue for understanding sexual selection (Darwin 1859; Andersson & Simmons 2006). Mating biases can arise from several sources and it is conceptually important to distinguish between them (Jennions & Petrie 1997). Most importantly, mating preferences can be separated into preference functions, i.e. the ranking order of stimuli, and choosiness, i.e. the investment into mating with the preferred stimulus (Jennions & Petrie 1997; Brooks & Endler 2001). To understand sexual selection it is important to disentangle these different sources and to study their transmission mechanisms. Estimates of the heritability of mating preferences are particularly desirable, since this is a critical assumption of

runaway selection (Fisher 1930; Lande 1981). Mating preferences might also be transmitted by cultural processes, in particular by sexual imprinting (Aoki *et al.* 2001), which so far has received mixed support (see below).

Substantial support for a genetic basis of preference functions comes from between-population differences in preferences and within-populations differences in preferences for dichotomous traits (Majerus *et al.* 1982; Houde & Endler 1990; Bakker & Pomiankowski 1995; Velthuis *et al.* 2005). However, it is not always clear how between-population differences translate into variation of preference functions within a single population (Chenoweth & Blows 2006). Within populations, there is very good evidence for heritable variation in choosiness (the range of stimuli that are accepted) or sexual responsiveness (the strength of response to sexual stimuli) (Collins & Cardé 1990; Bakker 1993; Bakker & Pomiankowski 1995; Brooks & Endler 2001; Brooks 2002; Rodríguez & Greenfield 2003). Although this might result in mating biases and is thus relevant for mate choice and sexual selection, it is not the same as quantifying the heritability of preference functions, since in order to measure preference functions, it is necessary to measure aspects of mate discrimination (Jennions & Petrie 1997).

The number of studies addressing the genetic basis of preference functions within populations is very limited. Evidence for heritable variation comes from selection lines in stalk-eyed flies and in guppies (Wilkinson & Reillo 1994; Brooks & Couldridge 1999). Other studies have tested for genetic effects on preferences and have found (Moore 1989; Charalambous *et al.* 1994; Houde 1994) or have not found significant effects (Johnson *et al.* 1993; Breden & Hornaday 1994; Ritchie *et al.* 2005). These studies, however, do not quantify the amount of heritable variation. A careful search for studies that estimated the heritability of preference functions revealed only seven studies that present heritability estimates (Table 1.1): Three of them showed significant heritability (point estimates for h^2 between 0.14 and 0.51), while the others were non-significant and mostly very low.

Importantly, Qvarnström *et al.* (2006b) found significant heritability of female realised pairing with respect to a male trait in the collared flycatcher, although this heritability was very low ($h^2 = 0.026$). However, such similarities between related females might also have arisen from sexual imprinting. Sexual imprinting is another form of vertical transmission of preferences, where preferences are formed during early development usually by using the own parents as models (Grant & Grant 1997; Aoki *et al.* 2001). There is very good evidence for sexual imprinting on heterospecific foster parents, morphs or novel-ornaments (e.g. Immelmann 1975; ten Cate & Bateson 1988; Qvarnström *et al.* 2004; Burley 2006), traits that are probably related to species recognition. The evidence for sexual imprinting on continuous variation within a single population (and thus not involving the categorisation of individuals into distinct classes) is limited and ambiguous (Bereczkei *et al.* 2004; Schielzeth *et al.* 2008b).

Table 1.1: *Published studies that present within-population heritability estimates of preference functions. We included only studies that analyse preference functions for traits that vary continuously within a population (although often only preferences for extremes were tested) and that estimate the heritability for the discrimination of mating stimuli (excluding estimates for the strength of a response to stimuli without discrimination).*

Study	Species	Preference for	Choice method	Manipulation of preferred trait	Heritability ($\pm$ SE)	Remarks
Collins & Cardé (1989)	Pink bollworm	pheromone composition (three component ratios)	sequential	yes	0.14 ± 0.05	
Jang & Greenfield (2000)	Moth	pulse rate and asynchrony interval of calls	simultanuous	yes	0.21 ± 0.13	
Iyengar *et al.* (2002)	Arctiid moth	body size	simultanuous	samples of defined differences	0.51 ± 0.11	sex chromosome linked inheritance
Gray & Cade (1999)	Field cricket	pulses per trill in calls	simultanuous	yes	0.34 ± 0.17	
Simmons (2004)	Field cricket	long chirp relative to short-chirp song elements	simultanuous	yes	< 0.00	
Hall *et al.* (2004)	Guppy	male attractiveness (as measured in choice chamber)	simultanuous	samples including extremes	-0.07 ± 0.13	selection lines (direct and indirect selection)
Brooks & Endler (2001)	Guppy	colouration & size (several measures) max for brightness contrast	simultanuous	no	0.10 ± 0.11	maximal heritability from a large range of tests

Most studies, in particular those on the heritable variation of preference functions, have focused on specific traits and have used manipulation (Collins & Cardé 1989; Gray & Cade 1999; Jang & Greenfield 2000; Simmons 2004; Ritchie *et al.* 2005) or have chosen extreme phenotypes (Houde 1994; Wilkinson & Reillo 1994; Brooks & Couldridge 1999; Hall *et al.* 2004) to increase the variance along a specific axis of ornamentation. This valuable approach, however, does not allow an understanding of sources of varia-

tion in preferences for potential mates in their full multidimensionality (Candolin 2003; Fawcett & Johnstone 2003). Hence, we have employed an experimental design that allows testing for the similarity in preferences between (a) genetic sisters and (b) foster sisters in a population of zebra finches, where males are likely to vary along multiple axes. A full-individual cross-fostering scheme ensured that all individuals grew up with only unrelated nest siblings and were raised by unrelated foster parents. This enabled us to disentangle genetic and early-rearing effects. For the first time in a population of animals, we quantify the genetic and early-rearing effects on mating preferences simultaneously. We focus on preference functions, but at the same time present results on between-individual variation in choice behaviour.

Methods

Subjects and housing

We used 176 female and 176 male zebra finches *Taeniopygia guttata castanotis* from a large captive population (for details on housing see Bolund *et al.* 2007). All individuals belong to a single generation, but were bred in two cohorts (September to November 2005 and April to June 2006). All individuals had been cross-fostered individually within 24h after egg-laying, which ensured that all broods consisted of only unrelated chicks and that all subjects were raised by foster parents unrelated to all nestlings (Schielzeth *et al.* 2008b). Juveniles were separated from their foster parents at 35 days of age and were kept in juvenile peer groups up to an age of c. 100 days (47% in unisexual peer groups, 53% in mixed-sex peer groups). Subjects were sexually mature at the time of testing (birds from 2005: 557 ± 21 days, mean $\pm$ SD, birds from 2006: 339 ± 21 days). Throughout the trial period, birds were housed in doublets of same-sexed individuals (but not with their genetic or foster sister).

Experimental design

We tested mating preferences of 44 pairs of genetic full-sib sisters that were raised apart (all from different families) and 44 pairs of unrelated foster sisters that grew up in the same brood (except for one pair, where foster sisters came from different clutches of the same foster pair). As stimulus birds we used a total of 176 males that were randomly assigned to duplets of one focal male and one opponent male (ensuring they were unrelated and unfamiliar to all females they were tested with). Focal males were always tested with the same opponent male. Male duplets were used as stimulus birds for four pairs of genetic sisters and four pairs of foster sisters. Hence, male pairs were used exactly 16 times and each female had exactly eight trials. Females had one trial per day

and the eight trials were run on four consecutive days followed by one day of break and another four trails on four consecutive days. The sequence of testing the females with male pairs was randomised. Males pairs were always tested in the same out of eight identical choice chambers, but the sides of the stimulus cages were randomly assigned to the two males.

Choice-chamber trials

We used a two-way choice chamber setup identical to the one described in Schielzeth *et al.* (2008b) (Figure 2.2 of page 34) except for two changes. First, the compartment of the accompanying female (as described in Schielzeth *et al.* 2008b) was empty, but inaccessible to the choosing female. Second, the compartments close to the male stimulus cages were equipped with two parallel perches to allow the female ritualised hopping. Presence in all three compartments (two close to the stimulus males and a neutral zone in the middle) was recorded automatically. We used eight identical choice chambers that allowed us running eight trials simultaneously. During trials, subjects had no visual contact to any individual that was not involved in the trial. Trials lasted one hour.

As described in Schielzeth *et al.* (2008b) we calculated the proportion of time spent with the focal male (time spent in the cage close to the focal male divided by the sum of the times spent in the outer cages) and used this as a measure of preferences. Time spent with males has been shown to correlated with sexual preferences (Witte 2006; Forstmeier 2007) and shows moderate, but significant repeatabilities when measured several weeks apart (0.26-0.29, Forstmeier & Birkhead 2004; Schielzeth *et al.* 2008b). We also analysed the similarity in preferences with respect to the identity of the preferred individual (the male a female spent most time with). Although a single second of difference in time allocation might, in the extreme case, decide about which male was the preferred one, this analysis is important to completely disentangle pure preferences from choice behaviour (the distribution of time between males). We also conducted an analysis, where we limited the dataset to trials in which females showed clear or very clear preferences (i.e. they spent at least 70% or 85% of their time with one male). This substantially reduces the number of data points, makes the design unbalanced and limits the analysis to a subset of females in the population, but including only clear decisions potentially reduces measurement error.

Furthermore, we calculated (a) the total number of registrations from the motion-sensitive sensors as a measure of hopping activity, (b) the number of transitions between end-cages close to males as a measure of the number of comparisons, (c) the total proportion of time close to any of the males as a measure of female interest in males, and (d) the absolute deviation of time allocation to the focal male from 0.5 (no discrimination) as a measure of clarity of the choice.

Data analysis

We used angular transformation ($y' = \arcsin(\sqrt[3]{y})$ on percent time spent with the focal male for further analysis. The number of transitions between males was log-transformed ($y' = \ln(y + 1)$). The percent time close to any male was transformed as $y' = y^5$. These transformations were applied to achieve better fit to normal distributions. We calculated the repeatability of male attractiveness as the variance component of focal male identity on time allocation to the focal male (assessed by eight females) in a random-intercept model (Schielzeth & Forstmeier 2009). In the absence of fixed factor predictors, variance components are the proportion of variance explained by a random-intercept effect relative to the total variance. We used likelihood-ratio tests to test for the significance of variance components.

To estimate the heritability and early-rearing effects on preferences, we calculated the correlation between the two females forming one pair (genetic sisters or foster sisters) assessing the same eight sets of males. We used the mean and the standard error of the population of correlation coefficients (one per pair of females) as an effect size estimate for the genetic and shared early-environmental effects on preferences. However, since there was some overall agreement between females on male attractiveness (see results), these estimates include effects of overall between-female agreement independent of relatedness. To control for this, we calculated the partial correlation coefficients for the preferences of sister pairs while controlling for the mean preference of the other 14 females that were tested with the same set of males. We then calculated the correlation across all pairs of sisters as described above. The population estimate for the correlation represents half the heritability of female preference functions (since in full-siblings 50% of the alleles are identical by decent) and the entire shared-environmental component. This estimate of heritability includes possible maternal effects and parts of dominance variance and epistatic interactions, but is independent of the early environment (as ensured by cross-fostering).

We analyzed the heritability and early-rearing effects on female behaviour in the choice chamber (female activity, number of comparisons, clarity of the choice, time spent with males) by variance component analysis. Models included the trait under consideration as a response and female identity, genetic sister pair identity, foster sister pair identity, male pair identity and choice-chamber identity as random-intercept effects. The variance component of genetic sister pair identity represents the within-full-sib repeatability. This is half the heritability of the respective trait (see above).

All calculations were done in R 2.8.0 (R Development Core Team 2008). We used the lmer function from the lme4 package (Bates *et al.* 2008) for variance component analyses.

Results

Individual concistency

The repeatability of male attractiveness was low but significant (variance component for male identity: 0.103 ± 0.001, LRT: $\chi^2_1 = 63.4$, $P < 10^{-14}$). We found significant within-female repeatability of time spent with males, the number of comparisons between males, female activity and clarity of choice (Table 1.2). All these traits had small male-pair identity and choice-chamber identity effects, although some were significant (Table 1.2).

Preference functions

The correlation in preferences between genetic sisters was low overall ($r = 0.12 \pm 0.07$, $N = 44$, $P = 0.087$, Figure 1.1), and even lower when controlling for overall between-female agreements on male attractiveness ($r = 0.05 \pm 0.07$, $N = 44$, $P = 0.42$). This results in a broad-sense heritability estimate for female mating preferences of $H^2 = 0.10 \pm 0.14$ (including maternal effects, parts of dominance and epistatic interactions, but no early-rearing effects). The correlation in preferences between unrelated foster sisters was low overall ($r = 0.12 \pm 0.06$, $N = 44$, $P = 0.051$, Figure 1.1), and even lower when controlling for overall between-female agreement on male attractiveness ($r = 0.05 \pm 0.07$, $N = 44$, $P = 0.52$).

We also analysed the agreement in choices between genetic sisters and foster sisters. Little more than 50% of all trials showed an agreement between females and both genetic sisters and foster sisters did not differ from unrelated females (Figure 1.2). When limiting the data to comparisons where both females spent more than 70% or more than 85% of their time with the preferred male, the agreement becomes larger, but genetic sisters and foster sisters did still not differ from unrelated females. In all three subsets, the agreement between foster sisters was slightly larger than between genetic sisters, although these differences were non-significant.

Choice-chamber behaviour

All traits showed some indication of genetic effects as measured by the similarity between genetic sisters (Table 1.2), but no indication of foster-environment effect as measured by the similarity between foster sisters (Table 1.2). Since the broad-sense heritability is twice the intra-class correlation coefficient for full-siblings, this results in estimated variance components of $H^2 = 0.10 - 0.30$ (including maternal effects, parts of dominance and epistatic interactions, but no early-rearing effects). All traits showed a

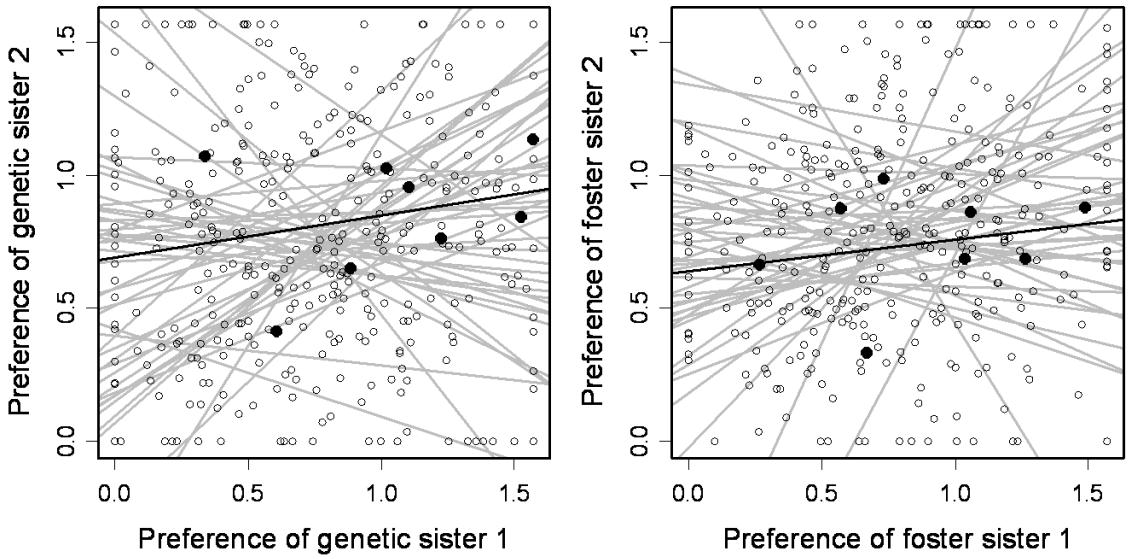

Figure 1.1: *Similarity in preferences between pairs of genetic sisters and pairs of foster sisters. Forty-four pairs of genetic sisters and 44 pairs of foster sisters were tested with eight sets of two males each. Regression lines are shown for each pair of sisters. The black data points and the solid black regression line highlight a typical example for the eight trials of one pair of sisters.*

large degree of between-female variation in behaviour after controlling for genetic and foster-environment effects (Table 1.2), which indicates non-shared environmental effects on choice-chamber behaviour.

Discussion

We estimated the similarity in preferences and choice behaviour between genetic sisters and foster sisters in a two-way choice chamber. In accordance with earlier work (Forstmeier & Birkhead 2004), the overall agreement between females on male attractiveness was low. The between-female agreement was slightly higher, when including only trials with very clear preferences than when including all trials (61% versus 55%, Figure 1.2). Genetic sisters and foster sisters did not show higher agreement than unrelated females. This indicates that heritability of and early-rearing effects on preference functions are very low. When analysing the strength of the preferences as in Figure 1.1 (a mixture of preference function and strength of choice), the broad-sense heritability was low and non-significant ($H^2 = 0.10 \pm 0.14$). At the same time, we found very consistent choice behaviour of individual females in the choice-chamber. Part of this between-individuals variation in behaviour was heritable (point estimates for H^2 between 0.10

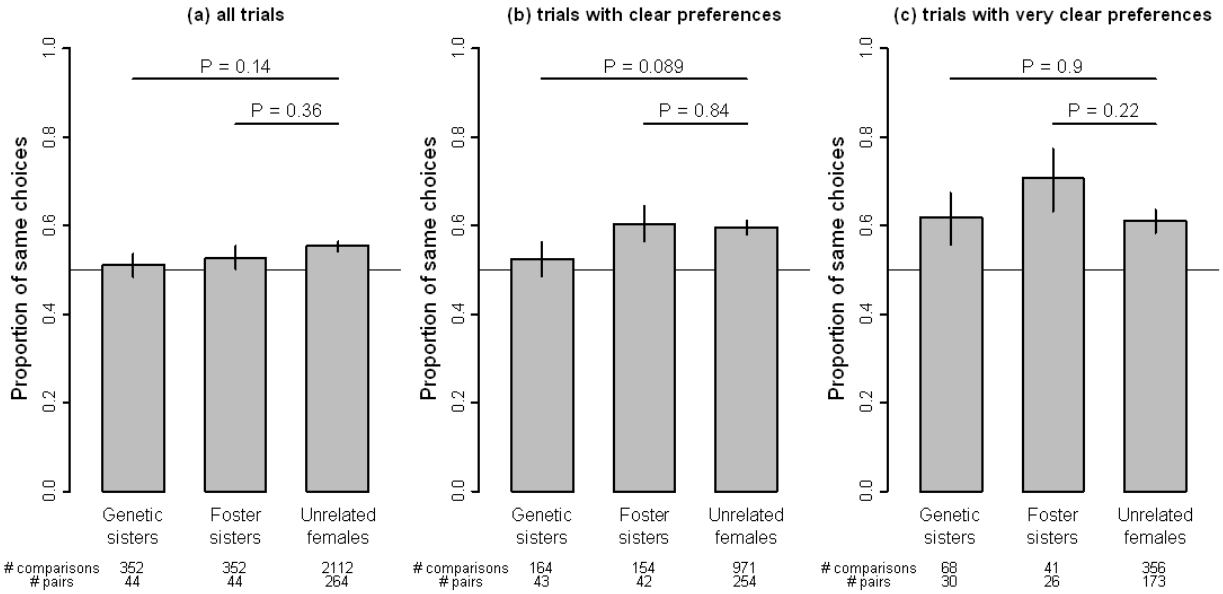

Figure 1.2: *Between-female agreement in mate choices in a two-way choice chamber. Each female had eight trials and proportion of agreements (identity of the male that a female spent the larger fraction of time with) was calculated between pairs of genetic sisters (N = 44 pairs), pairs of foster sisters (N = 44) and pairs of unrelated females (N = 264). The three plots show all trials (a), only trials with time allocation to the preferred male of > 70% (b), and only trials with time allocation to the preferred male of > 85% (c). Since limiting the comparisons to only clear choices means excluding trials with less clear choices, sample sizes vary among plots.*

and 0.30), whereas the shared environmental component was estimated to zero for all traits.

Seven published studies have estimated within-population heritability of preference functions for continuous variation, usually by measuring the relative time spent with males or the proportion of visits (Table 1.1). Our estimate of the heritability based on the proportion of time spent with males is very close to the median of these estimates (0.14 versus 0.10). However, the relative time allocation might include aspects of choice behaviour and thus might produce a somewhat higher heritability estimate (see below) as compared to pure preference functions. In our data, there was no evidence for a similarity in the outcome of choices between genetic sisters. Since the agreement between genetic sisters was even slightly lower than between unrelated females, this indicates that the heritability of preference functions was indeed very close to zero. It is hard to imagine processes that would make genetic sisters dissimilar in their preferences, hence the negative estimate is likely to be due to sampling variance alone.

Table 1.2: *Variance component (VC) analysis for female behaviour in the choice chamber. Likelihood-ratio tests were used for significance testing. There were 176 females (44 pairs of genetic sisters and 44 pairs of foster sisters) that had 8 trials each, 88 sets of males that had 16 trials each and 8 choice-chambers with 176 trials in each. The broad-sense heritability is twice the similarity between full-sibs (this estimate possibly includes maternal effects and part of dominance and epistatic interactions). The total female effect is the sum of genetic, foster environment and additional female identity effects. Add. = Additional*

	Clarity of choice			Time with males			Number of comparisons			Female activity		
	VC	χ^2_1	P	VC	χ^2_1	P	VC	χ^2_1	P	VC	χ^2_1	P
Genetic component	0.05	1.36	0.51	0.06	17.4	$<10^{-3}$	0.12	19.2	$<10^{-4}$	0.15	16.8	$<10^{-3}$
Early-rearing environment	0.00	0.00	1.00	0.00	0.0	1.00	0.00	0.00	1.00	0.00	0.0	0.99
Add. female component	0.18	13.5	$<10^{-3}$	0.43	22.9	$<10^{-5}$	0.45	20.5	$<10^{-5}$	0.35	19.5	$<10^{-4}$
Male-pair component	0.00	0.19	0.66	0.00	0.0	1.00	0.02	13.7	$<10^{-3}$	0.03	25.7	$<10^{-6}$
Cage component	0.00	0.00	0.98	0.01	16.8	$<10^{-4}$	0.02	17.4	$<10^{-4}$	0.03	13.7	$<10^{-3}$
Residual	0.77			0.50			0.39			0.44		
Broad-sense heritability	0.10			0.12			0.24			0.30		
Total female effect	0.23			0.50			0.57			0.50		

Although not significantly different from zero in our and several published studies (Table 1.1 and references in the introduction), we do not think that the heritability of preference functions is actually zero. The evidence from selection lines and quantitative genetics in insects and fish (Wilkinson & Reillo 1994; Brooks & Couldridge 1999) as well as between-population differences (e.g. Velthuis *et al.* 2005) give convincing evidence for non-zero heritabilities. However, the within-population heritable variation appears to be very low in most studies.

Brooks & Endler (2001) estimated the heritability of preference functions for a large number of traits in guppies. All of them were non-significant and mostly very low (max. $h^2 = 0.11$). However, they found significant heritability of responsiveness ($h^2 = 0.27 \pm 0.13$) and conclude that heritable variation in responsiveness might mask variation in preference functions and may be the most relevant source of between-individual variation in mating preferences. Our results support this suggestion, since we find highly

repeatable and also heritable variation of behaviour in the choice chamber. Although it is not clear, how the specific behaviours translate into mating behaviour in the wild, this might relate to differences in mate sampling. The high within-female repeatability clearly indicates individuality in choice behaviour.

Beside the potential for masking variation in preference functions, variation in choice behaviour might also be confused with variation in preference functions. For example, female sticklebacks show a preference for redder males, but there is heritable variation in the strength of discrimination (Bakker 1993). Females that show strong preferences had brothers with redder colouration compared to females that do not discriminate (Bakker 1993). Since the strength of preferences is an aspect of choosiness, this could potentially be explained by condition dependence in choosiness (Burley & Foster 2006). If condition is heritable and is also expressed in males by showing larger areas of red colouration, this might appear like a genetic correlation between a trait and a preference for this trait.

Our preference tests also allowed a strong test for sexual imprinting effects on mating preferences. Early-rearing effects as estimated from time allocation and between-foster sister agreement on attractiveness were very low and non-significant. This is in agreement with an earlier finding in our population (Schielzeth *et al.* 2008b). The finding we present here was derived from an independent set of experiments and uses a different approach. Hence, the only positive evidence for sexual imprinting on continuous variation to date stems from humans (Bereczkei *et al.* 2004). As long as there is no further evidence, we conclude that sexual imprinting is probably relevant for species recognition (e.g. Immelmann 1975; ten Cate & Bateson 1988; Qvarnström *et al.* 2004; Burley 2006) and also for sex recognition (ten Cate *et al.* 2006), but does not explain between-individual differences in mating preferences within a single population.

We conclude that empirical support for early-rearing effects on preferences is currently very limited and is unlikely to play an important role for variation in preferences within populations. There is more evidence for heritable variation of preference functions, although this is not always strictly separated from heritable variation of choosiness and responsiveness. Estimates are usually low and, hence, heritable variation is apparently not strong enough to fully explain between-individual differences in preference functions. However, we find strong evidence for individual and heritable components to choice behaviour. This is likely to produce patterns of non-random mating and influence the outcome of realised choices.

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Chapter 2

Sexual imprinting on continuous variation: do female zebra finches prefer or avoid unfamiliar sons of their foster parents?

Abstract

Sexual imprinting on discrete variation that serves the identification of species, morphs or sexes is well documented. By contrast, sexual imprinting on continuous variation leading to individual differences in mating preferences within a single species, morph and sex has been studied only once (in humans). We measured female preferences in a captive population of wild-type zebra finches. Individual cross-fostering ensured that all subjects grew up with unrelated foster parents and nest mates. Females from two cohorts ($N = 113$) were given a simultaneous choice between (two or four) unfamiliar males, one of which was a genetic son of their foster parents (SFP). We found no significant overall preference for the SFP (combined effect size $d = 0.14 \pm 0.15$). Additionally, we tested if foster parent traits could potentially explain between-female variation in preferences. However, neither the effectiveness of cooperation between the parents nor male contribution to parental care affected female preferences for the son of the foster father. We conclude that at least in zebra finches sexual imprinting is not a major source of between-individual variation in mating preferences.

Variation in mating preferences within species can arise from both genetic and non-genetic factors (Jennions & Petrie 1997). Sexual imprinting is a learning process by which mating preferences are formed during early development, usually by using the own parents as models (Bischof 2003). This process is often assumed to be one of the nongenetic factors that create between-individual differences in mating preferences (e.g. Owens *et al.* 1999; Aoki *et al.* 2001). The suggestion is based on an extrapolation from evidence for sexual imprinting on discrete variation to sexual imprinting on continuous variation. There is ample evidence for sexual imprinting on characters that are involved in the categorization of individuals into species, morphs or sexes (species and

morphs: Immelmann 1975; Sonnemann & Sjölander 1977; ten Cate *et al.* 1992; Kruijt & Meeuwissen 1993; Vos 1995; Bischof 1997; Slagsvold *et al.* 2002; Bischof 2003; artificial novel traits: ten Cate & Bateson 1988; Witte *et al.* 2000; Witte & Sawka 2003; Qvarnström *et al.* 2004; Burley 2006; sexes: ten Cate *et al.* 2006). By sharp contrast, there is only one study that has addressed sexual imprinting within a more or less homogenous population: human husbands resemble the foster fathers of adopted daughters (Bereczkei *et al.* 2004). Here, we present the first empirical test of sexual imprinting on continuous variation in a nonhuman animal.

Most studies addressing sexual imprinting have focused on positive sexual imprinting, i.e. a learned preference for phenotypes similar to an individual's parents. This can be considered a type of mate-choice copying both in the case of discrete characteristics and continuous variation. In contrast to classical mate-choice copying, where individuals copy the preferences for specific individuals (Pomiankowski 1990; Pruett-Jones 1992), sexual imprinting involves a generalization of a same-sex parent's mating decision to unfamiliar individuals. This can be referred to as generalized mate-choice copying. Similar forms of generalization have been demonstrated based on public information from peers (Godin *et al.* 2005; Swaddle *et al.* 2005), but the link to sexual imprinting has never been made.

Less often discussed under the label of sexual imprinting is negative sexual imprinting, i.e. a disassortative preference for phenotypes unlike the parents (Ihara & Feldman 2003). In this case, sexual imprinting would lead to inbreeding avoidance. In fact, sexual imprinting could be considered a special case of family phenotype matching (Komdeur & Hatchwell 1999; Tang-Martinez 2001; Nakagawa & Waas 2004), as parents are used as a template to generalize to unfamiliar kin. This sort of negative sexual imprinting has to our knowledge never been demonstrated empirically and has only rarely been discussed (but see Miller 1979; ten Cate & Vos 1999; Ihara & Feldman 2003; Mateo & Holmes 2004). Bateson (1982) found a preference for intermediate degrees of relatedness in the Japanese quail, but did not show that these preferences were learned. Nevertheless, a strong body of literature that focuses on kin recognition and inbreeding avoidance makes disassortative preferences based on sexual imprinting a sensible prediction for experiments on sexual imprinting within a relatively homogenous population.

In addition to pure strategies of avoidance or preference, we argue that the consequences of learning the parents' phenotypes may depend on environmental conditions. Individuals might use the parental phenotypes either as positive or negative models for their own mate choice, so that sexual imprinting could be used flexibly to result in assortative or disassortative preferences (ten Cate 1984; Oetting *et al.* 1995). For example, we expect individuals to show a stronger preference for phenotypes similar to their parents, if the cooperation between them was very effective. Similarly, we might expect a positive correlation between a male's contribution to parental care and the preference

for father-like phenotypes in his daughters. Females could benefit from such flexibility in their use of sexual imprinting by copying their mother's mate choice decision only (or more strongly so), if their father proved to be as a suitable partner in terms of contribution to parental care.

Here, we test for sexual imprinting within a single population of captive, wild-type zebra finches *Taeniopygia guttata*. Despite many reports of sexual imprinting in zebra finches on artificial new traits, colour morphs, foster species and sexually dimorphic traits (e.g. Immelmann 1975; ten Cate *et al.* 1992; Kruijt & Meeuwissen 1993; Vos 1995; Witte & Sawka 2003; Burley 2006; ten Cate *et al.* 2006), a similar test of imprinting on continuous variation has surprisingly never been reported. All individuals of our population had been cross-fostered individually, ensuring that all individuals were raised by unrelated foster parents together with only unrelated nest mates. Cross-fostered females were allowed to choose in a choice chamber between unfamiliar, cross-fostered males, with one of them being a genetic son of the female's foster parents (SFP). SFPs are similar to the female's foster parents (=their genetic parents) for all heritable traits. We expected one of two alternative outcomes: either (i) females would prefer the SFP, demonstrating positive sexual imprinting; or (ii) females would avoid the SFP, demonstrating negative sexual imprinting. Furthermore, we test if there is evidence for an adaptive flexibility in the imprinting mechanism by testing if the preference for the phenotype of the foster parents depended on the effectiveness of cooperation between foster parents or paternal investment in parental care.

Methods

Subjects

We selected subjects from two cohorts of wild-type zebra finches that were kept at the Max Planck Institute for Ornithology, Seewiesen, Germany (housing conditions described in Bolund *et al.* 2007). For tests in a four-way choice chamber, we used 64 females and 64 males that were bred in spring 2004 in Sheffield, UK (Forstmeier 2005) and transferred to Seewiesen in October 2004. This cohort of birds is referred to as the F_1 generation. For tests in a two-way choice chamber, we used 50 females and 70 males from the F_2 generation that were bred in autumn 2005 in Seewiesen. Prior to the testing, birds were kept in same-sex groups in cages that were set up in the experimental room.

All eggs were cross-fostered within 24 h of egg-laying, so that all eggs of a given clutch ended up with different fosters (Forstmeier 2005). Hence, all experimental birds were raised by unrelated parents together with unrelated nest mates. They were separated from their foster parents at the age of 35 days and subsequently held in unisexual

groups (F_1) or half-half in unisexual and mixed-sexed groups (F_2). Birds were used in a few behavioural experiments before testing for sexual imprinting (Forstmeier 2005), but had never been paired up for breeding. They were unfamiliar with all stimulus birds they met in the choice chamber trials.

Female subjects from the F_1 generation were tested only once. For every female ($N = 64$), we chose one genetic SFP that was unknown to her and that had not been raised by his genetic parents. From these matched pairs, we formed 16 groups of four females and four males so that all birds within groups were unrelated and unfamiliar to each other. We tested the preferences of each female for the four males in their group, using a four-way choice chamber (see below). The same set of males was therefore presented to four different females, with always a different male being the SFP. The positions of individual males as well as the position of the SFP rotated between trials in a fully balanced manner. Female subjects from the F_2 generation were tested in a two-way choice chamber (see below). They were tested repeatedly if more than one unfamiliar SFP was available in our population (28 females were used once, 17 twice and five thrice, a total of 50 females in 77 trials). Opponent stimulus birds were chosen at random from the pool of unfamiliar males (in total, 29 males were used once, 13 twice, 18 three, seven four, one five and two six times) and the position of the SFP was randomized.

Four-way choice chamber (F_1 generation)

Female preferences of the F_1 generation were measured in a four-way choice chamber apparatus (Forstmeier & Birkhead 2004; Figure 2.1). Birds were allowed to familiarize with the choice chamber for 24 h about two weeks before trials began. During acclimation, males were held singly in the end cages and females were held in groups of four birds in the central arena with the sexes separated by opaque dividers. Trials were run in January and February 2005 when subjects were 267 ± 35 (mean $\pm$ SD, range 210–330) days old. During a trial, the time spent on the perches in the corridors was recorded using photoelectric relays and log files of registrations were stored on a microcomputer.

Two trials were run each day. A new set of birds (four males to the end cages and the focal female to the central arena) was placed in the choice chamber at noon time, when the female and the stimulus males were separated by opaque dividers. After 1 h of pretrial, the dividers were pulled out and the trial began. Trials ended after 3 h. The dividers were put back, the males were rotated to a different position and the female was replaced by the second female of the group. After one night in the choice chamber (with individuals separated by opaque dividers), the second trial was run in the morning and the entire set of birds was removed at noon. After 24 h, the same set of males was put in the end cages again and trials with the third female and the fourth

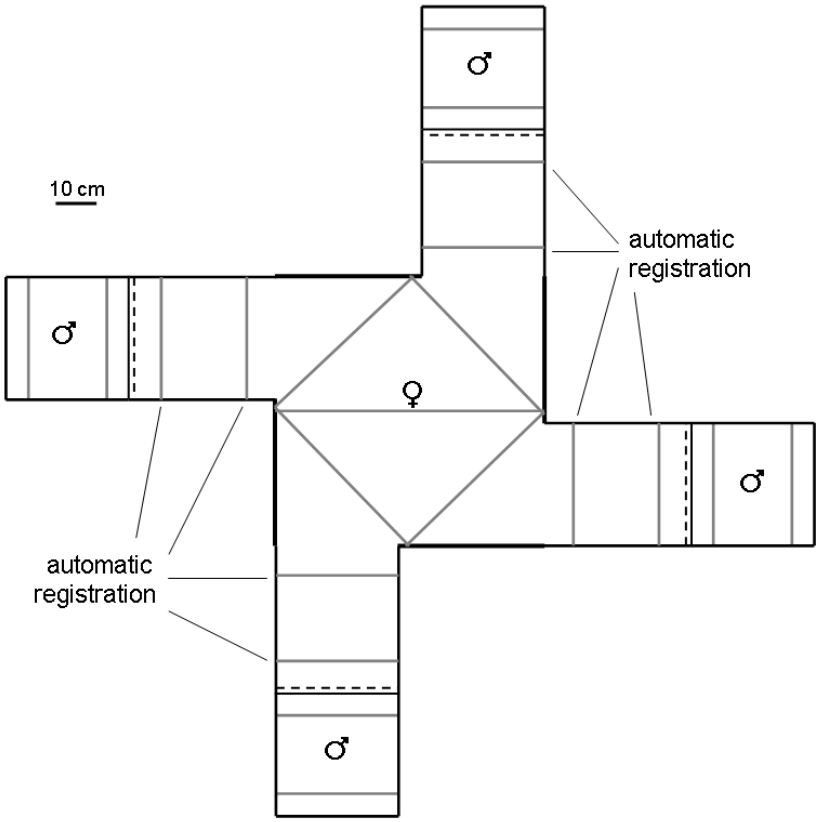

Figure 2.1: *4-way choice-chamber designs as used for the F_1 and F_2-2 generation. Solid black represent opaque walls, dashed lines show mesh wire. Perches are marked in grey. Solid and dotted lines between male stimulus and female cages signify an opaque separation in the lower 19 cm and mesh wire in the upper part.*

female were run in the afternoon and the following morning respectively. Hence, males had only one test of 3 h per day and the four tests with one set of males (and different females) were run over four consecutive days.

Two-way choice chamber (F_2 generation)

Female preferences in the F_2 generation were measured in a two-way choice chamber apparatus (Figure 2.2). Males were held in wire-mesh stimulus cages measuring $22 \times 36 \times 24$ cm at either side of the female cage. The female cage consisted of three compartments measuring $40 \times 40 \times 40$ cm each. About one-third of the central compartment was separated by Plexiglas and an accompanying female (the cage mate from the holding cages) was present there during trials. The accompanying female did not

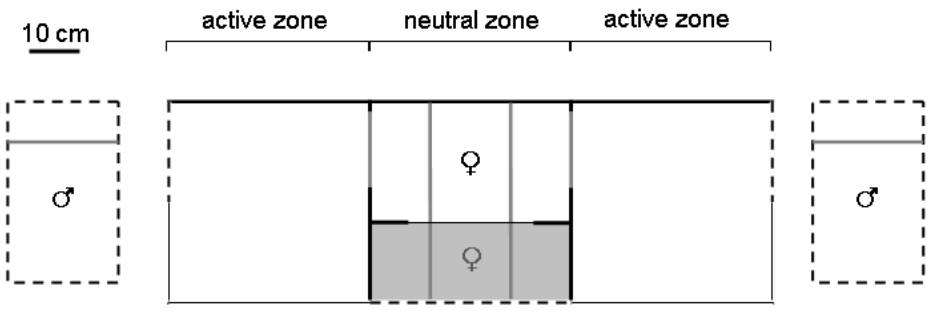

Figure 2.2: *The two-way choice apparatus used in mating preference trials. Dotted lines show wire mesh, thin black lines full-spectrum transparent (300–700 nm) Plexiglas Sunactive® GS and thick black lines opaque plastic walls. Perches are marked in grey. The grey area was occupied by an accompanying female; this area was separated by Plexiglas and not accessible to the choosing female. Time spent in the three cages was measured. The central compartment was treated as a neutral zone, the other two as active zones. Time spent on perches between compartments was counted half to each adjacent compartment.*

have visual contact with either of the males. She was introduced to separate socially motivated from sexually motivated association of the focal birds, so that the neutral zone was not a nonsocial environment. The choosing female had to pass through openings measuring 15×10 cm (width $\times$ height) to enter the compartments close to the stimulus males. Passage through these openings was recorded by photoelectric relays. Two movement-sensitive infrared sensors in each compartment recorded the choosing females whenever she was moving. All registrations were logged on a microcomputer.

All subjects were acclimated to the two-way choice chamber in unisexual groups some days before trials began. Trials were run from February to early May 2006 when subjects were 156 ± 24 days of age (mean $\pm$ SD, range 128-220). All subjects were used in one trial per day only. There was no pretrial period as in the four-way choice chamber, but subjects were given 5 min to settle (separated from each other by opaque dividers) before trials started. All except for two trials were repeated in late May 2006 (59 ± 29 days, mean $\pm$ SD after the first trial) to calculate the repeatability of female time allocation.

Measures of cooperation and paternal investment

In our population, there is large variation in chick growth during early development despite *ad libitum* food being available. We measured individual chicks to the nearest 0.1 g when they became 8 days old. Chick weights ranged between 1.6 and 12.2 g ($N = 829$). As all chicks grew up with randomly assigned foster pairs rather than

their genetic parents, we were able to decompose this variation into genetic determination (genetic parent effects) and early rearing effects (foster parent effects). We fitted a mixed-effects model to the data of individual chick weights, with rearing location (Sheffield vs. Seewiesen) and rearing environment (cage vs. aviary) as fixed-effects predictors and hatch order, genetic parent identity, genetic parent clutch identity, foster pair identity and foster clutch identity included as random effects. We included hatch order, as this controls for total brood size effects and individual starting condition most effectively (better than brood size alone). The model included all clutches from our population (not only the experimental foster families, $N = 829$ chicks in 302 foster clutches from 366 genetic clutches). The variance component (after controlling for hatch order and fixed effects) explained by genetic parent identity was 7, by genetic parent clutch identity 7%, by foster pair identity 24 and by foster clutch identity 0. Hence, about one-quarter of the variation in chick mass (after controlling for confounding effects) was caused by variation between foster pairs and chick mass was consistent in different broods from the same foster pair. We interpret this as a measure of the effectiveness of cooperation within the foster pair. Including egg volume as a covariate further improves the model fit. Egg volume shows mainly between-female variation, hence decreasing the genetic parent identity effect, while still reducing residual variance, so egg volume was included in the final model. To estimate the foster parent effect as precisely as possible, we used the best linear unbiased predictors (BLUPs) for pair identity as a predictor for the effectiveness of care within the pair bond.

Furthermore, we were interested in the relative male contribution to parental care. During daily nest checks, we recorded the presence of the partners in the nest box during incubation and the early nestling period and calculated the proportion of times the male was present (relative to at least one of the partners being present). We used arcsine-transformed proportions per clutch as data points and fitted a model with female identity, male identity and pair identity as random effects. The model contained data on 920 clutches from 361 pairs formed from a pool of 184 males and 182 females. Female identity explained virtually nothing of the variation in relative male contribution (variance component 0%). Male identity explained 35% of the variation and pair identity explained 8%. Hence, about one-third of the variation was due to between-male variation. We used the BLUPs for male identity as a measure of male contribution to parental care in further analysis.

Data analysis

We used female time allocation to the SFP (time near SFP/total time near any of the males) as the response variable in our analysis. We transformed these values to $y' = \arcsin(\sqrt[3]{y})$ for the four-way choice chamber data (Forstmeier & Birkhead 2004) and $y' =$

$\arcsin(\sqrt{y})$ for the two-way choice chamber data. One female from the four-way data set had to be excluded, because she did not enter any of the four corridors.

We used a Monte Carlo simulation to test if time allocation to the SFP differed from random expectation in the four-way choice chamber. Our test statistic was the mean time spent with the SFP across all trials. For each trial, we selected one of the four stimulus males at random and then calculated the mean time for these randomly selected males across all trials. This was repeated 10,000 times to give a distribution of the means that can be expected when the focal male is actually a randomly chosen individual. We compared our test statistic (the time allocation to the SFP) with this distribution of means from the randomized data. We calculated the effect size (Cohen's d) as the difference between the time allocation to the SFP and the nonfocal stimulus birds, standardized by the variation in time allocation to different stimulus males (Cohen 1988). Confidence intervals were estimated by bootstrapping, i.e. resampling trials with replacement and calculating d for 10,000 bootstrap samples. For better comparability between cohorts, we used the same procedures for the two-way choice chamber data. We combined the two effect size estimates by averaging their means and estimated the combined standard error as half the squared rooted sum of the squared standard errors.

Calculations of repeatabilities and their standard errors follow Becker (1984) and Lessells & Boag (1987). All calculations were conducted using R 2.6.0 (R Development Core Team 2007). The function lmer from the package lme4 (Bates 2007) was used for BLUPs estimates of chick mass and the function lme from the package nlme (Pinheiro *et al.* 2007) was used for mixed models of preference for the phenotype of the foster parents. All P-values presented are two tailed.

Results

The repeatability of females choosing between the same two males with about two months in between the trials was $r = 0.26 \pm 0.11$ (mean $\pm$ SE, $F_{74,75} = 1.72$, $P = 0.01$). Hence, to reduce measurement error, we averaged time allocation for the SFP between the repeated trials involving the same individuals and used these means in the following analyses. The repeatability of time allocation for different SFP assessed by the same female in the two-way choice chamber was $r = 0.23 \pm 0.17$ (mean $\pm$ SE, $F_{21,27} = 1.72$, $P = 0.093$).

The 63 females in the four-way choice chamber showed a nonsignificant trend towards a preference for the SFP (randomization test: $P = 0.092$, bootstrapped Cohen's d: 0.27 ± 0.20, mean $\pm$ SE, Figure 2.3a). The SFP achieved the highest time allocation in 19 of 63 trials (=30%), which is not significantly more than one-quarter (exact binomial test: $P = 0.38$). Among the 50 females in 77 trials in the two-way choice chamber,

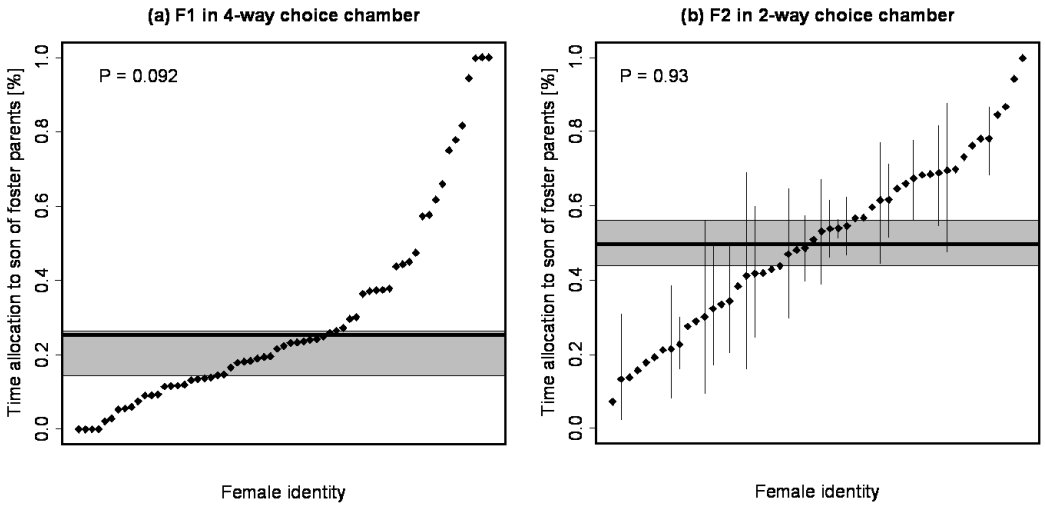

Figure 2.3: *Time allocation to the son of the foster parents (SFP) for (a) the F_1 generation in a four-way choice chamber; and (b) the F_2 generation in a two-way choice chamber. Dots and whiskers refer to individual females (ranked by their time allocation to the SFP, mean ± SE). The bold black line shows the empirical mean of the data points. The shaded grey area shows the 95% confidence interval for the expected mean as estimated from randomization of the data. The data for the F_2 generation are averages of two trials with the same set of males several weeks apart.*

there was no trend in the time allocation to the SFP (randomization test: $P = 0.93$, bootstrapped Cohen's d: 0.00 ± 0.22, mean ± SE, Figure 2.3b) and the SFP received the highest time allocation in 38 trials (=49%) (exact binomial test: $P = 1.0$). Time allocation to the SFP did not differ significantly between the two generations (one-way ANOVA after standardization for expected time allocation based on randomization: $F_{1,138} = 1.28$, $P = 0.26$). The overall effect of sexual imprinting on female time allocation in both cohorts was nonsignificant (combined Cohen's d: 0.14 ± 0.15, Fisher's combined probability: $P = 0.30$).

Variation in the effectiveness of cooperation in parental care and variation in paternal contribution to parental care are candidate predictors that might explain variation in preferences for the SFP. We used chick mass (BLUPs for foster pair identity) as a proxy for the effectiveness of cooperation. Time allocation to the SFP decreased with increasing weight of the chicks of the foster pair in the F_1 generation (mixed-effects model including foster pair identity as a random effect: $b = -0.23 \pm 0.09$, $F_{1,23} = 6.47$, $P = 0.018$, Figure 2.4a), but not in the F_2 generation (mixed model including foster pair identity and female identity within foster pair identity as random factors: $b = -0.01 \pm 0.06$,

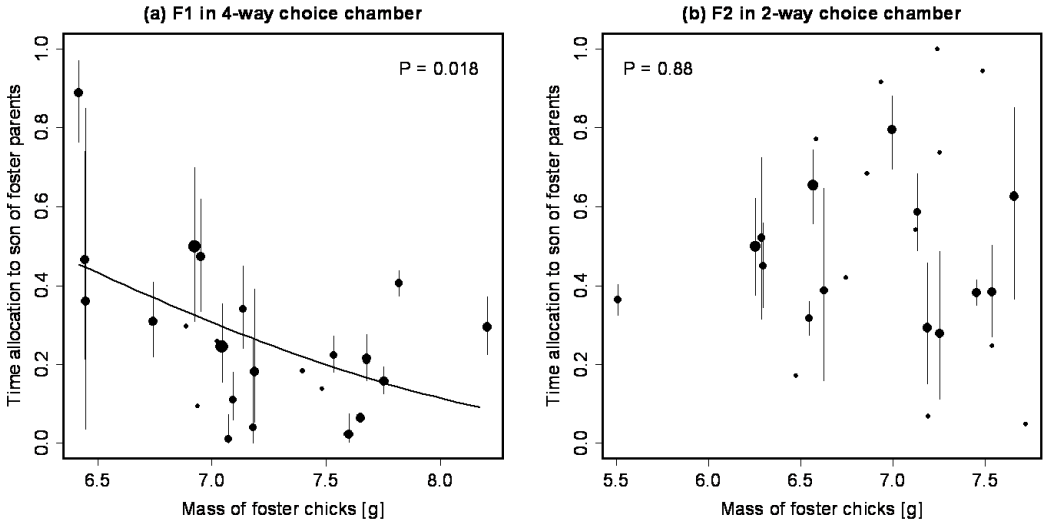

Figure 2.4: *Time allocation to the son of the foster parents in relation to a measure of the effectiveness of cooperation between foster parents (mean brood mass at day 8 after controlling for brood size) for (a) the F_1 generation in a four-way choice chamber and (b) the F_2 generation in a two-way choice chamber. Dots and lines show means and standard errors for females from the same foster parents. The size of the dots represents the number of females tested (small = 1, medium = 2, large = 3). The regression line shows the model fit of a mixed-effects model including foster environment as a random effect. The model was fitted on transformed values (values were back-transformed for illustration).*

$F_{1,24} = 0.02$, $P = 0.88$, Figure 2.4b). Female time allocation to the SFP did not depend on the individual female's own weight at day 8 in the F_1 generation (linear regression: $b = 0.00 \pm 0.03$, $F_{1,61} = 0.01$, $P = 0.92$) nor in the F_2 generation (mixed-effects model including female identity as a random effect: $b = 0.01 \pm 0.02$, $F_{1,47} = 0.34$, $P = 0.56$). Furthermore, time allocation to the SFP did not correlate with their father's relative contribution to parental care in the F_1 generation (mixed-effects model including foster pair identity as a random factor: $b = 0.52 \pm 0.48$, $F_{1,23} = 1.17$, $P = 0.29$) nor in the F_2 generation (mixed-effects model including foster pair identity and female identity nested within foster pair identity as a random factors: $b = -0.02 \pm 0.32$, $F_{1,24} = 0.003$, $P = 0.95$).

Discussion

Overall, we found neither a significant avoidance nor a significant preference for the SFP, although in one of the two experiments there was a weak trend towards a preference. Contrary to our expectations, the preference for the SFP was negatively related

to indicators of the effectiveness of cooperation between foster parents in one of the cohorts. As we did not find the same pattern in the second cohort and as it is against the expectation, this might represent a type I error. In both generations, the early rearing environment of the individual females (mass at day 8) did not explain variation in the time allocation to the SFP (i.e. variation in preference for the phenotype of the foster parents). Hence, overall we did not find evidence for adaptive between-individual variation in the tendency to use foster parents as models in mate choice.

Our experimental set-up tests for sexual imprinting on genetically determined traits, whereas the cross-fostering scheme prevented the transmission of learned traits from parents to their sons. Zebra finches usually (but not exclusively) learn their songs form their fathers (Zann 1996), so that sexual imprinting on song could lead to preferences for song phenotypes like (or unlike) their father's songs. Our experimental set-up did not allow detecting such preferences. Riebel & Smallegange (2003), however, tested for generalized preferences for the father's song specifically. They found a preference for the song of the father in females, but no generalization of these preferences to his tutees. As female zebra finches usually do not mate with their fathers, there is no indication for sexual imprinting on song that would generate between-individual differences in preferences.

Many studies demonstrating sexual imprinting on discontinuous variation (cited in the Introduction) show that this seems to be a common and widespread mechanism. Discrete variation can indicate genetic compatibility and sexual imprinting on such traits could help to mate with (genetically) compatible partners. A generalization of the sexual imprinting mechanism to continuous variation within a more or less homogenous population has never been tested in nonhuman animals, although it has been assumed to contribute to between-individual variation in mating preferences (Owens *et al.* 1999; Aoki *et al.* 2001). As our study could not demonstrate sexual imprinting in the zebra finch, humans are still the only species for which sexual imprinting within a nonpolymorphic population has been demonstrated (Bereczkei *et al.* 2004). At the moment, it would still be premature to assume that imprinting on continuous variation is a widespread phenomenon.

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Chapter 3

Assortative versus disassortative mating preferences of female zebra finches based on self-referent phenotype matching

Abstract

Kin recognition abilities allow individuals to treat relatives differently. In mate choice contexts, kin recognition can ensure individuals avoid the costs of inbreeding. However, there is also the potential for assortative preferences for genetic similarity as part of an optimal outbreeding strategy. We tested the kin recognition abilities of captive female zebra finches, Taeniopygia guttata*, in standard mate choice trials. A full individual cross-fostering scheme ensured that all subjects grew up in broods of only unrelated nestmates and were raised by unrelated foster parents. Therefore, individuals could use only self-referent phenotype matching (or recognition alleles), since exposure to genetic kin was excluded completely. Females were allowed the simultaneous choice between an unfamiliar genetic brother and unfamiliar, unrelated stimulus males. We used three cohorts of birds (199 females in total) that allowed three independent hypothesis tests. We found a significant avoidance of the unfamiliar brother in the first cohort, but could not replicate this finding in the other two cohorts. This was not easily explained by differences in the treatment of the cohorts, since the difference between cohorts was nonsignificant and testable differences in treatments did not show significant effects. The combined effect size was very low ($d = -0.048$) and nonsignificant. The initial finding of disassortative preferences may represent a type I error or there may be a true effect that is very weak and not easily reproducible. In any case, we did not find evidence of assortative preferences based on self-referent phenotype matching.*

Kin recognition abilities have potentially large benefits for the individual, for example by facilitating cooperation and nepotism (Komdeur & Hatchwell 1999; West Eberhard 1975). In social contexts, an individual can gain from helping relatives by increasing its inclusive fitness (Hamilton 1964a,b; West Eberhard 1975). When it comes to mate choice, individuals are more likely to avoid close kin, since inbreeding imposes costs of increased homozygosity and the expression of recessive deleterious alle-

les (Charlesworth & Charlesworth 1987; Pusey & Wolf 1996). The resulting inbreeding depression has been demonstrated in many cases (Armbruster & Reed 2005; Keller & Waller 2002; Kruuk *et al.* 2002; Pusey & Wolf 1996). Although there are also potential benefits of inbreeding (Kokko & Ots 2006), empirical evidence is in clear favour of inbreeding avoidance (Armbruster & Reed 2005; Boakes *et al.* 2007; Keller & Waller 2002; Kruuk *et al.* 2002; O'Grady *et al.* 2006; but see Pusey & Wolf 1996 for studies that failed to show it).

Outbreeding might also be costly, although there is only limited evidence for outbreeding costs within a more or less homogeneous population (Bateson 1978, 1980, 1982; Peer & Taborskyi 2005). In quails, *Coturnix japonica*, Bateson (1978, 1980, 1982) found a preference for distant kin over close kin as well as over nonkin and interpreted this as an optimal outbreeding strategy. Optimal outbreeding constitutes a balance between inbreeding and outbreeding avoidance. This could be achieved by using different mechanisms, one ensuring the avoidance of close inbreeding and another preferring similar to novel phenotypes (Bateson 1978). We would expect mechanisms that are reliable indicators of kinship (e.g. direct familiarization, see below) to be used for inbreeding avoidance, whereas other mechanisms could ensure assortative preferences for similar phenotypes. Hence, in the lack of full information about kinship it seems possible that individuals might show assortative preferences for kin.

Conceptually, five ways to assess genetic similarity and kinship can be distinguished based on differences in the mechanisms involved and/or on their consequences for behaviour: (1) spatial association (e.g. Beecher *et al.* 1981); (2) recognition of specific individuals by direct familiarization (e.g. Komdeur *et al.* 2004); (3) family phenotype matching that involves a generalization from familiar to unfamiliar individuals (e.g. Sharp *et al.* 2005); (4) self-referent phenotype matching that involves learning of an individual's own phenotype and a generalization to unfamiliar individuals (e.g. Hauber *et al.* 2000, sometimes called the 'armpit effect' Dawkins 1982); and (5) recognition alleles that allow intrinsic recognition without learning (Tang-Martinez 2001). Some authors conceptually lump family phenotype matching with self-referent phenotype matching since both are based on learning kin characteristics from familiar kin (including the focal subject itself, Blaustein 1983; Hauber & Sherman 2001; Heth *et al.* 1998). However, since self-referencing would work even without prior exposure to kin, it would, for example, enable birds hatched from dumped eggs to recognize unfamiliar kin whereas family phenotype matching would not (Hare *et al.* 2003; Hauber & Sherman 2000). Since brood parasitism is a relatively widespread phenomenon (e.g. Birkhead *et al.* 1990 for zebra finches, *Taeniopygia guttata*), we suggest it is better to treat self-referent phenotype matching as a distinct mechanism with its own specific consequences.

Our study focuses on kin recognition mechanisms without prior exposure to kin, separating self-referent phenotype matching and recognition alleles (4 and 5) from all

other mechanisms (1-3). For reasons of brevity, we use the term self-referent phenotype matching to refer unspecifically to mechanisms 4 and 5. These two mechanisms are rather difficult to separate, since it is necessary to exclude exposure to an individual's own phenotype completely to show convincingly that recognition alleles exist (Hauber & Sherman 2003). On the other hand, the existence of both these mechanisms is highly debated and hence needs experimental support (Hare *et al.* 2003; Hauber & Sherman 2000). The difficulty of excluding exposure to close kin completely (e.g. during gestation in mammals, Hare *et al.* 2003) often makes it difficult to rule out family phenotype matching. This problem can easily be overcome in birds, where it is possible to cross-foster eggs before the onset of embryo development (e.g. Komdeur *et al.* 2004; Petrie *et al.* 1999; Sharp *et al.* 2005).

We tested for self-referent phenotype matching by measuring mating preferences for unfamiliar brothers in a population of captive wild-type zebra finches. Cross-fostering of eggs before the onset of incubation allowed us to create broods of only unrelated chicks that were raised by unrelated foster parents. We let individually cross-fostered females choose between cross-fostered stimulus males, one of which was the female's unfamiliar brother. Since subjects were prevented from having prior contact with kin, a differential treatment of the unfamiliar brother in mate choice trials would give evidence for self-referent phenotype matching (or recognition alleles). Surprisingly, the opportunity for complete individual cross-fostering in birds does not seem to have been used previously for the study of self-referent phenotype matching in mate choice contexts. To our knowledge, the present study is the first to do so.

Previous studies on zebra finches have demonstrated preferences for familiar kin in pairing experiments (Slater & Clements 1981), a preference for same-sex cousins over same-sex nonkin in choice experiments (Burley *et al.* 1990, but no discrimination in intersexual choices) and no differential mating with respect to kinship in aviaries (Fetherston & Burley 1990; Schubert *et al.* 1989). None of these studies used cross-fostering. In the wild, sibling matings do occur but seem to be relatively rare (Zann 1996). Inbred zebra finches suffer costs of slower growth rates and lower survival during early development (Fetherston & Burley 1990). Although an avoidance of unfamiliar brothers in mating contexts would seem advantageous for reasons of inbreeding avoidance, the existing studies are more indicative of weak effects or even a preference for kin. In our study, by excluding familiarity with kin completely, we might expect assortative preferences to be expressed even more strongly.

Methods

We used a choice chamber paradigm to test for mating preferences. Preferences were measured as time spent close to individual males, since this measure has been shown to correlate with female responsiveness, solicitations to copulations and pairing in aviaries (Clayton 1990; Forstmeier 2007; Houtman 1992; Witte 2006). The time spent with focal males is significantly repeatable in our population when the same female is tested with the same set of stimulus males several weeks apart (Forstmeier & Birkhead 2004; Schielzeth *et al.* 2008). We used birds from three cohorts of captive zebra finches (referred to as F_1, F_2-1 and F_2-2, respectively) that were tested in either a four-way choice set-up (F_1 and F_2-2) or in a two-way choice set-up (F_2-1). We used the three cohorts for purposes of internal validation of our results by conducting three independent hypothesis tests. Specifically, after an initial significant finding in the F_1 cohort, we used new birds from the following two cohorts for replication purposes. The two-way choice set-up allowed us to conduct a larger number of tests and was introduced for this purpose only. Since findings from the F_1 and F_2-1 cohort differed and this might have been caused by differences in the choice chamber (two-way versus four-way), we again used the four-way choice chamber to test the F_2-2 cohort. The treatment of the three cohorts differed in further details that are beyond the scope of this study (Table 3.1).

Subjects and housing

All subjects originated from a large captive breeding population of wild-type zebra finches. The stock of zebra finches has been bred in captivity for several decades (Forstmeier *et al.* 2007b). We arbitrarily label the first generation in our breeding scheme the parental generation (P). The F_1 generation was bred from the P generation in spring 2004 in Sheffield, U.K., and has been held at the Max Planck Institute for Ornithology, Seewiesen, Germany, since October 2004. The F_2-1 cohort was bred in autumn 2005 and the F_2-2 cohort in spring 2006 at Seewiesen, both from the same pool of parents (mainly F_1 and some P generation birds). We used 64 females and 64 males from the F_1 cohort, 90 females and 122 males from the F_2-1 and 48 females and 48 males from the F_2-2 cohort. All females were tested with males of their own cohort. Subjects were individually marked with numbered metal rings. The study was approved by the animal care and ethics representative of the Max Planck Institute for Ornithology.

All eggs were cross-fostered individually within 24 h after egg laying, so that all subjects were raised by unrelated foster parents together with only unrelated nestmates (Forstmeier 2005). Young birds were separated from their foster parents at the age of 35 days and subsequently held in peer groups of different sizes. In the F_1 cohort, peer groups were exclusively unisexual. In the F_2-1 and F_2-2 cohorts, half of the peer groups

Table 3.1: *Differences in set-up and treatment of the three cohorts used in the kin recognition experiments.*

	F_1	F_2-1	F_2-2
Choice chamber type	4-way	2-way	4-way
Control for variation in male attractiveness	Balanced design	Randomisation	Balanced design
Number of individuals	64 females & 64 males	90 females & 122 males	45 female & 48 males
Multiple use of individuals	No	Yes	No
Age at testing (mean $\pm$ SD)	315 ± 30 days	163 ± 21 days	236 ± 28 days
Trial duration	3 h	1 h	3 h
Accompanying female in neutral zone	No	Yes (cage mate from holding cages)	No
Light conditions	Artificial light only	Artificial light plus natural daylight	Artificial light only
Temperature and humidity	Constant (24 ± 1 °C)	Variable (15-35 °C)	Constant (24 ± 1 °C)
Group size before testing	4 individuals (unisexual)	2 individuals (unisexual)	4 individuals (unisexual)
Rearing location	Sheffield	Seewiesen	Seewiesen
Early development of females (0-35 days)	Cages	Cages (82%) or aviaries (18%)	Cages (92%) or aviaries (8%)
Peer groups (35-100 days)	Unisexual	Unisexual (46%) or mixed-sexed (54%)	Unisexual (50%) or mixed-sexed (50%)

were unisexual and half were mixed-sexed (until around day 100, when all subjects were transferred to unisexual groups). There were five peer groups in the F_1 cohort, 25 in the F_2-1, and 12 in the F_2-2 cohort. Subjects were sexually mature at the time of testing (Table 3.1), but none of the subjects (neither males nor females) had been paired up for breeding before the trials. Prior to testing, subjects were housed in unisexual groups of two to four individuals (Table 3.1) that were set up in the experimental room.

We used genetic brothers as focal stimulus males in mate choice trials and unrelated males as nonfocal stimulus birds. The average inbreeding coefficient in our populations is $F = 0.03$ (Forstmeier *et al.* 2004), hence the average relatedness between unrelated individuals is about $r = 0.06$ and between unfamiliar siblings about $r = 0.54$. All birds involved in the same trial (the choosing female and the two to four stimulus males) were unfamiliar to each other, that is, they were raised by different foster pairs, did not share

the same peer group and had not met on any other occasion. All nonfocal stimulus birds were unrelated to the choosing female and unrelated to the focal stimulus male (the unfamiliar brother). They also did not share the rearing environment with them. None of the nonfocal stimulus males was related to the foster parents of the choosing female. However, one of the nonfocal stimulus males was a genetic half or full sib of nestmates of the choosing female in 19% of the trials in the F_1, 9% of the trials in the F_2-1 and 16% of the trials in the F_2-2. However, in all cases only one of the nestmates was related to the nonfocal stimulus birds and in most cases there was more than one nestmate (mean brood size $\pm$ SD: F_1: 3.9 $\pm$ 1.2, F_2-1: 3.4 $\pm$ 1.1, F_2-2: 3.3 $\pm$ 1.0). Females did not show a preference for or avoidance of these brothers of nestmates (randomization tests: $P = 0.57$ for F_1, $P = 0.16$ for F_2-1 and $P = 0.13$ for F_2-2; combined Cohen's d: $d = 0.049 \pm 0.12$; Fisher's combined probability: $P = 0.18$). The results presented in this paper did not change when we excluded all trials that involved brothers of nestmates.

Birds were held in a 14:10 h light:dark cycle of full-spectrum fluorescent light with a flicker frequency of 48 kHz (Osram Biolux L 36W/72-965). They were provided daily with fresh seed and water and had access to cuttlefish and grit ad libitum. Once a week, subjects received salad and multivitamin supplementation to the drinking water (for further details on housing, see Table 3.1 and Bolund *et al.* 2007).

Choice experiments

Four-way choice chamber

Female mating preferences of the F_1 and F_2-2 cohorts were measured in a four-way choice chamber apparatus (details in Forstmeier & Birkhead 2004, Figure 2.1 on page 33). In short, stimulus males were held in stimulus cages containing food and water. Females were placed in a large central compartment (the neutral zone) with food and water. They could approach the males via corridors that contained two perches. These perches were equipped with photoelectric relays and all registrations were logged on a microcomputer. Males could not see each other. All subjects were allowed to familiarize themselves with the choice chamber (females were separated from males by opaque dividers) for 4 h several days before trials began.

Subjects were randomly allocated to groups of four females and four males so that every male was the unfamiliar brother to exactly one of the females, while all the other birds were unrelated and unfamiliar. The same set of four males was presented sequentially to all four females, so that every male was the focal male (i.e. the unfamiliar brother) exactly once. Males were rotated among stimulus cages so that every individual male and the (changing) focal male were once in every position. For the F_2-2 we initially formed 12 groups with 48 females and 48 males, but three females had to be

excluded, because they had accidentally met one of the stimulus males in prior experiments. This left us with 45 females to be analysed for the F_2-2 cohort.

Choice chamber trials lasted 3 h and two trials were run per day. A new set of birds (four males in the stimulus cages and the first focal female in the central arena) was put in the choice chamber at noon. After 1 h of pretest with the males hidden behind opaque dividers, the dividers were removed manually. After 3 h of trial the dividers were replaced, the males were rotated to a different position and the first female was replaced by the second female of the group. After one night in the choice chamber, the set of birds had a second trial in the morning and was removed at noon. A different set of birds was tested next to give the previous set a break of 24 h, after which females 3 and 4 had their trials in the afternoon of day 3 and in the morning of day 4.

Two-way choice chamber

Preferences of F_2-1 females were measured in a two-way choice chamber apparatus (Figure 2.2 on page 34). Males were held in wire-mesh stimulus cages (22×36 cm and 24 cm high) at both sides of the female cage. The female cage consisted of three compartments measuring $40 \times 40 \times 40$ cm each. About one-third of the central compartment was separated off by Plexiglas and an accompanying female (the female cagemate from the holding cages) was present there during trials. The accompanying female could not see either of the males and was introduced to separate socially motivated from sexually motivated association of the focal birds, so that the neutral zone was not a nonsocial environment. The choosing female had to pass openings measuring 15×10 cm (width $\times$ height) to enter the compartments close to the stimulus males. Passage through these openings was recorded by photoelectric relays. Two movement- sensitive infrared sensors in each compartment recorded the choosing female whenever it was moving. All subjects were allowed to familiarize themselves with the choice chamber for 2 h several days before trials began. In a choice trial, a female was presented with two unfamiliar males, with one of them being an unfamiliar brother. Subjects were given 5 min to settle from handling before trials began. Trials ran for 1 h. Females were tested repeatedly when more than one unfamiliar brother was available in our population (34 females were used once, 23 twice, 14 three, 11 four and 8 five times, a total of 90 females in 206 trials) and males were used repeatedly as the unfamiliar brother to several females or as unrelated nonfocal males (19 males were used once, 22 twice, 29 three, 25 four, 8 five, 13 six, 4 seven and 2 eight times). All individuals had only one trial per day.

Data analysis

From the log files of the automated registration, we extracted the times spent on the perches in the corridors (four-way choice chamber, sum of the presence on both perches within a corridor) and the time spent in the end cages (two-way choice chamber, with the time spent in the opening weighted by half, see Figure 2.2 on page 34). The time spent in the central compartment was treated as neutral with respect to choice and was ignored in further analysis. We used time allocated to the focal male (time near focal male divided by the total time near any one male) as the response variable in our analysis. We transformed the four-way choice chamber data by $y' = \arcsin(\sqrt[3]{y})$ and the two-way choice chamber data by $y' = \arcsin(\sqrt{y})$ to achieve a better fit to normality. Owing to large sample sizes, one of the three distributions was significantly nonnormal (Shapiro-Wilk tests: F_1: $P = 0.07$; F_2-1: $P = 0.02$; F_2-2: $P = 0.18$), but all tests of main effects that we applied are robust to such minor deviations from normality.

Since time allocated to one male decreases the percentage of time spent with all three other males and angular transformation spreads the low values, the expected value for a randomly chosen male is lower than $\arcsin(\sqrt[3]{0.25})$. Hence, we used a Monte Carlo simulation to test whether time allocated to the unfamiliar brother differed from random expectation. In the simulation, one of the four males of each trial was chosen at random for every female and the mean time allocated to these randomly selected males over all females was calculated for each run. This was repeated 10,000 times and the empirical mean of time allocated to the unfamiliar brother was compared to the resultant distribution of simulated means. For better comparability between cohorts, we used the same procedure for the two-way choice chamber. Using a random-intercept model with female identity as a random factor instead of a randomization test for the two-way data set did not change the conclusions. Calculations were conducted using R 2.6.0 (R Development Core Team 2007).

We present the results separately for the three cohorts. To test whether the three cohorts differed significantly from each other, we scaled the empirical data by subtracting the mean and dividing by the mean standard deviation of the randomization runs. This centres the data to the same expected mean and scales the units to standard deviations. We used a one-way ANOVA with cohort as a three-level factor to test for differences between cohorts. This test contains pseudoreplication for the F_2-1 data, since many females were tested more than once. We also conducted a more conservative approach by averaging time allocated to the unfamiliar brother within females and using these averages in the ANOVA. We used bootstrapping on standardized values to estimate effect sizes for the three cohorts (Cohen's d, Nakagawa & Cuthill 2007).

Two approaches allowed us to combine the results into an overall conclusion. First, we calculated Fisher's combined probability (Quinn & Keough 2002, page 50). Since

one of the estimates was marginally positive, while the other two were negative, we transformed the two-tailed P value for the positive estimate to $P' = 2 * (1 - P/2)$. This yields a comparable two-tailed probability for the negative effect ($P' > 1$). Second, we used Bayesian inference on standardized data to estimate the posterior distribution of the intercept (i.e. the effect of the unfamiliar brother) combining the results from all three cohorts. Standardization (see above) ensured that the intercept was expressed in units of standard deviations and can be interpreted as a standardized effect size estimate (Cohen's d, Nakagawa & Cuthill 2007). We used the estimates of the F_1 cohort as a prior for the F_2-1 and the posterior distribution as a new prior for the F_2-2. Since the F_2-1 data were pseudoreplicated within females, we included a normally distributed female identity effect. We used three chains with automatically generated initial values and allowed a burn-in phase of 2,000 iterations. Models converged well and showed a good mixture of the chains. We sampled from the posterior distribution for the following 10,000 iterations. The Bayesian approach is an efficient way to combine evidence from different experiments, by appropriately weighting prior information (evidence from previous experiments) with new evidence (new data from new experiments; McCarthy 2007). Calculations were conducted using WinBUGS 1.4 (Medical Research Council Biostatistics Unit, Cambridge, www.mrc-bsu.cam.ac.uk/bugs).

Results

In 64 trials conducted with the F_1 cohort, females showed a significant avoidance of the unfamiliar brother (randomization test: $P = 0.011$, $d \pm$ SE = -0.30 ± 0.12; Figure 3.1). In both series of trials with the F_2 generation, the results were clearly nonsignificant (F_2-1: $P = 0.60$, $d = -0.03 \pm 0.07$; F_2-2: $P = 0.67$, $d = 0.06 \pm 0.14$; Figure 3.1). Despite these differences in estimates, cohorts did not differ significantly from each other (one-way ANOVA: $F_{2,312} = 2.20$, $P = 0.11$). Combining the results in a Bayesian framework by using the results of the F_1 tests as a prior distribution for the F_2-1 data and using the posterior distribution as a prior for the F_2-2 led to an estimated effect size of $d = -0.048 \pm 0.044$ (mean $\pm$ SD, 95% credibility interval [-0.135, 0.038]). Fisher's combined probability for the overall effect was $P = 0.15$.

The same result was reflected in the number of cases in which the unfamiliar brother achieved the highest time allocation from a specific female. This was the case in nine of 64 trials for the F_1 (expected 16 cases; binomial test: $P = 0.043$), 93 of 206 trials for the F_2-1 (expected 103 cases; binomial test: $P = 0.19$) and 13 of 45 trials for the F_2-2 (expected 11.25 cases; binomial test: $P = 0.61$).

Females did not differ consistently in their time allocation to the unfamiliar brother in the F_2-1 cohort where repeated measures of individual females with different unfa-

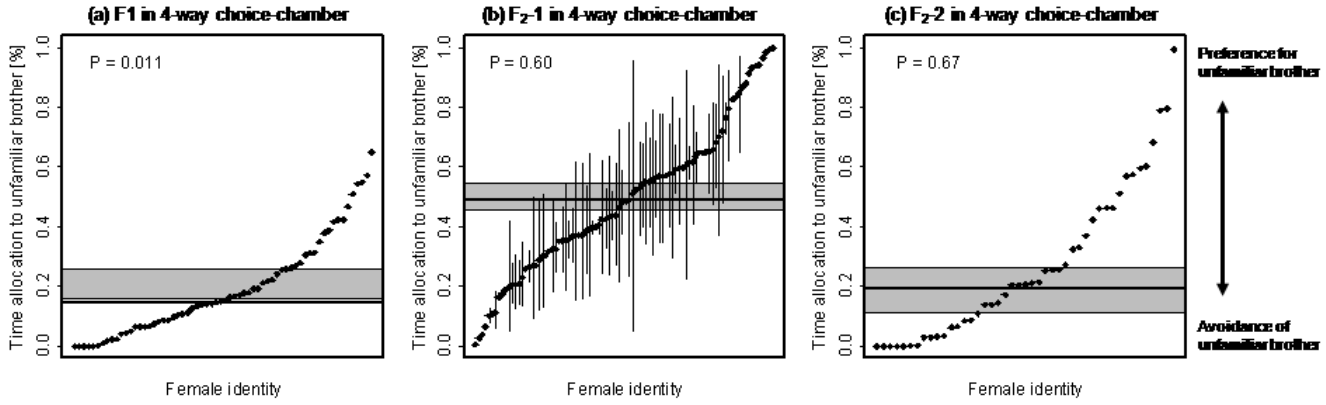

Figure 3.1: *Results of the unfamiliar brother experiments for three cohorts of females: (a) 64 females of the F_1 cohort in 2005 in a four-way choice chamber; (b) 90 females of the F_2-1 cohort in 2006 in a two-way choice chamber; and (c) 45 females of the F_2-2 cohort in 2007 in a four-way choice chamber. Female identity is ranked by time allocated to the unfamiliar brother. In (b) dots refer to mean time allocated to unfamiliar brothers by the same female, while thin lines show standard errors for multiply tested females. The shaded grey area shows the 95% confidence interval of the randomization test. The bold line shows the empirical mean over all trials. Calculations were done on transformed values and back-transformed for display.*

miliar brothers were available (variance component in random-intercept model $< 10^{-8}$). Furthermore, there was no effect of family identity on discrimination against unfamiliar brothers (variance component of family identity in a random-intercept model: 10^{-5}).

Of those factors that differed between cohorts (Table 3.1) a few could be tested specifically. There was no effect of either female age (ANCOVA controlling for cohort: $F_{1,132} = 0.22$, $P = 0.64$), cage versus aviary rearing (two-way ANOVA controlling for cohort: $F_{1,132} = 0.53$, $P = 0.47$) or of mixed- versus same-sex peer groups (two-way ANOVA controlling for cohort: $F_{1,132} = 0.11$, $P = 0.74$).

Discussion

We found a significant avoidance of unfamiliar brothers among 64 females, but could not replicate this finding in two follow-up experiments. This is not easily explained by changes in the experimental design or rearing conditions, since the outcomes of the three experiments did not differ significantly from each other and testable differences in rearing conditions did not show significant effects. The combined effect size ranged from –0.14 to +0.04 standard deviations (95% Bayesian credibility interval) and Fisher's combined probability was nonsignificant. It is possible that our initial finding repre-

sents a type I error, although the experiment was specifically designed, involved only one statistical test and gave a P value of 0.011. It is also possible that there is a true effect, but that it is very weak and not easily reproducible. In any case, our estimated effect was slightly negative; hence there was no evidence for assortative preferences. Our results do not allow us to conclude whether self-referent phenotype matching is possible in zebra finches, since subjects may have recognized kin, but not discriminated them in our experimental situation.

In our setting, only heritable traits could be used for kin recognition. These traits would have to be expressed in both sexes (so that females could match their own trait value to the trait value of stimulus males) or a template of the trait has to be inherited by the female, while being expressed in the male. Zebra finches show facial plumage patterns that are expressed in males and females and that are highly heritable (Burley & Bartels 1990). The question is how females would be able to assess their own facial pattern to evaluate its similarity to other birds' facial patterns. For most of their lives subjects involved in our study did not have the opportunity to see their mirror images (e.g. neither water dispensers nor bathing cups produced useful reflections). However, for periods of a few weeks all subjects had been housed in aviaries with dark bathing cups that produced some useful reflections. In the wild, individuals are likely to find water ponds that provide reflection images. It seems possible that the limited access to reflecting surfaces in our study is responsible for the weakness of the effect.

Calls and song are also candidate cues for kin recognition. Calls of females are innate, whereas male calls and songs are learned (Zann 1996). Nevertheless, despite cross-fostering and learning from nonkin, several male call characteristics show significant heritability and these components also show significant genetic correlations between the sexes (Forstmeier *et al.* in press). Given the higher heritability of female call traits, it seems easier for males to recognize unfamiliar sisters than for females to recognize unfamiliar brothers. Males that are not provided with tutors produce calls that are similar to female calls (Price 1979; Zann 1985). Males may have an inherited template of their family-specific female call and could thus recognize their sisters. The observed very weak avoidance of unfamiliar brothers could have been caused by male discrimination against unfamiliar sisters (for example by singing less to them) rather than vice versa.

Behavioural traits are also candidates for kin recognition. Personality traits, for instance, are often heritable (van Oers *et al.* 2004a) and individuals could match their own behavioural tendencies with the behaviour of unfamiliar individuals. The assessment and matching of personality traits might need more time and/or space, so that females could not match personality traits accurately in our experimental situation.

Another potentially useful cue for kin recognition could be odour cues, possibly linked to MHC alleles (Zelano & Edwards 2002). In both our choice chamber settings, females were able to approach males to within approximately 10 cm, being separated

only by wire mesh. Thus, it is unlikely that the potential for self-referent phenotype matching using odour cues was prevented in our study.

Under more natural conditions without cross-fostering, young birds would usually be exposed to kin during early development (parents and siblings). Zebra finches in the wild would have more information about kinship and genetic similarity, since they would not have to rely on self-referencing, but could resort to family phenotype matching using true kin for a template. In our cross-fostering scheme, however, young birds faced increased variation within broods, since all chicks were unrelated to each other. This would make family phenotype matching probably a more confusing than revealing mechanism, if used under these conditions. Other zebra finch studies (without cross-fostering) did not find kin discrimination in mate choice contexts (Fetherston & Burley 1990; Schubert *et al.* 1989) or found evidence for assortative preferences based on direct familiarization (Slater & Clements 1981). We conclude that based on self-referent phenotype matching alone, zebra finches show at best a weak avoidance of close kin in mate choice contexts. Furthermore, despite one study showing a preference for familiar kin (Slater & Clements 1981), there is overall very limited support for assortative mating preferences with respect to genetic similarity in zebra finches.

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Chapter 4

Variation in personality traits and their relevance for sexual selection: a study on captive zebra finches

Abstract

Individual differences in personality traits have often been described, but their evolutionary significance is still debated. In particular the link between personality traits and sexual selection has hardly been studied empirically. We scored approach to novel objects and hopping activity of 530 captive zebra finches. Scores were highly repeatable and approach scores showed substantial additive genetic variation ($h^2 = 0.43$). We measured reproductive success, promiscuity and extra-pair paternity rates under aviary conditions and calculated linear and non-linear selection differentials based on fertilization success as well as effects on chick rearing success of pairs. Approach to novel objects and hopping activity had little influence on these components of reproductive success. However, we found that the social environment (manipulated operational sex-ratios) influenced the correlation between personality traits and extra-pair paternity. This effect was stronger in males than in females. We conclude that despite the lack of differences in overall reproductive success, approach to novel-objects and activity might reflect variation in reproductive strategies.

In humans and in other animal species individuals from the same population differ consistently in their behaviour towards the environment, and suites of traits describing these behaviours have been termed coping styles, behavioural syndromes, personality traits or temperament (Wilson *et al.* 1994; Wilson 1998; Bolnick *et al.* 2003; Bell 2007; Réale *et al.* 2007; in this paper we use the term 'personality'). Personality traits are characterized by high within-individual consistency and low context-specificity (Sih *et al.* 2004a,b). This makes them particularly interesting for evolutionary ecologists, because they might mediate trade-offs and explain maladaptive behaviour (Bell 2007).

A central issue in the field of animal personality research is how variation in personality traits is maintained within a population (Dall *et al.* 2004; Bell 2007; Réale *et al.*

2007; Stamps 2007; Wolf *et al.* 2007). Several studies have shown a significant additive genetic basis of animal personalities (Bouchard & Loehlin 2001; Drent *et al.* 2003; van Oers *et al.* 2004a,b, 2005; Sinn *et al.* 2006), which implies the opportunity for evolutionary change (Rice 2004). Relatively few studies, however, have explored the covariance between personality traits and fitness (reviewed in Dingemanse & Réale 2005; Sinn *et al.* 2006; Réale *et al.* 2007; Smith & Blumstein 2008). Our understanding of fitness consequences is still very limited and clear predictions of how and when personality traits should affect fitness are largely lacking (Bell 2007).

Assuming that variation in personality traits is not just scatter around a single optimum (Wilson 1998), there are two main reasons that would create and maintain personality variation. Firstly, there might be disruptive selection or other complex fitness surfaces. In this case, variation would be created due to selection for different optima. Complex fitness surfaces might arise, for example, if the combination of personality traits within pair-bonds matters for reproductive performance (Both *et al.* 2005). Secondly, fluctuating selection pressures in a variable environment might maintain variation (Dingemanse *et al.* 2004; Boon *et al.* 2007). Such fluctuation may be variability in food availability, predator pressures or population structure.

The zebra finch is a highly gregarious passerine, who's group compositions change frequently (Zann 1996). Hence, the group structure constitutes a fluctuating social environment that has the potential to impose fluctuating selection pressures. We studied the fitness consequences of two personality traits in captive zebra finches held under a constant physical environment but in different social environments. These environments were characterized by variation in the operational sex-ratio, which leads to variation in intra-sexual competition for mates (Burley & Calkins 1999). We present evidence that the sex-ratio treatments influenced reproductive behaviour in our population. If the personality traits we measured are important for mating success and reproduction and are maintained by fluctuation in the social environment, we expect different behavioural types to be most successful under different sex-ratio treatments.

As personality traits, we quantified the overall activity of individuals and their approach to a novel object that was presented in a familiar environment (Verbeek *et al.* 1994). First, we test whether these traits are repeatable within individuals (between different novel-objects, and between years). Furthermore, we test whether hopping activity in the experiment correlates with hopping activity in other situations. The approach to a novel object may reflect 'novelty seeking', and constitute a mixture of exploratory behaviour and boldness (Réale *et al.* 2007). In the wild, this might relate to the exploitation of food and water resources, to defence against predators, dispersal and dominance (Réale *et al.* 2007), but potentially also to the response to new potential mates. In our captive situation, where there are no predators and food is highly predictable and *ad libitum*, novelty seeking might influence the frequency of interactions with individuals

other than the mate and consequently lead to higher promiscuity in explorative individuals. The link between personality traits and sexual selection is not highly obvious, but has been suggested repeatedly (e.g. Dingemanse & Réale 2005; Réale *et al.* 2007). For example, Réale *et al.* (2007) suggest that boldness as measured in novel-object experimental setups influences mating success (an aspect of sexual selection) via dominance structures in the population. Empirical data, in particular for the link between personality and sexual selection, is lacking. This makes it difficult, to make clear-cut predictions.

The first aim of our study was to estimate proximate sources of variation in personality traits by quantifying additive genetic, maternal and early-environmental effects using animal models (Kruuk 2004). The second aim was to explore the covariance between personality traits in both sexes and several components of fitness, including a measure of brood quality that reflects parental quality. We did this by calculating standardized linear and non-linear selection differentials (Arnold & Wade 1984; Brodie *et al.* 1995). Linear selection differentials measure the directional selection effects on the phenotype (for larger or smaller trait values), while non-linear selection differentials capture selection on the variance in the trait value (disruptive versus stabilizing selection). Finally, we explored how personality traits influence levels of extra-pair paternity (number of genetic partners and proportion of extra-pair eggs laid/sired). For all traits we tested whether the sex-ratio treatments changed the relationship between personality traits and fitness, i.e. whether the performance of personality phenotypes depended on the social environment.

Methods

Subjects and housing

We studied wild-type zebra finches from a large captive breeding population held at the Max Planck Institute for Ornithology in Seewiesen, Germany. Although genetic diversity is somewhat lower in captive populations compared to wild populations, our population does not show substantially reduced gentic diversity (Forstmeier *et al.* 2007b). Subjects belonged to two generations (referred to as F_1 and F_2). Eggs had been cross-fostered individually within 24 h after laying, so that all subjects grew up with unrelated nest mates and were reared by unrelated foster parents (Schielzeth *et al.* 2008b). Subjects were separated from their foster parents and siblings at day 35 (day 0 is hatching date), when young zebra finches become independent from their parents, and held in (same-sex or mixed-sex) peer groups between day 35 and day 100 (see Schielzeth *et al.* 2008b).

We measured the approach to a novel object and activity of 156 males and 131 females from the F_1 generation and of 125 males and 118 females from the F_2 generation. Birds from the F_1 generation were 358 ± 32 days of age (mean $\pm$ SD; range: 287-417) at the start of testing and birds from the F_2 generation were 412 ± 22 days (range: 360-453). Only subjects from the F_1 generation were involved in the aviary breeding experiment (in total 68 males and 71 females). Most of these birds were tested again for the two personality traits after two breeding seasons (62 males and 57 females, 985 ± 29 days of age, range: 930-1,036).

Novel object experiments

Setup of novel object experiments

We tested subjects by presenting them novel objects in their familiar environments. Before the trials, two same-sex individuals were held in a cage ($120 \times 45 \times 40$ cm, width $\times$ height $\times$ depth) with two wooden perches in each of two compartments. We provided food, water and grit *ad libitum* in one of the compartments (A); the other compartment (B) was empty except for the two perches. We allowed subjects to familiarize themselves with the cage for 3-4 days before testing them with novel objects. We housed birds in same-sex pairs before and between trials to avoid exposing these highly social birds to potentially stressful long-term isolation. Nevertheless, we tested subjects individually to minimize confounding effects of interactions during trials.

Just before trials, we gently chased birds (without handling), one to compartment A and one to an identical third, adjacent compartment (C). We then used opaque dividers to separate the three compartments (A, B and C). At the start of the trial, we placed a novel object at the far end of compartment B and removed the divider between A and B so that the focal bird in compartment A had access to the novel object in compartment B (compartments A and B were the familiar home cage). After the 5 min trial, we replaced the dividers and swapped the position of the two subjects (in compartments A and C) without handling. Then, we tested the second subject in the same way. We randomized the sequence of testing of the two birds, and the orientation of the compartments.

We tested all individuals with two types of novel objects. On day 1 we presented a bouquet of dry perennial herbs (height c. 25 cm, width at top c. 12 cm), which was placed upright in the far corner of compartment B. On day 3 we presented half an apple placed below the outer perch of compartment B with the cutting edge showing upwards. None of the subjects had previously had any contact with these kinds of objects, but all subjects had been kept in aviaries with natural tree branches for a few weeks. We additionally tested all individuals from the F_2 generation and all repeated trials with the F_1 generation (119 individuals) with a flashy colored toy ball (blue and orange, 7 cm in

diameter). This was done to assess whether the reaction to a highly artificial novel object (ball) correlated with the reaction to more natural objects used in the first round (herbs and apple).

Data recording and analysis

During trials we recorded all changes in position (between the perches, the floors of the two compartments and the slat between compartments; i.e. seven positions in total), using The Observer 4.1 software (Noldus Inc., The Netherlands) for live scoring. From the resulting log files we calculated the time spent in each of the seven positions.

We calculated an approach score that integrated approach distance and duration as follows. Each position was assigned a weight that expressed the relative distance to the novel object and this time was multiplied by the time spent in this position (in seconds). The weights for the bouquet and the ball trials were: far perch in compartment A = 1, close perch in A = 2, far perch in compartment B = 3, close perch in B = 4, floor of compartment A = 1.5, slat between A and B = 2.5 and floor of B = 3.5. In the apple trials the scores were the same except for the floor in compartment B (with a weight of 4 instead of 3.5), because the apple itself was placed on the floor. To reward early approach more than late approach, we weighted the first half (first 2.5 min) of the trial twice as much as the second half. We took the square-root of these weighted sums to reduce the skew in the distribution. We refer to the averages of the scores from the two novel object experiments as the approach score of an individual.

As a measure of hopping activity we calculated the total number of movements between positions. This number was log-transformed ($y' = \ln(y + 1)$). We refer to the average of the scores from the two experiments as the hopping activity score of an individual.

Repeatabilities of personality scores and their standard errors were calculated following Lessells & Boag (1987) and Becker (1984).

Quantitative genetics

We used animal models in REML-VCE 4.2.5 (Groeneveld 1998) to partition the phenotypic variance in approach score and hopping activity into additive genetic, maternal and early environmental effects. We included (a) a pedigree that identifies the ancestors of each bird in the grand-parent, parent, F_1 and F_2 generation ($N = 1,066$ individuals) to estimate additive genetic components, (b) mother identity to estimate maternal effects (additional to the additive genetic contribution of the mother, $N = 114$ mothers), (c) foster parent identity to estimate rearing environment effects ($N = 128$ foster families) and (d) peer group identity to estimate peer group effects ($N = 59$ peer groups). By

calculating expected repeatabilities from multiple measurements (Falconer & Mackay 1996, pp. 139), we further decomposed the residual variance into (e) measurement error and object-specificity, $ME = [(1 - R_{obj})/n_{obj}]/[(1 - R_{obj})/n_{obj} + R_{obj}]$, where R_{obj} is the between-object repeatability and n_{obj} is the number of objects, (f) behavioural flexibility, $BF = ME - [(1 - R_{years})/n_{years}]/[(1 - R_{years})/n_{years} + R_{years}]$ where R_{years} is the between-year repeatability and nyears is the number of trials, and (g) non-shared environmental effects, $NE = Residual - ME - BF$.

Reproductive success

Aviary breeding

We allowed 63 male and 63 female zebra finches to breed under semi-natural conditions in aviaries (birds that died were replaced, so the total number of individuals involved was 68 males and 71 females). All analyses were restricted to birds that were in the aviaries for at least one complete breeding round. We used three sex-ratio treatments (population sex-ratio 0.4, 0.5 and 0.6) with three replicate aviaries for each treatment (Figure 4.1) and allowed two breeding rounds of three months each (30th August – 22nd November 2005 and 31st March – 26th June 2006). We swapped subjects between breeding rounds so that each subject experienced two of the three treatments (except for nine males and nine females that stayed in the male-biased and female-biased treatment, respectively).

We randomly assigned birds to aviaries, ensuring that genetic or foster siblings did not share the same aviary. Variance and range of personality scores were similar for all three treatments in males (approach score: equal sex-ratio 7.02 ± 0.86 (mean $\pm$ SD), male-biased 7.23 ± 0.78, female-biased 6.94 ± 0.81; hopping activity: equal sex-ratio 2.39 ± 0.81, male-biased 2.44 ± 0.80, female-biased 2.27 ± 0.72) and in females (approach score: equal sex-ratio 7.21 ± 0.93, male-biased 7.27 ± 0.81, female-biased 7.22 ± 0.83; hopping activity: equal sex-ratio 2.48 ± 0.99, male-biased 2.38 ± 0.92, female-biased 2.31 ± 0.87). All tests for differences between treatments were non-significant (all $P > 0.12$).

In total, experimental birds laid 801 fertile eggs in 2005 and 821 in 2006. We cross-fostered eggs to caged foster pairs within 24 h hours after they were laid. We sampled blood from all parents and offspring and took tissue samples from all dead chicks and embryos. We genotyped all samples using 10 microsatellite makers (Forstmeier *et al.* 2007a) and assigned parentage by exclusion. We sampled 96.3% of all fertile eggs and unambiguously assigned 99.9% of these to a genetic father and a genetic mother. Additionally, we sampled 175 eggs from 19 females for hormonal analyses (for another study) and 170 of these were assigned to their putative parents (based on genetic parentage assignment from the remaining eggs of the clutch). We tested the validity of this assign-

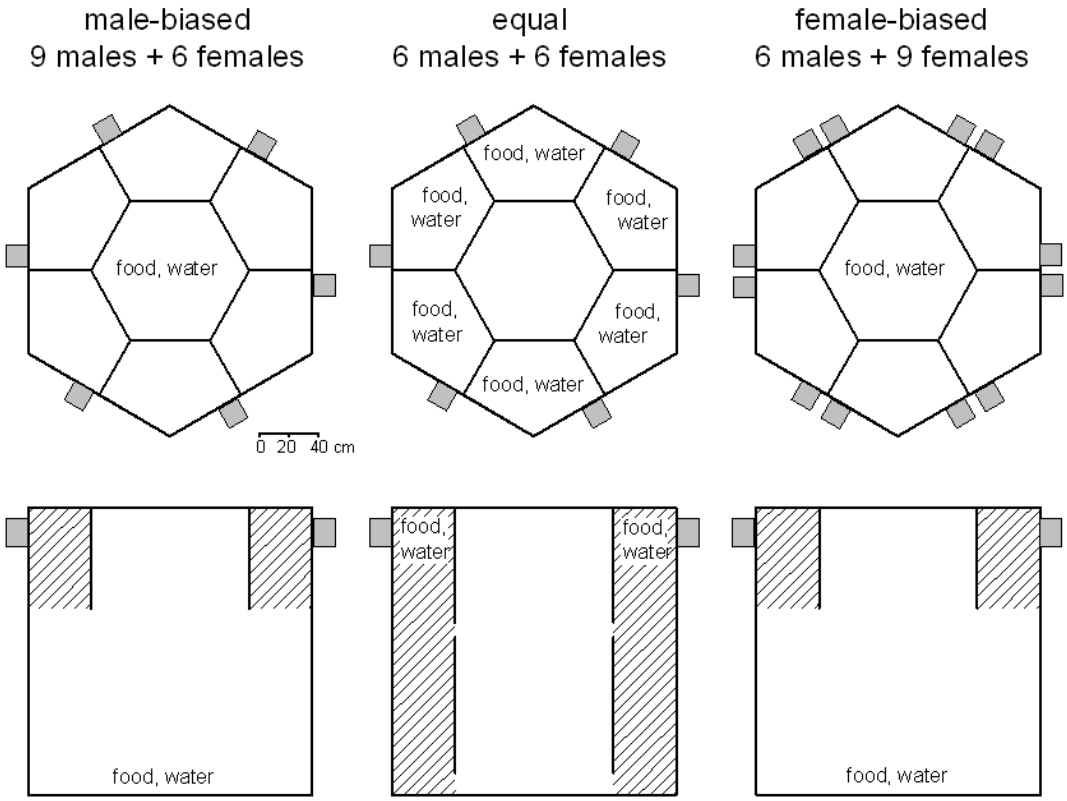

Figure 4.1: *Setup of the breeding aviaries. Zebra finches were allowed to breed in hexagonal aviaries under three sex-ratio treatments. The treatments also differed in spatial separation, distribution of food and water and supply of nest boxes. The upper row shows a top view of the aviaries. The lower row shows a cross-section that illustrates the spatial separation. Hatched sections indicate the separation between breeding compartments (by mesh wire); vertical lines indicate the separation between the outer breeding compartments and the central compartment. Nest boxes are shown as grey squares.*

ment by simulating the same assignment rules for clutches with complete parentage information and found that 14% of the assigned eggs were misassigned (1.2% of the total). This introduces some measurement error, but is still preferable over the exclusion of these eggs, since this would introduce bias against some individuals. Hence, including the hormone eggs, the analyses are based on a total of 1,727 eggs.

Fitness components and selection differentials

In the aviary breeding experiment we measured two independent components of fitness.

Female fecundity and male fertilization success. We counted the number of fertile eggs produced (females) or fertilized (males). We used relative fecundity and fertilization success (absolute values divided by the mean of all individuals in the same aviary) to calculate standardized selection differentials by regressing these measures of relative fitness on standardized personality scores (Arnold & Wade 1984; Brodie *et al.* 1995). The slope of the linear term of the personality scores represents the linear selection differential S (directional selection), while the slope of the quadratic term represents the non-linear selection differential C (positive = disruptive, negative = stabilizing selection). Models controlled for individual identity as a random-intercept effect (N for males = 52, 35 and 34, N for females = 34, 35 and 50, sample sizes for the male-biased, equal sex-ratio and female-biased treatment, respectively).

Chick rearing success. Pairs that occupied nest boxes received 2–3 eggs from randomly selected caged pairs (every egg from a different pair) after they had laid eggs themselves. On day 8 post-hatching, we measured chick mass. Mass at day 8 varies greatly between individual chicks and a large proportion of this variation can be explained by foster pair identity (24%, Schielzeth *et al.* 2008b). We used best linear unbiased predictors (BLUPs) for each aviary pair within seasons controlling for hatch order, egg volume, genetic parent identity and genetic clutch identity (for details see Schielzeth *et al.* 2008b). We calculated standardized slopes by regressing these BLUPs (standardized to mean of zero and unit of variance) on male and female personality scores (linear, quadratic and including the interaction between male and female score). These standardized slopes represent the differential success of low-scoring versus high-scoring individuals (linear term), intermediate versus extremes (quadratic term) and assortatively versus disassortatively paired individuals (interaction). Models controlled for pair identity as a random-intercept effect (N for pairs for the three treatments: 24, 19 and 25).

Extra-pair paternity

We measured two traits that represent reproductive strategies.

Promiscuity. We counted the number of partners with whom a particular individual produced eggs within a given breeding season. We used a Poisson model with log link to model the number of mating partners for all individuals (sample sizes as for fecundity and fertilization success). Models controlled for individual identity as a random-intercept effect.

Levels of extra-pair paternity. For all monogamous pairs that were paired for an entire season, we calculated the number of eggs sired within the pair-bond and the number of eggs sired outside the pair-bond separately for the two sexes. We used a Binomial model with logit link to model the proportion of extra-pair eggs (N = 26, 29 and 27 for the male-biased, equal sex-ratio and female-biased treatment, respectively). Models controlled for individual identity as a random-intercept effect.

Statistical analysis

Generalized linear mixed-effect models were fitted in R 2.7.2 (R Development Core Team 2008) using the function lmer from the lme4 package (Bates 2007). P values for the selection differentials were calculated by MCMC sampling using the function pvals.fnc from the languageR package (Baayen 2008). The significance of treatment effects on slopes was determined by likelihood ratio tests. We denote linear standardized slopes as b_x, 2nd degree polynomials as b_{x^2} and interaction term estimates as b_{int}.

Results

Behavioural response to the novel object

Zebra finches appeared to realize the novelty of the situation immediately after trials started. Many individuals leaned forward inspecting the object from a distance. Individuals then typically started hopping back and forth between the perches in cage A. In 61% of the trials, subjects entered the cage B (where the novel-object was placed). Subjects typically entered cage B only briefly at a time, but many started hopping back and forth between perches in cage A and in cage B. Only nine individuals made physical contact with the novel object.

Zebra finches made 0-192 hops during their five-minute trials. Although the distribution of the number of hops was highly right-skewed, the distribution of the log-transformed values was continuous and unimodal (Figure 4.2). The approach scores varied between 5.5 and 11.2 (possible range: 5.5–42.4) and showed a continuous and unimodal distribution (Figure 4.2). Mean approach scores and hopping activity were significantly positively correlated ($r = 0.34$, $N = 530$, $P < 10^{-15}$, Figure 4.2). Sexes did not differ in their approach scores ($F_{1,528} = 0.01$, $P = 0.93$) or in hopping activity ($F_{1,528} = 0.26$, $P = 0.61$).

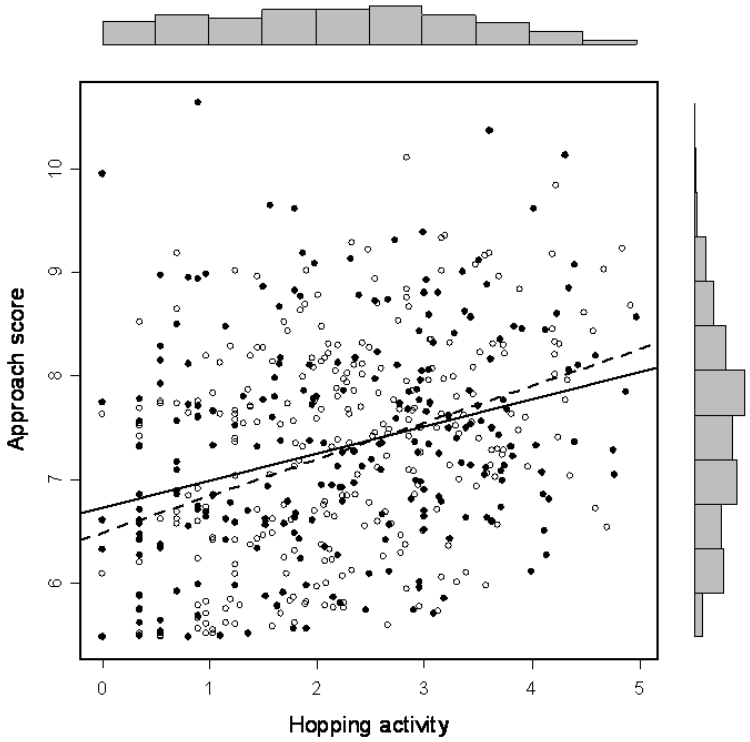

Figure 4.2: *Correlation between approach to novel object and hopping activity scores (transformed values, see Methods). Open symbols refer to males, filled symbols to females. The lines show regression lines for males (dashed) and for females (continuous).*

Repeatability of measurements and consistency across context

Subjects achieved slightly lower approach scores (Cohen's $d = -0.10 \pm 0.06$) and lower hopping activity ($d = -0.11 \pm 0.06$) in the trials with the herbs as novel object than in those with the apple. Scores measured later with the flashy colored ball as novel object correlated significantly with scores measured when presenting more natural objects (approach score: flower-ball $r = 0.49$, $P < 10^{-15}$, apple-ball $r = 0.57$, $P < 10^{-15}$, hopping activity score: flower-ball $r = 0.36$, $P < 10^{-12}$, apple-ball $r = 0.49$, $P < 10^{-15}$, all $N = 362$).

The between-object repeatability was high for both approach score ($R = 0.46 \pm 0.03$, $P < 10^{-34}$) and hopping activity ($R = 0.36 \pm 0.03$, $P < 10^{-21}$; both based on $N = 530$ individuals measured with two novel objects). The between-year repeatability (i.e. after averaging between the two objects) was also high for both approach score ($R = 0.46 \pm 0.07$, $P < 10^{-7}$) and hopping activity ($R = 0.48 \pm 0.07$, $P < 10^{-7}$; both based on $N = 119$ individuals measured twice with a 1.5 years interval). To reduce measurement

error and because the majority of individuals involved in the breeding experiment were tested twice, we used mean scores for all individuals in all further analyses.

To test for the context-generality of activity, we correlated hopping activity in the novel-object trials with hopping activity in choice-chamber trials (Schielzeth *et al.* 2008a,b). Hopping activity in the novel object trials correlated significantly with hopping activity in trials in which birds were presented with groups of males or females in a 2-way choice-chamber (both sexes combined, $r = 0.23$, $N = 287$, $P < 10^{-4}$, own unpublished data). In females, hopping activity in novel-object trials correlated weakly and non-significantly with hopping activity close to males in a four-way choice-chamber (hops per minute spent in front of males; $r = 0.12$, $N = 231$, $P = 0.079$) and with the number of movements between males in a 2-way choice-chamber ($r = 0.13$, $N = 89$, $P = 0.24$).

Quantitative genetics of personality scores

Approach scores showed a substantial amount of heritable variation (43%), whereas the heritability estimate for hopping activity was low with a large standard error (Table 4.1). Foster environment and peer group effects were close to zero for the approach scores and somewhat higher, though non-significant, for hopping activity. Maternal effect estimates were effectively zero for both traits. Based on between-object and between-year repeatabilities we separated the residual variance into variance due to the individual-specific environment (consistent between-individual differences not explained by any of the aforementioned sources), long-term behavioural flexibility (within-individual variation between years) and measurement error (including short-term flexibility and object specificity). Both traits showed low behavioural flexibility between years (after accounting for measurement error within years) and some non-shared environmental component (Table 4.1).

Personality scores and number of fertilized eggs

Female fecundity ranged between 0 and 37 and was very similar between the three treatments (mean $\pm$ SD: male-biased 13.0 ± 5.7, equal sex-ratio 14.3 ± 7.2, female-biased 14.9 ± 7.1). Male fertilization success ranged from 0–54 and increased strongly with declining sex-ratio (mean $\pm$ SD: male-biased 8.7 ± 8.6, equal sex-ratio 14.3 ± 10.5, female-biased 21.9 ± 12.8). For males, the opportunity for selection (variance in relative fitness) based on the number of fertilized eggs differed significantly among treatments (mean $\pm$ SE: male-biased: $I = 1.1 \pm 0.11$, equal sex-ratio: $I = 0.62 \pm 0.29$, female-biased: $I = 0.37 \pm 0.09$; $F_{2,15} = 3.8$, $P = 0.046$). In females, the opportunity for selection was overall lower and did not vary much between the treatments (male-biased: $I = 0.19 \pm 0.09$, equal sex ratio: $I = 0.27 \pm 0.06$, female-biased $I = 0.24 \pm 0.07$; $F_{2,15} = 0.30$, $P = 0.74$).

Table 4.1: *Variance component analysis of the approach score and hopping activity. Additive genetic, maternal, foster environment and peer group effects were estimated from an animal model with four generations of pedigree information. Variance components and their standard errors are shown.*

	Approach score	Hopping activity
Additive genetic effects	0.43 ± 0.19	0.08 ± 0.13
Maternal effects	0.00 ± 0.00	0.00 ± 0.00
Foster environment effects	0.00 ± 0.00	0.14 ± 0.10
Peer group effects	0.02 ± 0.04	0.20 ± 0.11
Residual	0.55 ± 0.19	0.59 ± 0.17
Non-shared environment (lasting effects)	0.12	0.12
Behavioral flexibility over time	0.06	0.00
Object specificity and measurement error	0.37	0.47

Selection differentials for approach score were low and non-significant in males (overall $S = -0.04 \pm 0.08$, $P = 0.80$, overall $C = 0.01 \pm 0.08$, $P = 0.99$). In females there was a non-significant trend that might indicate weak disruptive selection (overall $S = 0.04 \pm 0.05$, $P = 0.34$, overall $C = -0.06 \pm 0.03$, $P = 0.08$). Selection differentials for hopping activity were low and non-significant in males (overall $S = 0.08 \pm 0.09$, $P = 0.16$, overall $C = 0.07 \pm 0.09$, $P = 0.33$) and in females (overall $S = 0.03 \pm 0.04$, $P = 0.33$, overall $C = -0.02 \pm 0.04$, $P = 0.57$).

Selection differentials differed little among the three sex-ratio treatments with the exception of the selection differential on hopping activity in females that showed a trend that was mainly caused by borderline-significant differences in the linear selection differential. In the equal sex-ratio and the female-biased treatment females with high hopping activity tended to produce more eggs (though non-significantly so), whereas in the male-biased treatment females with low hopping activity tended to lay more eggs (Figure 4.3).

Personality scores and chick-rearing success

Our sex-ratio treatment did not affect the average mass of chicks at day 8 (mean $\pm$ SE, male-biased: 7.78 ± 0.10, equal sex-ratio: 7.93 ± 0.10, female-biased: 7.90 ± 0.08; $F_{2,65} = 0.77$, $P = 0.47$), or the variation in chick mass between pairs (Levene test: $F_{2,65} = 0.54$, $P = 0.58$).

Overall, approach scores were only weak predictors of chick rearing success in males ($b_x = 0.17 \pm 0.17$, $P = 0.32$, $b_{x^2} = 0.25 \pm 0.17$, $P = 0.18$), and in females ($b_x = 0.04 \pm 0.15$, $P = 0.76$, $b_{x^2} = 0.16 \pm 0.14$, $P = 0.25$). There was no evidence for an interaction between male and female approach scores to explain chick-rearing success ($b_{int} = -0.01 \pm 0.27$,

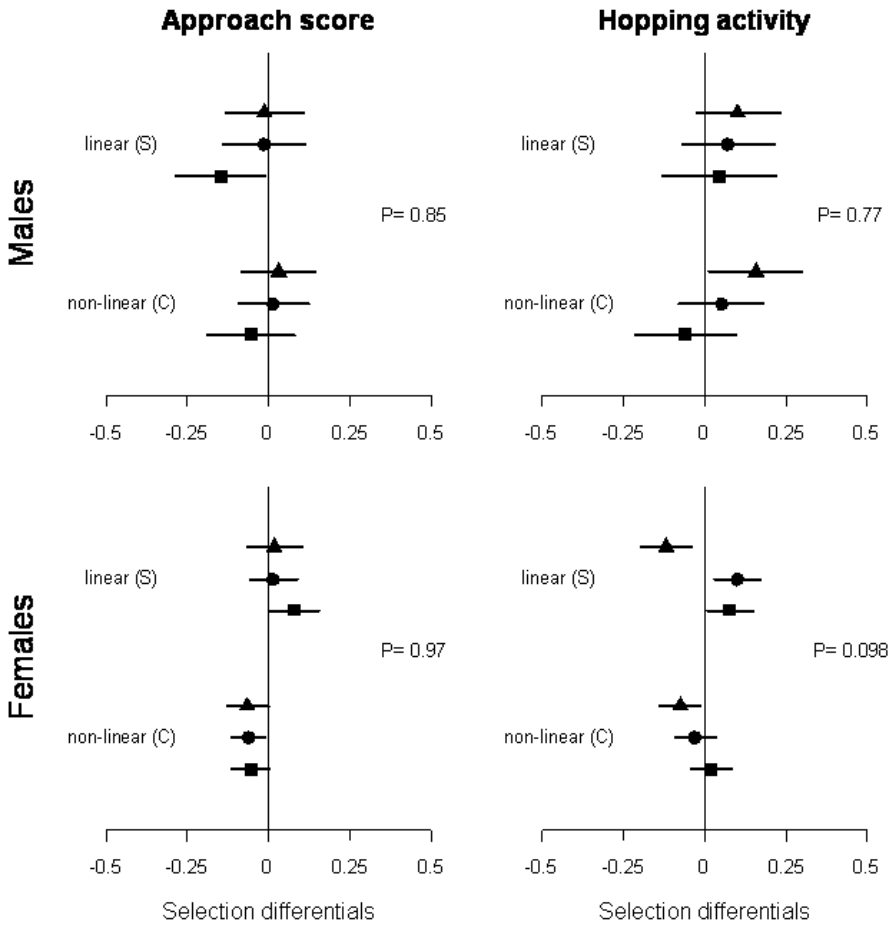

Figure 4.3: *Linear and non-linear selection differentials (means ± SE) on personality traits based on total fertilization success (in males) and fecundity (in females). S = linear selection differential (directional selection), C = non-linear selection differential (selection on the variance, i.e. $C > 0$ means disruptive selection, $C < 0$ means stabilizing selection). The three sex-ratio treatments are marked as male-biased (triangles), equal (circles), and female-biased (squares). Differences between treatments (in the linear and quadratic term combined) were tested by likelihood-ratio test.*

$P = 0.97$). Similarly, hopping activity was only a weak predictor of chick rearing success in males ($b_x = 0.13 \pm 0.19$, $P = 0.62$, $b_{x^2} = -0.04 \pm 0.16$, $P = 0.76$), and in females ($b_x = 0.14 \pm 0.17$, $P = 0.41$, $b_{x^2} = -0.12 \pm 0.13$, $P = 0.46$). However, there was a trend for disassortatively mated pairs to have higher chick rearing success ($b_{int} = -0.33 \pm 0.16$, $P = 0.059$).

There were no significant differences between treatments in how approach score and hopping activity affected chick-rearing success (Figure 4.4).

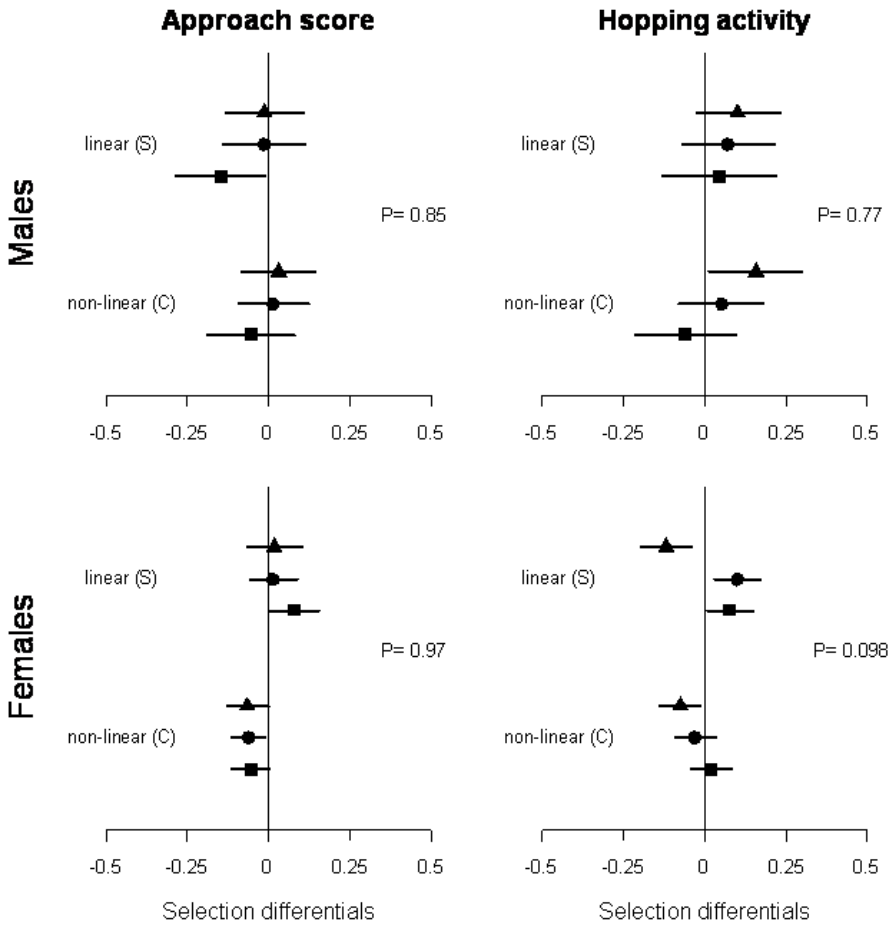

Figure 4.4: *Standardized coefficients (means ± SE) for personality traits as predictors of rearing success measured as mean mass of chicks at day 8. Standardized coefficients represent the change in rearing success in response to changes in the personality trait, both in units of standard deviation. The three sex-ratio treatments are marked as male-biased (triangles), equal (circles), and female-biased (squares). Differences between treatments were tested by likelihood-ratio test. Hence, P values test the between-treatment differences in curves separately for male contribution, female contribution (both with linear and quadratic term combined) and the interaction between the sexes. The linear term captures directional effects, while the quadratic term captures nonlinear effects ($b_{x^2} > 0$ means extremes produces higher-weight offspring, $b_{x^2} < 0$ means intermediates produce higher-weight offspring) and the interaction measures the non-additive interaction between male and female scores ($b_{int} > 0$ means assortatively mated pairs produce higher-weight offspring, $b_{int} < 0$ means disassortatively mated pairs produces higher-weight offspring).*

Personality scores and number of partners

Females laid eggs that were sired by 1–4 (mean: 2.1) partners, except for one individual that reproduced with 5 males in the polygyny treatment. The number of sires did not differ among treatments (Poisson model, LRT: $chi_2^2 = 0.11$, $P = 0.95$). In males, the number of females with whom they sired eggs differed significantly between treatments (male-biased: 0–5, mean = 1.4; equal sex-ratio: 0–6, mean = 2.0; female-biased: $0 - 7$, mean = 3.3; Poisson model, LRT: $\chi_2^2 = 31.28$, $P < 10^{-6}$).

Overall, the effect of approach scores on the number of partners was low and clearly non-significant in males ($b_x = 0.04 \pm 0.09$, $P = 0.61$, $b_{x^2} = -0.09 \pm 0.08$, $P = 0.27$), and in females ($b_x = -0.02 \pm 0.07$, $P = 0.72$, $b_{x^2} = -0.01 \pm 0.05$, $P = 0.81$). Similarly, hopping activity did not explain variation in the number of partners, neither in males ($b_x = 0.10 \pm 0.09$, $P = 0.28$, $b_{x^2} = -0.01 \pm 0.09$, $P = 0.92$), nor in females ($b_x = -0.02 \pm 0.06$, $P = 0.71$, $b_{x^2} = -0.03 \pm 0.06$, $P = 0.59$).

The effect of approach score differed little between treatments, although there were indications that intermediate males had more (genetic) partners in the female-biased treatment, but not in the other treatments ($P = 0.067$, Figure 4.5). Treatments differed little with respect to the influence of hopping activity.

Personality scores and extra-pair paternity

In females, the proportion of eggs sired by extra-pair males (levels of extra-pair paternity in females) did not differ between treatments (means: male-biased: 0.19, equal sex-ratio: 0.14, male-biased: 0.18; $F_{2,81} = 0.19$, $P = 0.83$, tested after angular transformation), whereas in males the proportion of eggs sired with extra-pair females (levels of extra-pair paternity in males) did differ between treatments (means: male-biased: 0.10, equal sex-ratio: 0.14, female-biased: 0.38; $F_{2,79} = 5.89$, $P = 0.041$).

Overall, approach score was only a weak predictor of levels of extra-pair paternity rate in males ($b_x = 0.28 \pm 0.19$, $P = 0.13$, $b_{x^2} = 0.23 \pm 0.19$, $P = 0.23$) and in females ($b_x = -0.43 \pm 0.40$, $P = 0.28$, $b_{x^2} = -0.35 \pm 0.29$, $P = 0.20$). Similarly, hopping activity was a weak predictor of levels of extra-pair paternity rate in males ($b_x = 0.13 \pm 0.22$, $P = 0.56$, $b_{x^2} = -0.18 \pm 0.19$, $P = 0.36$) and in females ($b_x = -0.34 \pm 0.36$, $P = 0.36$, $b_{x^2} = -0.39 \pm 0.30$, $P = 0.20$).

There were substantial differences between treatments in the effect of personality traits on levels of extra-pair paternity. Males with intermediate approach scores showed higher levels of extra-pair paternity in the female-biased treatment (the one with the highest level of extra-pair paternity), whereas in the equal sex-ratio treatment males with extreme approach scores tended to have higher levels of extra-pair paternity ($P < 0.001$, Figure 4.6). Males with high hopping activity showed highest levels of extra-pair

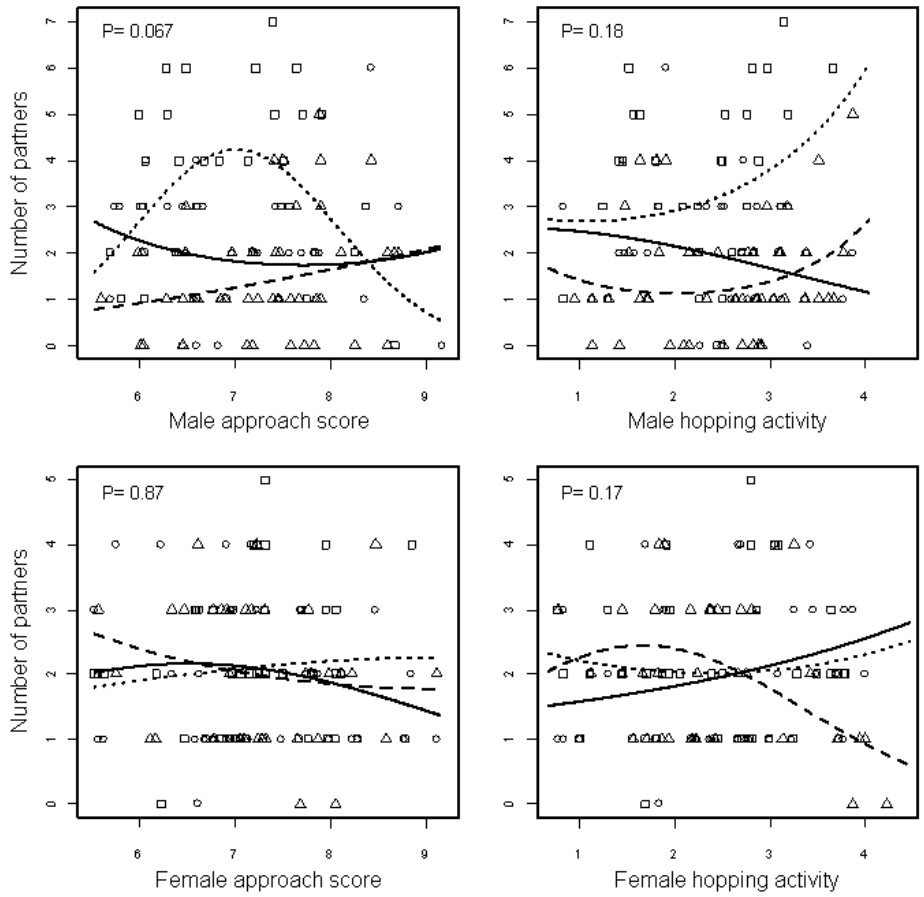

Figure 4.5: *The number of (genetic) partners in aviaries as a function of personality traits (first and second polynomial). Functions were calculated for a Poisson model and back-transformed for display. The three sex-ratio treatments are marked as male-biased (triangles and dashed lines), equal (circles and continuous lines), and female-biased (squares and dotted lines). Differences between treatments (in the linear and quadratic term combined) were tested by likelihood-ratio test.*

paternity in the female-biased treatment, but less so in the other treatments ($P = 0.0037$, Figure 4.6). In females, differences between treatments were less strong. Females with high approach scores had lower levels of extra-pair paternity in the equal sex ratio and in the male-biased treatment, but not in the female-biased treatment ($P = 0.014$, Figure 4.6). Similarly, females of intermediate hopping activity tended to have higher levels of extra-pair paternity in the female-biased treatment, but not in the other two treatments ($P = 0.068$, Figure 4.6).

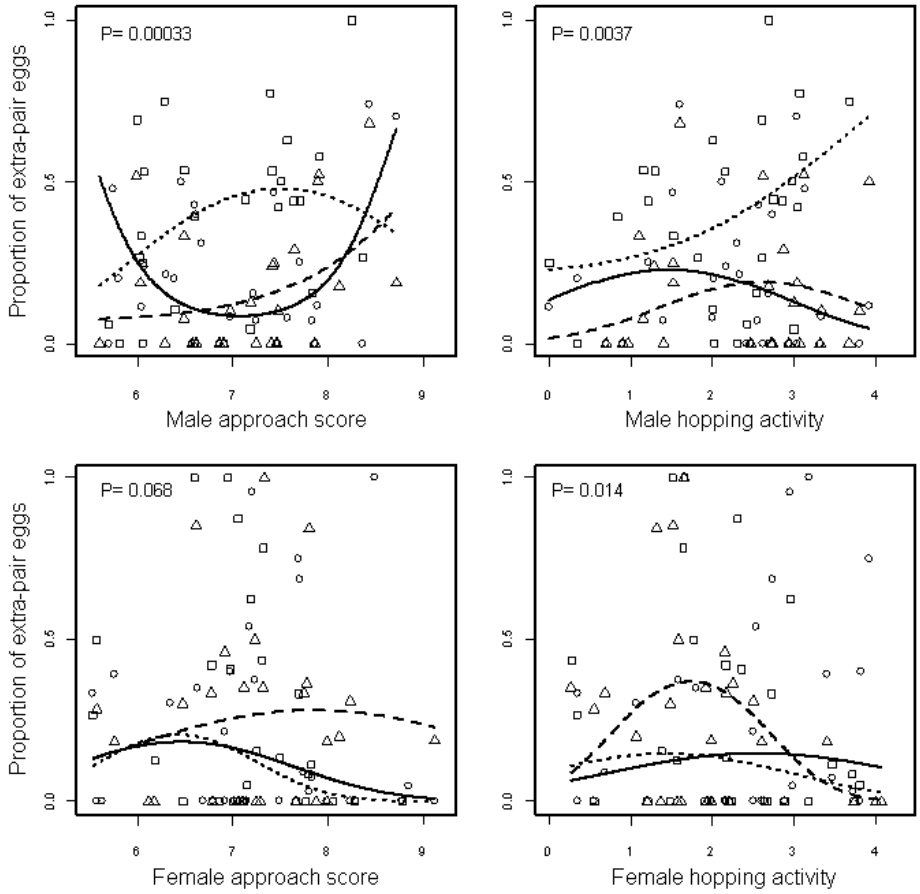

Figure 4.6: *Levels of extra-pair paternity in males (proportion of all fertilized eggs sired with extra-pair females) and in females (proportion of all laid eggs sired by extra-pair males) in aviaries as a function of personality traits (first and second polynomial). Functions were calculated for a binomial model and back-transformed for display. The three sex-ratio treatments are marked as male-biased (triangles and dashed lines), equal (circles and continuous lines), and female-biased (squares and dotted lines). Differences between treatments (in the linear and quadratic term combined) were tested by likelihood-ratio test.*

Discussion

Our study explores the consequences of personality traits for reproductive performance under semi-natural breeding conditions in aviaries. Firstly, we show that approach score and hopping activity as measured in the novel-object experimental setup, were consistent within individuals even over extended periods of time and that there were in-

dications of context-generality. Secondly, we show that approach to the novel-object had an additive genetic basis. Thirdly, we show that our sex-ratio treatment was effective in manipulating reproductive performance of males, but less so of females. Fourthly, we found little evidence for overall fitness consequences under semi-natural aviary breeding conditions. Lastly, we found that the sex-ratio treatments affected the relationship between personality scores and extra-pair paternity, but not overall fertilization or chick-rearing success. This suggests that variation in personality traits might reflect variation in reproductive strategies that depend on the social environment. In the following, we will discuss these points in turn.

Our experimental setup enabled us to measure approach score and hopping activity in a repeatable way. Consistency over time (high repeatability between years) is a critical attribute of personality traits (Réale *et al.* 2007). Strong correlations between different novel objects showed that the traits we measured were not highly context-specific. Correlations of hopping activity with other measures of activity were low, but positive. This indicates that hopping activity is somewhat context-specific to the novel-object situation. Approach score and hopping activity scores were correlated, at least partly because they were measured in the same trial. Nevertheless, we find it appropriate to include both traits in the analysis, since they represent two distinct and interpretable modes of reaction to the novel object.

We found a significant additive genetic component to approach scores, combined with low shared-environment effects. This is consistent with other studies that demonstrated substantial heritability of boldness in a novel-object and/or novel-environment setup (Dingemanse *et al.* 2002; Daniewski & Jezierski 2003; Drent *et al.* 2003). In contrast, hopping activity showed low heritability and a higher environmental component (both shared and non-shared), partly caused by early-environment effects (foster environment and peer groups during youth). Hence brood and peer mates seem to converge to some degree on a common activity-response to a novel situation, possible due to behavioural copying. Similarly, in the threespined stickleback, the heritability of activity in an unfamiliar environment was relatively low ($h^2 = 0.05$ and 0.16 in two populations, Bell 2005), although the same study also found a low heritability of boldness under predation risk ($h^2 = 0.002$ and 0.04). The fact that approach to a novel-object and hopping activity showed very different heritabilities, despite being measured in the same trail, indicates that they indeed represent independent axis of behaviour.

Our sex-ratio treatment was successful in manipulating the opportunity for selection in males. The more competitive the environment (i.e. the more male-biased the sex-ratio), the larger the between-male variation in fertilization success. On the other hand, our treatments did not affect chick-rearing success. In agreement with this, zebra finch males increased nest defense in a male-biased treatment, but did not alter their parental-care behaviour (Burley & Calkins 1999). This suggests that the sex-ratio treat-

ment influenced mainly sexual selection (intra-sexual competition and possibly mate choice) and less so natural selection (parental qualities). The absence of an effect on chick-rearing success may be a consequence of the aviary conditions, with *ad libitum* access to high-quality food (see also below). In females, the treatment did not influence the opportunity for selection as measured by the number of fertile eggs they produced and it had little influence on the number of genetic partners and the proportion of eggs sired by extra-pair males. In contrast, in males, the treatment clearly affected the level of promiscuity: in the female-biased treatment males sired eggs with more females and overall they had a higher extra-pair siring success. In summary, these data show that the sex-ratio treatment had a profound effect on male, but little effect on female reproductive success.

Overall, the two personality traits we measured in the novel-object situation were only weak predictors of fertilization success/fecundity and chick-rearing success. Pairs mated disassortatively for hopping activity reared slightly heavier chicks. Although this effect is relatively weak, it might indicate that chicks reared by parents that behaviourally complement each other receive better care. In contrast, in a field study on great tits, Both *et al.* (2005) showed that assortative pairs of extreme exploratory behaviour produced chicks of higher weight. Although the mechanism behind these findings remains unknown, one could have expected the opposite pattern. This is because behavioural synchronization may be more advantageous in zebra finch pairs, which often forage together (Zann 1996), compared to great tits, where individuals mostly forage alone. There were little indications that the sex-ratio treatment affected the correlations between personality traits and reproductive success.

The weak effects of personality traits on fitness in this study might be a consequence of the *ad libitum* food availability. This is particularly likely for the effect on parental qualities. Differences in parental quality due to more or less effective cooperation might show up only if resources are limited or if resource availability varies in space and/or time (Dingemanse *et al.* 2004; Boon *et al.* 2007). Studying the effectiveness of cooperation between pairs of different personalities in variable environments may be a promising avenue to find effects of novelty seeking and activity on reproductive success and to understand the underlying causes of these effects (Dingemanse *et al.* 2004; Both *et al.* 2005).

The treatment had a more profound effect on the correlations between personality traits and levels of extra-pair paternity than on the correlations between personality traits and overall reproductive success (see above). In the high competition (male-biased) treatment, males with the highest scores for approach to the novel object sired the highest proportion of extra-pair eggs. However, in the female-biased treatment, males with intermediate scores had higher extra-pair success and in the equal sex-ratio treatment males with extreme scores had highest success (Figure 4.6). Despite the posi-

tive correlation between approach score and activity, the treatment effects on the correlation between activity and male extra-pair success showed different patterns. Here, high activity males were more successful in the female-biased treatment. More active males may simply attempt to acquire extra-pair copulations more frequently, which may lead to more success in the female-biased treatment, whereas in the male-biased treatment, bolder (higher novelty-seeking scores) males may be more successful in competition with other males. In a number of species, exploration and boldness are correlated with aggression (e.g. in crickets: Kortet & Hedrick 2007, sticklebacks: Bell 2005, great tit: Verbeek *et al.* 1996) and this might explain the success of individuals with high approach scores in the male-biased treatment. However, increased aggression does not always translate into higher dominance ranks (great tit: Verbeek *et al.* 1999).

The effects of the social environment on the correlations between personality scores and reproductive performance were weaker in females, perhaps because our sex-ratio treatment was less successful in manipulating female reproductive success and behaviour in a measurable way. Clearly, however, there was no positive correlation between female approach to novel-objects and extra-pair paternity as might have been expected (see Introduction).

In summary, most effects we found were relatively weak and given the large number of parameters we estimated, these effects should not be over-interpreted. However, it is remarkable that differences between treatments are apparent when considering extra-pair paternity, but not when looking at overall success (in producing eggs or in rearing chicks). Hence, it seems likely that the personality traits we studied are related to variation in reproductive strategies. Interestingly, these effects depend on the social environment, as shown by the effects of the social environment on the correlations between personality traits and extra-pair paternity rates. It remains to be seen whether personality traits mainly influence performance in intra-sexual competition or whether they also influence (extra-pair) mate choice. Females guppies prefer bold males over shy males (Godin & Dugatkin 1996), but otherwise studies on mating preferences in relation to novelty-seeking on activity are lacking.

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General discussion

In this thesis, I address four topics that have the potential to explain between-individual differences in mating preferences. Chapters 1, 2 and 3 focus mainly on the proximate causes of variation in preference functions that could potentially ensure genetic and/or behavioural compatibility. The genetic and early-environmental effects on reference template formation were generally weak and not strong enough to fully explain the large between-individual variation in preferences present in our population of zebra finches. There seems to be a tendency for inbreeding avoidance via self-referent phenotype matching, but this effect is weak and not easily reproducible (Chapter 3). However, Martin (2008) found a borderline significant trend for an avoidance of sibling mating based on self-referent phenotype matching in a more recent aviary breeding experiment in our zebra finch population. This provides evidence that the initial finding presented in Chapter 3 was not simply a type I error. Nevertheless, template formation based on self-referent phenotype matching certainly explains only very little of the between-individual differences in preferences in our zebra finch population.

Using two different approaches I found no evidence for variation in preference functions based on sexual imprinting (Chapters 1 and 2). There are several reasons why my studies might have failed to show evidence for sexual imprinting. My two studies use completely different sets of birds and a different experimental design, but of course the birds stem from the same captive population and were exposed to a similar breeding regime; hence, they are not completely independent. For example, all female subjects were separated from their parents at day 35. The sensitive phase of song learning starts already around day 25 (Roper & Zann 2006), but song learning continues after day 35 and it is likely that female preferences for specific songs also develop after day 35. Although Riebel & Smallegange (2003) did not find sexual imprinting on parental song in zebra finches, it is possible that female zebra finches need both, the visual and acoustic stimulus. Since Riebel & Smallegange (2003) focused exclusively on sexual imprinting

on acoustic stimuli while my studies primarily focused on imprinting on visual (and behavioural) stimuli (Chapters 1 and 2), no study has ever tested for the possibility of interaction effects between acoustic and visual stimuli. It is also possible that the importance of sexual imprinting differs between sexes (ten Cate 1985; Witte & Sawka 2003) and that I have missed effects when focussing on sexual imprinting in females. It would be interesting to test, if males sexually imprint on their mothers on the fine scale of within-population variation (imprinting on the coarse level of species, morphs and novel ornaments has been demostrated for male zebra finches in a number of studies, Bischof & Clayton 1991; Kruijt & Meeuwissen 1993; Vos *et al.* 1993; Oetting *et al.* 1995; Oetting & Bischof 1996, but see Witte & Sawka 2003 and Witte & Caspers 2006).

Despite these potential reasons that might produce non-significant findings, there is overall (including the published literature) only very limited empirical evidence for sexual imprinting being an important factor for generating within-population variation in preference. The published evidence shows that sexual imprinting serves outbreeding avoidance on a very coarse (between-population) level (Irwin & Price 1999; ten Cate & Vos 1999; Hauber *et al.* 2001; Grant & Grant 2002; Ihara & Feldman 2003), but does not necessarily generate within-population variation in preferences (Chapters 1 and 2). This suggests that sexual imprinting is mainly relevant for the categorisation of individuals (species recognition) (see Chapter 2 for references and discussion) and less so for the formation of mating preferences in a more or less homogenous population. The only positive evidence for the latter comes from a study on humans, which shows that the husbands of daughters raised by unrelated foster parents show a detectable facial similarity to the women's foster fathers (Bereczkei *et al.* 2004). My studies on zebra finches illustrate that this does not necessarily generalise to other species. Unfortunately, a more recent study that aimed at pinpointing the cues for sexual imprinting in humans has been retracted by the authors (Bereczkei *et al.* 2009).

In order to plan further studies on sexual imprinting it is worth considering under which ecological conditions one would expect sexual imprinting to be an adaptive mechanism for forming mating preferences. Such considerations will help to search for candidate model species. One particular advantage of sexual imprinting is that it could serve behavioural compatibility. If effective cooperation between partners is important for effective reproduction, it might be advantageous to adjust one's own behaviour and to avoid behavioural conflict. Sexual imprinting could potentiall ensure mating with behavioural phenotypes that a subject is already familiar with (having experiences the behaviour of its parents). This could potentially save time and avoid conflict. Therefore, it seems most promising to test for sexual imprinting in species with bi-parental care and scope for behavioural compatibility issues. Alternatively, sexual imprinting could primarily serve genetic compatibility. This explanation, however, is somewhat at odds with the kin recognition studies that would suggest disassortative preferences based on

sexual imprinting (a special case of family phenotype matching), because inbreeding is an extreme case of genetic (in)compatibility. This rests on the assumption that inbreeding is costly, which seems to be the case in a large number of species studied (Pusey & Wolf 1996) including the zebra finch (Bolund *et al.* in prep.). Hence, I suspect that behavioural compatibility (rather than genetic compatibility) is the more likely cause that might drive sexual imprinting as an adaptive strategy. Therefore, it seems not very surprising that the only positive evidence stems from humans (Bereczkei *et al.* 2004), since humans seem to be a prime candidate for such studies mainly due to their long-term partnerships and bi-parental care. More studies on sexual imprinting in other species are clearly needed and are most promising in species in which cooperation is essential for raising offspring successfully.

One of my studies (Chapter 1) was testing not only for sexual imprinting but more generally for early rearing effects that would produce a similarity in preferences between foster sisters. There is some evidence that early developmental stress affects the attractiveness of zebra finch males to females (de Kogel & Prijs 1996; Blount *et al.* 2003a; Spencer *et al.* 2003; Naguib *et al.* 2004; Spencer *et al.* 2005). The idea that only high quality individuals are sufficiently able to buffer against harsh early rearing conditions has been termed the developmental stress hypothesis (Nowicki *et al.* 1998). In our population, we have tested for effects of early conditions on adult traits in males and females. We found that while the morphology was affected (stressed individuals stayed smaller), there were no measureable effects on male sexual ornaments (Bolund *et al.* submitted). Interestingly, however, male fertilisation was significantly impaired in individuals that had experienced harsh rearing conditions (Bolund *et al.* submitted). There is not much published work that empirically tests, if early-conditions influence mating preferences (Jennions & Petrie 1997). Early conditions are more likely to affect choosiness rather than the preference functions themselves. Nevertheless, it is possible that subjects that had experienced harsh conditions during early life would prefer partners of high parental quality over partners of high genetic quality. This might produce differences between individuals, if these two axes are sufficiently independent. My mate choice study focused on shared effects that would produce similarities between females when they were raised in the same brood (Chapter 1). However, some of the early rearing effects are non-shared. For example, Bolund *et al.* (submitted) used non-shared variation to test for life-time consequences of early rearing conditions. It is possible that such non-shared rearing conditions would produce systematic patterns in female mating preferences.

Interestingly, I found that the within-population heritability of preference functions was relatively low (Chapter 1). This finding is important, since the heritability of mating preferences is a critical assumption of models of evolution. For example, the Fisherian runaway process requires a heritability of preferences to form a genetic correlation be-

tween a trait and a preference for the trait (Fisher 1930; Lande 1981). My finding is in general agreement with previous studies that found partly significant, partly non-significant but mostly low heritabilities (see references in Chapter 1, in particular Table 1.1). Nevertheless, there is evidence for a non-zero heritability of preference functions from selection lines (Wilkinson & Reillo 1994; Brooks & Couldridge 1999). This underlines that even a low amount of heritable variation can have a significant effect on evolution. Although the evolutionary significance of heritable variation in preferences is not in doubt, heritable variation in preferences apparently does not explain much of the between-individual variation in preferences within populations.

When measuring the similarity in preferences between full-sib sisters (Chapter 1), this estimate includes similarity due to additive genetic effects (in full-sibling 50% of the alleles are identical by decent, Lynch & Walsh 1998), but also due to dominance and epistatic interactions (full-siblings share 25% of the dominance and epistatic interaction effects, Lynch and Walsh 1998) and maternal effects (full-siblings share 100% of the prenatal maternal effects, Mousseau & Fox 1998; Qvarnström & Price 2001). This seems like a disadvantage of my study. However, the low similarity between genetic sisters shows that it would be almost impossible to accurately separate additive genetic effects, non-additive genetic effects and maternal effects with a tractable number of birds. Hence, my experimental design allows concluding that all of these effects have to be very low, since even the combined effect was low and non-significant.

A separation of additive genetic, non-additive genetic and maternal effects would require a half-sib full-sib experimental design and a large number of females. Most of the studies that quantified the heritability of preference functions were conducted using insects that allow a larger number of individuals to be tested (Collins & Cardé 1989; Gray & Cade 1999; Jang & Greenfield 2000; Iyengar *et al.* 2002; Simmons 2004; Table 1.1). My study is only the third study that measured the heritability of preference functions in a vertebrate species and the first one that did so in birds. This is certainly partly due to the difficulty in measuring mating preferences precisely (repeated measures are required due to relatively low repeatabilities) and large numbers of individuals are needed to accurately quantify low heritabilities. While the estimate for the heritability of preference functions was low, there was good evidence for a heritable component to choice behaviour (Chapter 1, see discussion below). Genetic sisters behaved similarly in the choice chamber, but they did not (at least not to a significant degree) agree on which males they preferred.

It is possible that template formation effects would be more easily detectable when focussing on single traits instead of studying preferences at a full multidimensional scale (see Introduction). This is an approach that many of the previous studies on zebra finches have taken (Burley 1986b; Burley & Coopersmith 1987; Burley 1988; Clayton & Prove 1989; Collins *et al.* 1994; Swaddle & Cuthill 1994a; Collins 1999; Gil *et al.* 1999;

Rutstein *et al.* 2004; Gorman *et al.* 2005; Gilbert *et al.* 2006; Williamson *et al.* 2006). However, my results show that effects found by means of single-trait manipulation do not play a substantial role in explaining within-population variation in preferences. The strength of my approach is that it puts mating preferences into a very natural setting, where individuals differ in multiple aspects and individuals have to make their choices between potential mates that vary in a large number of traits.

Multidimensional signals might be either redundant ('backup-signals') or convey multiple messages (Johnstone 1996; Candolin 2003). In the case of redundant signals, multiple traits would map on one (or few) principle axes of attractiveness. Since every single trait can be expected to contain some environmental noise to its signalling function, redundant signals would reduce assessment error and hence facilitate mate choice (Johnstone 1996). This is most likely to produce clearer preferences with less assessment error and hence a more uniform preference in the population (if reference templates are largely shared between individuals) or more easily identifiable differences between individuals (if reference templates are largely non-shared). However, empirical data suggest that different signals are often uncorrelated (Candolin 2003). Alternatively, if multiple traits convey multiple messages (Johnstone 1996; Candolin 2003; Scheuber *et al.* 2003a,b, 2004), the situation is more likely to produce between-individual differences in preferences. If there are multiple peaks in the multidimensional attractiveness surface, individuals might differ in their choice of peaks. This leaves us with the question which proximate mechanisms make individuals prefer different optima; and my results show that it is difficult to pinpoint them.

There are several reasons for condition-dependent differences in choosiness and sampling strategies. Individuals in poorer condition can be expected to be less choosy (Burley & Foster 2006), because investment into mate choice trades off with other necessities. However, if the costs of choice are relatively low and marginal benefits are larger for individuals in less good condition, individuals in poorer condition might actually be choosier because they benefit more (Buchholz 2004). In our laboratory population of zebra finches, food was available *ad libitum* and environmental conditions were highly constant. Therefore, condition-dependence of choosiness is likely to be less important than under harsher environmental conditions. Interestingly, female choice behaviour in the choice chamber was significantly affected by inbreeding (Bolund *et al.* in prep.). Inbred females were less active in the choice chamber and their time allocation deviated more from an even time allocation to all males, i.e. they spent relatively more time with their preferred male. This indicates that inbreeding affects choosiness in zebra finches. Individuals are also likely to differ in their preferences depending on the context of mate choice. For example, individuals might differ in their preferences for social partners versus extra-pair partners (Candolin 2003). All mate choice experiments presented in Chapters 1, 2 and 3 were conducted with unpaired individuals at similar ages and

with similar past experience. This was done to control for differences in context as far as possible. Although short-term effects might produce differences in state (like condition or motivation), my experimental standardisation helped avoiding possible biases that could arise from context-dependent mate choice.

Wagner (1998) has argued that choice-chamber experiments with simultaneous presentation of stimuli entails a mixture of preference functions and sampling behaviour in the data. Part of the between-female differences in the distribution of time among males could be due to differences in sampling behaviour. But even when looking at the outcome of choices rather than the proportion of times spent with males, there was little agreement between females (Chapter 1). Therefore, it is unlikely that my experimental setup as such might have produced a biased pattern of between-individual variation in preferences. Nevertheless, it is a valid argument that mate choice studies have to make sure not to mix up preference functions and sampling behaviour or choosiness. For example, Bakker (1993) found that sisters of brightly coloured male guppies had a stronger preference for bright males than sisters of duller males. This is a frequently cited example for heritability of preferences and for a genetic covariance between female preferences and male attractiveness. However, the data shows that females either preferred bright males or they did not discriminate between males. Female preferences were measured as the proportion of displays towards the brighter one of two stimulus males and all females showed 40-100% of their display to the bright males. A few females that preferred the duller male in one out of two trials were excluded from the analysis. Such a finding is probably better interpreted as between-female variation in choosiness rather than variation in preference functions, since all females preferred the bright male (more or less clearly) and none of the females clearly preferred a dull male. Hence the axis of variation is 'weak preferences' to 'strong preferences' (i.e. a measure of choosiness) rather than 'preference for dull' versus 'preference for bright' males (which would indicate variation in preference functions).

One potential reason why it turned out difficult to pinpoint variation in preference functions might lie in the choice of a domesticated stock of zebra finches. It seems possible that zebra finches have been unintentionally selected to become less choosy during domestication. If breeders have force-paired zebra finches for a large number of generations and have used offspring from pairs that were fastest to reproduce, then the stock of domestic zebra finches would consist largely of descendants from fast-breeding, less choosy individuals. This would be particularly problematic if birds were bred under cage conditions, while breeding in aviaries would have left some possibilities for mate choice. Unfortunately, the (artificial) selection regimes that our stock was exposed to during domestication are unknown. Reassuringly, however, Rutstein *et al.* (2007) did not find systematic differences in repeatability of choices between wild-caught and domestic zebra finches. Furthermore, Tschirren *et al.* (2009) found that the effects of early

rearing conditions on life history traits were remarkably similar between domesticated and wild-caught zebra finches. Although this does not rule out differential selection with respect to choosiness, it shows that basal life history traits are relatively stable and were not strongly altered by domestication.

It has been suggested that between-individual variation in responsiveness, choosiness and sampling behaviour are more important than between-individual variation in preference functions (Brooks & Endler 2001; Brooks 2002). This is supported by high individual consistency of behaviour in the choice-chamber (Chapter 1). There are several reasons why individuals would differ in choice behaviour and investment into mate choice (Figures 1 and 2). If there are alternative behavioural strategies with equal net benefits, specialisation on sampling strategies might be beneficial, because it tends to reduce costs by increased efficiency (Bolnick *et al.* 2003). In this context, it seems promising to study the between-individual differences in mating strategies under a personality framework as a 'mate-choice behavioural syndrome' (Sih *et al.* 2004a,b).

It is difficult to say what the consistent between-individual and heritable differences in choice behaviour in the choice chamber (Chapter 1) would mean in a wild situation. I found individuality in the clarity of choice, which might indicate variation in choosiness. Individuals that distribute their time more unevenly between the two males can be considered to be choosier than individuals that distribute their time more evenly (see discussion of the findings of Bakker 1993 above). However, some of the females that showed very extreme values of time spent with individual males might actually be considered less choosy, since they did not make much effort in assessing both males. Hence, the interpretation of clarity of choice is slightly ambiguous. Furthermore, I found individuality in the number of comparisons females made between males. Individuals that made many comparisons would be considered choosier than individuals that made few comparisons. However, aspects of activity, a very basic axis of variation in behaviour (Réale *et al.* 2007), might also contribute to the variation in the number of comparisons. Hence, this interpretation is also not unambiguous. Finally, I found heritable variation and individuality in overall activity in the choice chamber. Interestingly the overall activity was independent of the number of comparisons. Still both of them might be related to variation in activity in other contexts (Réale *et al.* 2007).

Chapter 4 addresses issues of behavioural compatibility in an aviary breeding environment. My findings give no evidence for pair-combination effects on rearing success with respect to two personality traits (novelty-seeking and activity). This study tested only for very specific aspects of behavioural compatibility. One possible approach to address behavioural compatibility more comprehensively would be to estimate the interaction variance of chick-rearing success in pairs. This could be done by repeatedly measuring chick-rearing success from a pool of males and a pool of females including changes of partners (preferentially multiple times). This would be a reliable method

to separate individual components (individual repeatabilities) from pair-combination effects (compatibility). Such follow-up experiment would be desirable. Chapter 4 addresses the importance of two specific personality axes: novelty-seeking and activity. I investigated proximate and ultimate causes of these personality traits, since independent of behavioural compatibility issues it is important to understand the origin and maintenance of variation in personality traits (Réale *et al.* 2007).

Now, what produces between-individual differences in preferences? I have made another preliminary experiment that gives some hint that familiarity might be important for mate choice in zebra finches. I have conducted a new series of 4-way choice chamber trials like the ones done with the son of the foster parents (Chapter 2) and the unfamiliar brother (Chapter 3). Contrary to all other studies presented in this thesis, subjects were not cross-fostered. Hence subjects grew up with sibling nest mates (mostly full-sibs and some half-sibs due to extra-pair paternity) and were allowed to stay with their sibling nest mates through puberty up to around day 100. In the 4-way choice chamber I used as stimulus males (a) a full-sibling nest mate, (b) a distantly related familiar aviary mate that females shared the natal aviary and the peer group with (up to around day 100) and (c-d) two unfamiliar and unrelated same-aged males. This allowed me to test if female zebra finches discriminate against the familiar full-sib brother, when they had the possibility to follow him from the nest till after the postjuvenile moult and song development (direct familiarisation). At the same time, the experimental design allowed measuring the preference for the familiar aviary mate over unfamiliar males.

I tested a total of 36 females. Surprisingly, these females did not show a significant avoidance of the familiar nest brother, as could be predicted from reasons of inbreeding avoidance based on direct familiarisation (Figure 5a). This was evident when analysing the relative time spent with the familiar nest brother as well as when analysing the number of trials, in which the nest brother received the largest amount of time by his sister (8 out 36 trials, with an expectation of 6 out of 36). Interestingly, among the 36 females there was a highly significant tendency to prefer the unfamiliar nest mate over the other males (Figure 5b). Again, this finding was consistent when analysing the relative time allocation and when analysing the number of times the familiar aviary mate received largest amount of time by the female (17 out 36 trials, with an expectation of 6 out of 36).

These new results suggest that familiarity might play an important role in forming mating preferences in female zebra finches. Females might benefit from choosing familiar males for reasons of behavioural compatibility. This might be the case for two reasons: First, partners that are familiar with each other might cooperate more quickly and effectively than unfamiliar individuals, since they already know each other's behavioural types. This implies behavioural predictability. Second, and possibly more

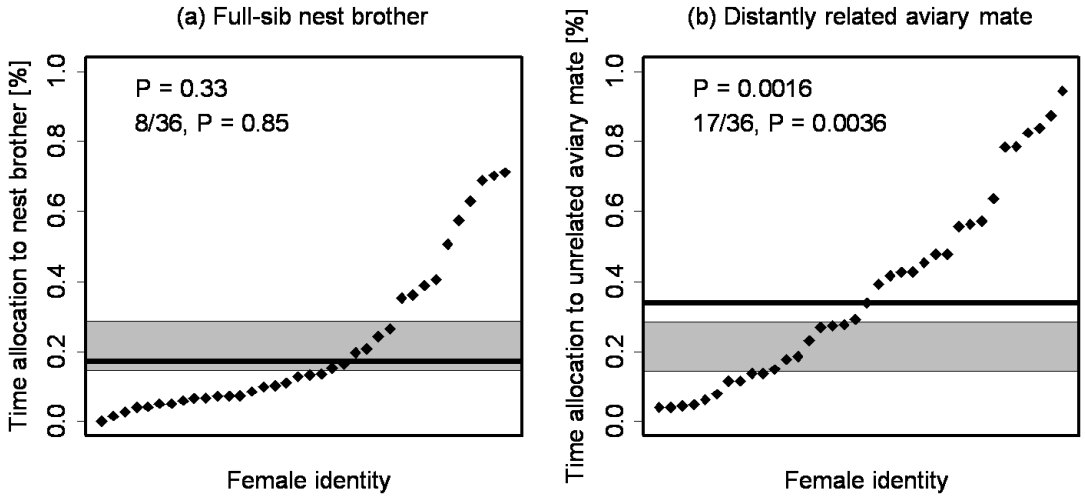

Figure 5: *Time allocation to (a) a familiar nest brother and (b) a familiar, more distantly related aviary mate in a 4-way choice chamber. Thirty-six females were tested with a familiar nest brother, a familiar, distantly related aviary mate and two unfamiliar, unrelated males. The bold black line shows the empirical mean of the data points. The shaded grey area shows the 95% confidence interval for the expected mean as estimated from randomization of the data. The upper P value in each plot shows the randomisation P value based on the relative time allocation. The ratio and the lower P value show the number of trials in which the nest brother and the familiar aviary mate achieve the highest time allocation within a trial (Binomial test against an expectation of 1/4).*

importantly, choosing familiar individuals as partners might reduce the risk of choosing an uncooperative partner. Subjects know each other from previous encounters and might thus have more profound information about each other's qualities. The detailed reasons are of course speculative and will need more exploration.

There is one important caveat that hampers a clear interpretation of my new finding. Due to the particular breeding design (that was implemented for reasons beyond the scope of my thesis), the aviary mate was at the same time more closely related to the choosing female than any of the unfamiliar males (Martin 2008). Actually, the familiar aviary mate shared all grandparents with the choosing female and was thus more closely related than a cousin. Hence, familiarity is confounded with relatedness in this preliminary experiment. An improved experimental design would need trials where females could choose between (a) familiar, unrelated individuals, (b) familiar, related individuals, (c) unfamiliar, related individuals and (d) unfamiliar, unrelated individuals. However, this requires a breeding design that we did not have in place at the time of

testing. But clearly there is scope for further mate choice trials to clarify the familiarity issue, particularly since my initial results seem promising.

The starting point of my thesis work was the surprising between-individual variation in mating preferences that was found in many animal populations (Bakker & Pomiankowski 1995; Jennions & Petrie 1997; Widemo & Sæther 1999; Brooks & Endler 2001; Brooks 2002). It is obviously important to show that individuals do not only differ in individual choice trials, but that they differ consistently. The estimation of repeatability is an essential tool to quantify the relative contribution of between-individual to total phenotypic variation (Nakagawa & Schielzeth submitted). In our population of zebra finches we found a significant within-female repeatability of choice-chamber attractiveness of 0.26–0.29 (Forstmeier & Birkhead 2004, Chapter 2). This means that individual preferences as measured in the choice chamber are not just random noise, but individuals show significant consistency. The unrepeatable part of choice-chamber preferences contains measurement error, individual flexibility in preferences and changes in male condition between trials.

It remains challenging to understand the origin of the repeatable between-individual variation in mating preferences. Between-individual differences in choosiness and sampling strategies seem to play a role and these characteristics are likely to be responsible for the outcome of choices that ultimately matter for sexual selection (Qvarnström *et al.* 2006a,b). Hence, studying the individuality in mate choice behaviour seems promising for providing new insights. There is relatively little scope for genetic compatibility issues independent of the inbreeding-outbreeding axis of relatedness (Tregenza & Wedell 2000). For theoretical and empirical reasons, genetic compatibility issues are most likely to be relevant at the extreme end points: inbreeding and hybridisation. Preliminary results show that familiarity might be involved in producing differences in preferences between individuals. However, even familiarity does not explain why individuals do not agree in their preferences when given the choice between unfamiliar individuals (as in Chapters 1, 2 and 3). Hence, although my results constitute a step forward in understanding the causes of between-individual differences in mating preferences, large parts of the phenomenon remain puzzling.

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Summary

Mate choice, the realisation of mating preferences, influences the course of evolution via sexual selection. There are a number of reasons why individuals would benefit from choosing a suitable partner, most of which predict population-wide preferences with little between-individual differences. For example, this applies to good-gene indicator and 'sexy-son' traits. However, recent studies show that in many animal populations individuals differ substantially in their mating preferences. It is important for our comprehension of sexual selection to understand the proximate and ultimate causes of between-individual variation in mating preferences.

There are some adaptive explanations why individuals within a single population would differ in their mating preferences. Genetic incompatibility introduces an axis of genetic quality that is not universal within a population, since it involves the interaction of male and female genomes. Current evidence for genetic incompatibility is strong for outbreeding and inbreeding depression. Hence, individuals benefit from species recognition mechanisms that prevent hybridisation as well as from kin recognition that prevents inbreeding. Although theoretically possible, evidences for genetic incompatibility independent of relatedness are scarce. Compatibility might also be behavioural, since the combination of partners within a pair bond might influence their parental success. To date, studies on behavioural compatibility are very limited and both assortative as well as disassortative mating seem theoretically possible.

A topical issue in evolutionary ecology is the origin of mating preferences from heritable variation or (learnt) early-rearing effects. I have quantified the relative importance of both by comparing the similarity of preferences between genetic sisters that were brought up by different unrelated foster parents and the similarity of preferences between unrelated females that were brought up by the same foster parents (Chapter 1). There was low overall agreement between females on male attractiveness, but remarkably there was also low agreement between genetic sisters and between foster sisters. This shows that heritable variation as well as shared early-rearing effects are very low.

One particular aspect of early-rearing effects that would make (foster) sisters similar to each other in their preferences is sexual imprinting. Sexual imprinting is the forma-

tion of mating preferences during early development by using the own (foster) parents as models. I tested specifically for sexual imprinting by measuring the preferences of cross-fostered daughters for an unfamiliar son of the foster parents (Chapter 2). Since a biological son of the foster parents is similar to the foster parents for all heritable traits, (positive) sexual imprinting would induce (foster) daughters to prefer him as a mating partner. However, female zebra finches did not prefer (nor avoid) the son of the foster parents in choice trials. This shows that sexual imprinting does not play an important role for the formation of mating preferences within a single population. With the current state of evidence, a generalisation from studies showing sexual imprinting on traits involved in the categorisation of individuals (e.g. for species recognition) seems to be premature.

Since incestuous mating is, in most cases, clearly disadvantageous, the ability to recognise and avoid unfamiliar kin as mating partners seems particularly beneficial. I have applied a strict individual cross-fostering regime that excluded contact with kin during early development. As adults, females were tested for their preferences for unfamiliar brothers (Chapter 3). When contact with kin is excluded, individuals can only resort on self-referent phenotype matching to recognise kin. I found a significant avoidance of unfamiliar brothers in one series of tests, while subsequent trials did not show the same pattern. It seems that kin avoidance based on self-referent phenotype matching is relatively weak and not easily measurable.

All the topics described so far address the proximate mechanisms that would produce differences in preferences. I have also tested for behavioural compatibility on the ultimate level, by measuring reproductive success (an important component of fitness) with respect to two important personality traits; i.e. novelty-seeking and activity (Chapter 4). Such personality traits are assumed to be relatively context-general and individual-specific axes of behaviour. I found no consistent personality-related differences in reproductive success and no pair-combination effects on chick-rearing success. However, personality traits were related to reproductive strategies and this effect depended on the social environment.

Collectively, my experiments show that the origin of variation in preferences is not easily understood by heritable variation or (learnt) early-rearing effects. This applies at least for preference functions ('what to prefer'), when tested within the full multidimensionality of within-population variation in traits. Interestingly, however, the data show substantial between-individual variation in choice behaviour (Chapters 1 and 4), parts of which are due to heritable variation (Chapter 1). This means that not only are individuals consistent in their choice behaviour, but also relatives are similar to each other in how they behave in mate-choice contexts. Hence, individuals appear to differ in choosiness and/or sampling behaviour ('how to choose'), which is likely to play an important role in generating between-individual differences in mating patterns.

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Behavioural Biology Group, Prof. Johan Bolhuis, Utrecht: invited talk
141st Annual Meeting of the Deutsche Ornithologen-Gesellschaft (DO-G), Bremen: talk and statistical consulting service

2007

140th Annual Meeting of the Deutsche Ornithologen-Gesellschaft (DO-G), Giessen: talk and statistical consulting service

2006

11th Congress of the International Society for Behavioural Ecology (ISBE), Tours: talk
26th International Ornithological Congress (IOC), Hamburg: poster

2003

136th Annual Meeting of the Deutsche Ornithologen-Gesellschaft (DO-G), Halberstadt: talk and poster
7th Annual Meeting of the Deutsche Gesellschaft für Orthopterologie (DGfO), Münster: talk and poster

Publications in peer-reviewed journals

Submitted

Schielzeth, H., Bolund, E., Kempenaers, B. & Forstmeier, W. (submitted). Variation in personality traits and their relevance for sexual selection in captive zebra finches.

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