

Compatibility benefits of social and extra-pair mate choice in the zebra finch

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Table of Contents

General introduction	3
Chapter 1 Does hatching failure breed infidelity?	31
Chapter 2 Revisiting the evidence for inbreeding avoidance in the zebra finch	43
Chapter 3 Fitness consequences of mate choice for compatibility in a socially monogamous species	53
General discussion	83
Summary	97
Acknowledgments	99
Author Contributions	101
Addresses of Co-Authors	103
<i>Curriculum vitae</i>	105
Statutory Declaration and Statement	107

General introduction

Sexual selection

Sexual selection, as Darwin already stated in 1871 (p. 256), is “that kind of selection, which [...] depends on the advantage which certain individuals have over other individuals of the same sex and species in exclusive relation to reproduction.” This evolutionary force was proposed to explain the evolution of secondary sexual traits which deviate from their presumed naturally selected optimum, while allowing the bearer of those traits to leave more copies of its alleles in future generations.

Typically, sexual selection is divided into intra- and inter-sexual selection (Danchin & Cézilly 2007; Brooks & Griffith 2010; Davies *et al.* 2012). Intra-sexual selection is the competition that occurs within one sex for access to the gametes of the other sex. This form of sexual selection has been put forward to explain the evolution of traits like armaments or traits involved in sperm competition. Inter-sexual selection is, respectively, a reduction of the set of potential mates imposed by one sex on the other according to certain traits. Such mating biases can result from passive processes where, for instance, individuals evolve traits that increase their likelihood of being detected by members of the other sex (Wiley & Poston 1996), but can also results from mate choice in the narrow sense, where mating preferences exerted by the choosing sex induce the non-random mating (Jennions & Petrie 1997; Kokko *et al.* 2003). Inter-sexual selection has been suggested to promote the evolution of ornaments.

The intensity of both intra- and inter-sexual selections can be quantified by measuring the variance in mating success within each sex. Usually, intra-sexual competition is most intense in males who show greater variance in reproductive success than females, as male (but not female) mean reproductive success increases with mating success (Bateman principle, Bateman 1948), while mate choice is expressed by females who also usually invests the most in each individual offspring (Trivers 1972). This asymmetry has its origins in anisogamy and is preserved in amniotes where the certainty of paternity is obscured due to internal fertilization. However, this pattern can be modulated by a variety of factors, for instance by the sex-specific costs of breeding, mating, competing, and caring in terms of reproduction and survival (Kokko & Monaghan 2001; Kokko *et al.* 2006; Kokko *et al.* 2012), which may result in biases of the adult sex-ratio of the whole population (Kokko & Jennions 2008), or the part of it that is available for mating (i.e. operational sex ratio, Emlen & Oring 1977). For example, necessity of biparental care for offspring survival can constrain mating behaviour and lead to strict monogamy, a system where male and female sex roles are much more symmetrical. However, caring and mating are not mutually exclusive in many socially monogamous species, and extra-pair mating can reinstate a greater between individual variance in reproductive success in males *via* female extra-pair mate choice and

male-male competition (Westneat *et al.* 1990; Webster *et al.* 1995; but see Whittingham & Dunn 2005).

Evolution of mate choice

Direct selection

Mating preferences allowing individuals to gain direct benefits from being choosy could logically evolve under direct selection (Kirkpatrick & Ryan 1991; Møller & Jennions 2001). Direct benefits associated to the quality of the partner include nuptial gifts (Vahed 1998), fertilization insurance (Arnqvist & Nilsson 2000; Hasson & Stone 2009), nest site and feeding territory (Alatalo *et al.* 1986), protection against harassment by other member of the opposite sex (Clutton-brock & Parker 1995), defence against predators (Rodriguez-Munoz *et al.* 2011), reproductive investments such as fecundity (Berglund *et al.* 1986), and parental care (Hill 1991).

Preferences can also have been selected for directly by natural selection, rather than by sexual selection, shaping the sensory system of individuals, for instance to optimize their foraging success. Mating preferences can then result from such sensory biases which leaves the opportunity for the competing sex to exploit them (Ryan 1990; Endler & Basolo 1998; Ryan 1998; Ryan & Cummings 2013). For instance, guppies (*Poecilia reticulata*) may benefit from an increased sensitivity to orange to optimize foraging on orange items (Rodd *et al.* 2002). This might have explained the evolution of male orange coloration whose intensity is subject to directional female choice (Rodd *et al.* 2002; but see Grether *et al.* 2005). Interestingly, pre-existing biases in the sensory system might be present in a species, like the preference for red in three and nine-spined stickleback (*Gasterosteus aculeatus* and *Pungitius pungitius* respectively), but might not (yet) be exploited by males in the context of mate choice: only males of three-spined stickleback have developed red throat and jaw, which females have been shown to prefer (Smith *et al.* 2004).

Simultaneously, there could be direct sexual selection acting on mating preferences that diminish the direct cost of mating such as the transfer of diseases or parasites. Sexual conflict theory extends on this idea, by including all direct costs that one sex, usually males, can impose on the other sex while attempting to increase its own fitness. For instance, when sexes differ in their optimal mating rate, males could develop traits that exploit the sensory biases of females, who could then develop resistance in an antagonistic co-evolutionary arm-race (chase-away hypothesis, Holland & Rice 1998; Arnqvist 2006) or become indifferent (Rowe *et al.* 2005). Interestingly, even if females lose the preference for an ornament, it could still persist in males only to reach a minimum threshold of stimulation for females (Holland & Rice 1998).

Indirect selection

Indirect benefits associated with the quality of the partner include all the additive genetic effects that can be passed on to the offspring and confer them a higher relative fitness, either by increasing their viability or their mating success. Under the ‘good genes’ hypothesis, preferences are expected to target traits that are indicators of the heritable viability of the potential mate (Møller & Alatalo 1999). The honesty of the signal could be insured when only individuals of high quality can afford to develop or maintain the trait, i.e. whose expression is constituting a handicap (Zahavi 1975), or if the trait is condition-dependent (e.g. immunocompetence hypothesis, Hamilton & Zuk 1982; Folstad & Karter 1992), or, more generally, if the marginal improvement in fitness (benefits in terms of fecundity minus the costs in terms of viability) per unit of display is greater in high quality individuals (Getty 2006).

Moreover, as soon as mating preferences exist for a trait that is genetically determined, either to obtain ‘good gene’ benefits, or due to sensory biases or even drift, the alleles for the mating preference and those for the display associate more often than expected by chance because choosy females mate more often with males with better displays (Kokko *et al.* 2006). This can result in a significant positive genetic covariance between the preference and the display. If this covariance is high, a rapid co-evolution of exaggerated display and preference can occur; this is known as the Fisherian runaway process (Lande 1981; Andersson 1994). In this situation the preference would be indirectly selected in a positive feedback loop, and when females do not gain any direct or ‘good gene’ benefits from having an even stronger preference, but only benefit from having attractive sons, this is referred to as the ‘sexy-son’ hypothesis (Lande 1981; Andersson 1994; Kokko *et al.* 2002).

The lek paradox

If females show consistent preferences to mate with one or few males, presumably only to acquire heritable genetic benefits for their offspring, directional selection is expected to lead to the depletion of additive genetic variation, yet this variation is a prerequisite for such benefits to occur (Kirkpatrick & Ryan 1991). This conundrum is known as the ‘lek paradox’. Some mechanisms (e.g. sexually selected mutation rates or modifiers, Pomiankowski & Møller 1995; Cotton & Pomiankowski 2007; Petrie & Roberts 2007), or specific situations (e.g. small or structured populations in which non-additive genetic variation can be inherited, Reid 2007; Neff & Pitcher 2008; Fromhage *et al.* 2009), have been suggested to maintain genetic variation despite such type of selection, but no consensus has emerged yet (Rowe & Houle 1996; Lehmann *et al.* 2007; Kokko & Heubel 2008; Kotiaho *et al.* 2008). However, if females varied in their choice, the lek paradox would not apply.

Relative mating preferences

Variation in mate choice has often been seen as the result of individual variation in sensitivity to the costs of mate choice, arising from ontogeny, physical condition or environmental situation (Jennions & Petrie 1997; Hunt *et al.* 2005; Cotton *et al.* 2006). When the costs of mate choice cannot be afforded by certain individuals, their choosiness, that is to say their willingness to invest time and energy in assessing mates, is expected to be reduced, leading to a different outcome of mate choice for different individuals (Burley & Foster 2006). Alternatively, individuals of poor competitive abilities could evolve preferences for low-quality individuals, for example to avoid the costs of competition (Fawcett & Johnstone 2003; Härdling & Kokko 2005). In addition, if attractiveness is multidimensional because there are different benefits to be optimised (e.g. direct *versus* indirect benefits, Candolin 2003), and if there is condition or context-dependent variation in needs, this would also affect how individuals rank opposite-sex stimuli (Cotton *et al.* 2006). In all cases, if mate choice is condition dependent, the assumption is again that members of the choosing sex agree on who would be the ideal partner if they were not constrained in their choice. Simply, when the context (genetically or environmentally determined) does not allow them to afford the costs of searching for, mating with or maintaining a pair-bond with the ideal partner, they are expected to follow a best-of-a-bad-job strategy.

Variation in mate choice between individuals could also be the result of adaptive variation in mating preferences, but this possibility has usually only been briefly mentioned in the major text books of the field (Krebs & Davies 1993; Andersson 1994; Ryan 1997; Danchin & Cézilly 2007; Brooks & Griffith 2010; Davies *et al.* 2012). Yet, studies looking specifically at the repeatability of choice actually show consistent differences between individuals (guppy (*Poecilia reticulata*), Godin & Dugatkin 1995; Brooks & Endler 2001; zebra finch (*Taenopygia guttata*), Forstmeier & Birkhead 2004; sand goby (*Pomatoschistus minutus*), Lehtonen & Lindstrom 2008). As most of these studies were carried out on individuals born in the laboratory in standardized conditions, it has been argued that these results suggest differences in mating preferences rather than a consistent non-heritable difference in individual condition (Widemo & Saether 1999). This interpretation gets further support from the fact that Forstmeier & Birkhead (2004) found no repeatability in female preferences with regard to male attractiveness. In other words, there was no evidence that some females (putatively in high condition) consistently preferred the males that were overall the most attractive, while others (putatively in poor condition) consistently preferred the less attractive males. This suggests that intrinsic mating biases could be different for each individual. Perhaps, individually-specific mating preferences have been somewhat overlooked because, unless they lead to disruptive selection and speciation in case of strong assortative mating (Butlin & Tregenza 1997), they would only reduce the strength of (directional) sexual selection. Moreover, this raises the important question of the origin and maintenance of such variation in mating biases. However, mate choice, whether leading to

directional sexual selection or not, should *a priori* evolve if discriminate mating increases mating rate with individuals that confer greater benefits per mating (Jennions & Kokko 2010). As presented before, the benefits of mate choice can be direct, when offspring quality or quantity is increased due to the partner's behaviour, or indirect, when offspring quality is improved by the genetic contribution of the partner. In the following, I will consider the benefits that arise from the compatibility of the two partners as opposed to the benefits linked to the absolute quality of the partner (Figure 1).

Indirect compatibility benefits

Several cases where the interaction between the parental genomes affects offspring fitness have been documented. For instance, non-additive genetic effects can occur from recessive deleterious alleles, selfish genetic elements, chromosome inversion polymorphisms, heterozygote advantages, and from complexes of co-adapted genes (Zeh 1997; Tregenza & Wedell 2000; Neff & Pitcher 2005; Kempenaers 2007). The most often documented case of genetic incompatibility is the case of homozygote disadvantage which is more likely to occur in offspring of related individuals (inbreeding depression). This implies either that heterozygosity is advantageous *per se* (i.e. due to overdominance and heterosis) or that rare recessive deleterious alleles get expressed (Charlesworth & Charlesworth 1987). The potential mechanisms of inbreeding avoidance have been the subject of numerous studies (Pusey & Wolf 1996; Komdeur & Hatchwell 1999; Ward & Hart 2003; Weddle *et al.* 2013), although important questions remain, especially in birds, as to whether individuals are able to recognize genetic similarity *per se*, for instance by self-referent phenotype matching, or only recognize kin by direct familiarization (Nakagawa & Waas 2004). **Chapter 2** of this thesis addresses the question of inbreeding avoidance and recognition mechanisms.

In order to evolve, mate choice for genetic compatibility as such (including incompatibilities between unrelated individuals), requires the (in)compatibility causing loci to be tightly linked (e.g. *via* pleiotropy) to a detectable phenotype, and it further requires a mechanism ensuring the appropriate assortative or disassortative preference, which can happen for instance when recombination between the choice- and compatibility-causing alleles is suppressed (Tregenza & Wedell 2000). Potentially because of the complexity of such mechanism to occur, but, likely, also because the mechanisms that underlie genetic incompatibilities are difficult to study, only a handful of such cases have accumulated substantial evidence (reviewed in Butlin & Tregenza 1997; Tregenza & Wedell 2000; Neff & Pitcher 2005; Kempenaers 2007; Mays *et al.* 2008).

For instance, in mice (*Mus domesticus*), a large chromosome segment known as the *t*-complex, contains both the major histocompatibility complex (MHC) - which plays a central role in immunological self/non-self-recognition and is thought to be associated with specific odours - and a lethal recessive mutation. The lethal *t*-allele persists because it causes segregation distortion in males, with heterozygous males passing on the *t*-allele to

more than 90% of their offspring. Heterozygote females show greater avoidance of heterozygous males than homozygous wild type females, indicating that genetic compatibility could influence mate choice, and might do so potentially *via* detection of odours (reviewed in Lindholm *et al.* 2013). Similarly, in mice and humans, MHC-disassortative mating preferences are suspected to have evolved to confer resistance against infectious diseases in the progeny by increasing their MHC-heterozygosity (Wedekind *et al.* 1995; Penn & Potts 1998; Penn & Potts 1999; Tregenza & Wedell 2000; Penn 2002). The benefits associated to MHC heterozygosity could be due to overdominance effects, that is when heterozygotes are superior because they produce a greater variety of gene products, or due to rare allele advantages, such that MHC alleles are in a cyclical frequency-dependent coevolutionary arms race with pathogens (Penn & Potts 1999; Tregenza & Wedell 2000). Nevertheless, a recent meta-analysis only provides mixed support for the role of MHC dissimilarity in mate choice in vertebrates animals: no effect was found overall, but the hypothesis was supported when MHC dissimilarity was characterised at multiple loci (Kamiya *et al.* 2014).

Finally, occasional observations that multiple or extra-pair mating improves embryo or offspring viability in polyandrous females are consistent with the genetic compatibility hypothesis. In such type of mating, males typically only transfer sperm and therefore, female can only obtain potential indirect genetic benefits that increase the fitness of the resulting offspring. In a study on the bluethroat (*Luscinia svecica*), extra-pair young showed a greater immune response than the maternal half-sibs, which suggests that female extra-pair behaviour could have evolved to increase the overall genetic quality of offspring; but extra-pair young also showed a greater immune response than the paternal half-sibs, and this suggests that both the extra-pair males and the females gained genetic compatibility benefits (Johnsen *et al.* 2000). Extra-pair matings in birds have also been suggested to be a potential way of compensating for genetic similarity (i.e. overall similarity due to relatedness or MHC similarity specifically) between social partners (Blomqvist *et al.* 2002; Foerster *et al.* 2003; Freeman-Gallant *et al.* 2003; Freeman-Gallant *et al.* 2006). Nevertheless, overall, the evidence regarding female choice for genetic compatibility in avian species is mixed (Mays *et al.* 2008; Slatyer *et al.* 2012; Forstmeier *et al.* 2014), and experimental work demonstrating which factor reliably predicts extra-pair paternity is visibly lacking (Petrie & Kempenaers 1998; Griffith *et al.* 2002; Westneat & Stewart 2003; Arnqvist & Kirkpatrick 2005; Kempenaers & Schlicht 2010). **Chapter 1** present an experimental test of the hypothesis under which females seek extra-pair copulations to compensate for their genetic incompatibility with their social partner.

In those previous examples, the recognition mechanisms allowing compatible mating are still largely unknown and hence often remain hypothetical. In addition to precopulatory mate choice, postcopulatory prezygotic mechanisms, known as female cryptic choice, have been suggested as a potential way to bias egg fertilization by sperm of different individuals (Tregenza & Wedell 2000; Neff & Pitcher 2005; Kempenaers 2007; Mays *et al.* 2008). While

some behavioural postcopulatory mechanisms have been shown (e.g. ejection of the sperm of subdominant males in the feral fowl (*Gallus gallus domesticus*) (Pizzari & Birkhead 2000) and potentially of related males (Pizzari *et al.* 2004)), physiological mechanisms involving the interaction between sperm and female reproductive tract are still subject of debate (Kempnaers 2007; Bretman *et al.* 2009; Løvlie *et al.* 2013). For instance, since sperm express MHC proteins on their membranes, it has been suggested that the mechanism that ensures mate choice for dissimilar MHC could potentially occur at the gametic level. Nevertheless, in one study on mice, preferences were reversed in cross-fostered individuals, and therefore mating biases appear to be learned rather than physiological (Penn & Potts 1998). Interestingly, existing studies that explicitly control for prefertilization behaviours through artificial insemination found no paternity bias toward non-related males or MHC dissimilar sires (mallard (*Anas platyrhynchos*), Denk *et al.* 2005; guppy, Evans *et al.* 2008; red junglefowl (*Gallus gallus*), Løvlie *et al.* 2013), which suggest that cell-cell interaction might not be the most common mechanism.

Overall, there is a noticeable lack of studies designed to measure compatible genes benefits while fully controlling for ‘good genes’ effects and direct effects such as maternal investment (Tregenza & Wedell 2000; Neff & Pitcher 2005; Kempnaers 2007; Mays *et al.* 2008). Variation in fitness can readily be partitioned into additive (male and female intrinsic quality) and non-additive (male x female interaction) genetic effects in breeding designs where all crosses between a set of males and a set of females are made (North Carolina Design II), but this has only typically been used in external fertilizers where perfectly balanced crossbreeding is more easily performed (e.g. Wedekind *et al.* 2001; Rodríguez-Muñoz & Tregenza 2009; Johnson & Brockmann 2013). By extension, the percentage of variance in individual breeding success explained by female, male and pair identities can be obtained from repeated measurement of individual fitness reached with the same and with other partners, even in unbalanced designs, when analyzed by mixed effect models with those levels of replication entered as random effects (Lynch & Walsh 1998); but this, again, has been underexploited so far (but see animal models in e.g. Bolund *et al.* 2011; Reid & Sardell 2012). **Chapter 1** and **3** of this thesis present such analyses to measure the effects of pair combination on the fate of fertilized eggs and offspring.

Direct compatibility benefits

Synergistic advantages could emerge from the combination of both parents’ behaviours. Mate choice for behavioural compatibility might be especially important in species with intense bi-parental brood care and with long-lasting monogamous pair bonds, like humans or most bird species. Indeed, historically, the first studies that suggested the existence of behavioural compatibility benefits are those that reported a positive correlation between breeding success and pair bond duration in long-lived socially monogamous birds (Black 1996; Ens *et al.* 1996; Black 2001). The ‘mate familiarity’ hypothesis proposes that repeated breeding with the same mate allows fine tuning of behaviour between the male and female,

enhancing coordination in shared breeding activities such as territory acquisition, anti-predator defence, breeding site preparation, and parental care, which could in turn translates into improved breeding success (Black 1996). This therefore refers to a long term adjustment of within-pair behaviour. Nevertheless, as those studies have been purely observational, this effect could not be disentangled from age, experience or quality. More recent studies intended specifically to tackle this issue. In one study on a long-lived seabirds, the kittiwake (*Rissa tridactyla*), only specific samples were analysed where confounding factors were not applicable, and in this study, Naves et al. (2007) found no effect of familiarity *per se* on breeding success. Yet, in the blue-footed booby (*Sula nebouxii*), a separation of the different factors was possible and, there, a higher reproductive success with pair bond duration was found, independent of age or experience (Sánchez-Macouzet *et al.* 2014). Finally, two experiments where pair-bond duration was manipulated reported higher reproductive performance in more familiar pairs (van de Pol *et al.* 2006; Griggio & Hoi 2011). Therefore, altogether, it seems possible that mates could increase their behavioural compatibility over time, but it is not clear whether pairs could vary for instance in the speed of their improvement by picking a particularly compatible partner already during mate choice.

More recently, with the increase of interest in consistent behavioural differences between individuals (Sih *et al.* 2004; Réale *et al.* 2007; Réale *et al.* 2010) - for which adaptive explanations have been proposed but are still subject of empirical and theoretical work (Dall *et al.* 2004; Dingemanse & Wolf 2010; Wolf & Weissing 2012; Dingemanse & Wolf 2013) - it has been suggested that individuals could readily choose each other based on their respective personality, which would determine their behavioural compatibility (Schuett *et al.* 2010). Possibly, individuals that show similar behavioural types, or otherwise similar plasticity (and therefore predictability), could be better at negotiating or coordinating their actions, and could therefore have reduced conflicts over parental care and an increase in reproductive success (Royle *et al.* 2010; Schuett *et al.* 2010). Indeed, it has sometimes been observed in great tits (*Parus major*) that pairs assortatively paired for exploration score had greater reproductive success (Dingemanse *et al.* 2004; Both *et al.* 2005; but see Mutzel *et al.* 2013). However, in a year with low availability of food, individuals of intermediate behavioural phenotype produced more recruits, and in this population, the pattern of mating was disassortative (Dingemanse *et al.* 2004). Based on those findings, it has then been suggested that disassortative mating for behavioural type could instead be favoured in order to produce intermediate phenotypes that could have, overall, a lower variance in lifetime reproductive success (Dingemanse *et al.* 2004; Both *et al.* 2005). This interpretation has also been used for the observation that female great tits from assortative pairs were more likely to have extra-pair young (van Oers *et al.* 2008). Therefore, according to that hypothesis, disassortative mating for behavioural types is associated to genetic rather than behavioural compatibility benefits. Moreover, disassortative pairs for behavioural type could be better at sharing tasks and complement each other by having a larger behavioural

repertoire, for instance, to be able to ensure good foraging success in unpredictable environments, but this has not been proposed so far. Usually, assortative mating for personality is presented as the default expectation regarding behavioural compatibility (Dingemanse *et al.* 2004; Both *et al.* 2005; Schuett *et al.* 2010; Schuett *et al.* 2011a; Gabriel & Black 2012), maybe because it complements the hypothesis of mate familiarity provided that same behavioural tendencies lead to more time spent together and therefore more experience with each other (Gabriel & Black 2012).

To a large extent, the hypotheses behind mate choice for compatible personality traits are still in the process of maturation and are not clearly defined, potentially also because the expectations could depend on environmental fluctuations (Dingemanse *et al.* 2004). Moreover, if assortative pairing for behavioural type is found in the wild like in the Steller's jays (*Cyanocitta stelleri*) (Gabriel & Black 2012), this does not necessarily mean that individuals expressed assortative preferences. This pattern could result from a passive process of mate choice, if individuals of same personality are more likely to encounter each other; or from temporal autocorrelation between the measurements of the personality of an individual and its mate; or from the convergence of behavioural tendencies of partners over time; or from shared environmental effects. In captivity, unpaired individuals can be evaluated for their behavioural profiles and then assessed for their mating preferences and their reproductive success. Interestingly, several studies performed in captivity found that only individuals of one extreme behavioural type (e.g. 'fast' explorer') expressed assortative preferences while individuals of the other extreme behavioural type did not (convict cichlid (*Cichlasoma nigrofasciata*), Budaev *et al.* 1999; great tit, Groothuis & Carere 2005; zebra finch, Schuett *et al.* 2011b). Although this pattern has typically been presented as evidence for mate choice for compatible (i.e. similar) behavioural types (Groothuis & Carere 2005; Schuett *et al.* 2010), if one personality trait is an indicator of quality (as suggested for boldness in the guppy, Godin & Dugatkin 1996), this outcome could also be explained by competition for the best quality individuals, and by the evolution of 'prudent' choice as mentioned earlier (Fawcett & Johnstone 2003; Härdling & Kokko 2005). Finally, two studies measured several personality traits of unpaired zebra finches before measuring their reproductive success with respect to the interaction between the personalities of the pair members (Schielzeth *et al.* 2011; Schuett *et al.* 2011a). In the study of Schuett *et al.* (2011a), where pairs were assigned randomly to each other, pair assortment for some of the personality traits measured (exploration tendencies) affected some measure of reproductive success (although with only 5 tests significant out of about 100), but this effect was not found in a subsequent replicate. In the study of Schielzeth *et al.* (2011), pairs freely chose each other, and the observed pairing was random with regard to the two personality traits measured (novelty-seeking and activity). Most importantly, there was no evidence for pair combination effects on rearing success with respect to those personality traits (Schielzeth *et al.* 2011).

To conclude about the studies relating behavioural compatibility with animal personality, there seems to be so far no clear hypothesis or consistent evidence on which pair combination of personality traits should affect reproductive success and how. The experimental approach where personality is measured before pair assignment and assessment of their reproductive success, especially associated to cross-fostering to disentangle genetic from rearing environment effects, is relevant to measure some of the potential behavioural compatibility benefits. Nevertheless, at the current stage, we do not know *a priori* what traits might be important, and this leaves the experimenter with the need of testing all independent personality axes, or ‘behavioural syndromes’ (which are, more precisely, suites of correlated personality traits (Sih *et al.* 2004)).

Alternatively to this approach, some studies have carried out behavioural observations to try to directly quantify pair compatibility as a composite of proximity, behavioural synchrony, allopreening, copulation frequency and aggression between partners (Spoon *et al.* 2004, 2006, 2007). In those studies on cockatiels (*Nymphicus hollandicus*), highly behaviourally compatible partners exhibited more coordinated incubation, had higher reproductive success (Spoon *et al.* 2006), and were less likely to engage in extra-pair copulation (Spoon *et al.* 2007) than individuals in disharmonious pairs. Overall, such type of observational data characterising concrete pair behaviours that could induce fitness differences is almost completely lacking. In the experiment presented in **Chapter 3**, such observations have been carried out with the purpose of directly evaluating pair compatibility. While pair compatibility, as measured above, could well be the result of combination of personality types, and pair incompatibility, the result of a lack of plasticity and possibility of adjustment, pair harmony could alternatively be the result of individual differences in ‘motivation’ to breed with a particular partner. A higher individual commitment in a partnership and investment in reproduction could indeed result from a greater stimulation of the sensory system by the partner (for instance mediated by pair display, Bolund *et al.* 2012; Servedio *et al.* 2013). This phenomenon could be fully idiosyncratic, and potentially reciprocal, if there is 1) individual variation in sensory systems that is not strongly enough selected against (i.e. if optimal stimulation is reached, despite this variation, for the most common situation for which the sensory system had been designed, Arak & Enquist 1993), and 2) if the cost of choosiness for a fitting stimulus is low (e.g. if there is a large availability of partners and fast assessment). If certain combinations of pairs are more effective at stimulating one another’s reproductive investment, for instance in term of sperm allocation, maternal investment into the egg, and parental care, this would also lead to direct benefits in term of reproductive success. To the best of my knowledge, this hypothesis has not been suggested so far; but the evolutionary consequences of non-adaptive variation could well be next in the historical chronology of factors to be considered, that is, shared optimal behaviours (e.g. Charnov 1976), adaptive individual variation (e.g. Dingemanse & Wolf 2010), and non-adaptive behaviours resulting from evolutionary constraints (e.g. Forstmeier *et al.* 2014).

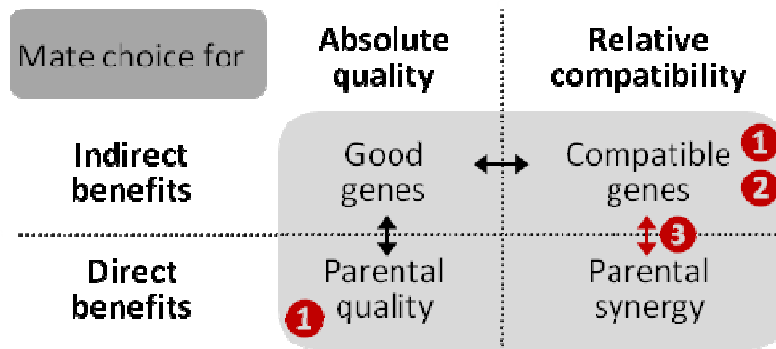


Figure 1. To date, the central debate on the benefits of mate choice has been about (i) the relative importance of direct vs indirect fitness benefits arising from the overall quality of the chosen partner (i.e. good parent vs good genes, vertical black arrow ; Møller & Jennions 2001; Maklakov & Arnqvist 2009), and more recently about (ii) the relative importance of the two types of indirect benefits, namely good genes vs compatible genes (horizontal black arrow ; Mays & Hill 2004; Neff & Pitcher 2005; Kotiaho & Puurtinen 2007; Cutrera et al. 2012). Contrastingly, the direct benefits arising from the compatibility between the two partners (bottom right), although suggested in species where breeding success increases with pair bond duration (Ens et al. 1996; Black 2001; van de Pol et al. 2006; Griggio & Hoi 2011; Sánchez-Macouzet et al. 2014; but see Naves et al. 2007), have only rarely been considered (Delesalle 1986; Spoon et al. 2006, 2007; Schuett et al. 2010; Fox & Millam 2014). The first chapter ① aims at testing whether female extra-pair behaviour evolved to compensate against a low hatching success reached with their social partner, that is to say whether female extra-pair mate choice target fertility benefits and/or compatible genes benefits. The second chapter ② tests whether siblings who grew up together avoid choosing each other as social partner; in other words, if they avoid inbreeding depression by choosing a genetically more compatible partner. The third chapter ③ aims at measuring the fitness consequence of mate choice for compatibility while experimentally controlling for effects of overall quality. Moreover, the study disentangles direct from indirect benefits of mate choice for compatibility (red vertical arrow).

The zebra finch mating preferences

The zebra finch

The zebra finch has been a model organism in studies on sexual selection for more than 30 years, and it is the species that I used throughout my PhD research. The use of model organisms allows assessing the reliability and robustness of findings through replication, and therefore gives the opportunity of getting a realistic understanding of biological phenomena, at least for the species concerned.

Zebra finches are small passerine birds from the dry biomes of central Australia. They are highly gregarious and mobile, forming large flocks all year round and constituting

an apparently panmictic population (Zann 1996; Forstmeier *et al.* 2007b). They are granivorous and they readily start breeding as soon as conditions are suitable, apparently when unpredictable rainfalls start up seed production (Zann 1996). In addition to being opportunistic and colonial breeders, zebra finches form life-long socially monogamous pair bonds. The species is sexually dimorphic regarding plumage and vocalizations. Males have colourful ornaments such as orange cheeks, brown flanks with white spots, a black breast band underneath a black-and-white striped breast, and a shiny red beak, while females only exhibit a plumage with different shades of grey, and an orange beak (Figure 2). Moreover, males, but not females, produce a courtship song that is highly consistent within an individual and highly variable between individuals (Vignal *et al.* 2008).



Figure 2. Photograph of a male (left) courting a female (right) zebra finch.

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Zebra finches are easily held in captivity and have been domesticated in Europe for more than 100 years. It is possible that during the process of domestication, female choosiness has been selected against due to breeding designs that did not allow free choice. For my research, I had the opportunity of working with a population derived from wild-caught zebra finches whose captive-bred offspring were imported to Germany in 1992, and had bred in outdoor aviaries for maximally 10 generations. This population (population #4 in Forstmeier *et al.* 2007b) is genetically still closely related to the wild Australian population, but has lost some of its genetic variability (heterozygosity at 10 microsatellite markers is 83% compared to 93% in the wild, Forstmeier *et al.* 2007a). This low extent of domestication, combined with the fact that birds are held in large semi-outdoor aviaries allowing conspecific interactions, appears to be the best opportunity available in Europe to work in controlled conditions with the least compromise regarding the expression of natural behaviours. Although the life-history trade-offs that underlie the development of phenotypic traits may be fundamentally the same in wild, captive wild-caught, and domesticated populations, these populations seem to differ at least in some behavioural traits (Tschirren *et al.* 2009; Mainwaring *et al.* 2010; Gilby *et al.* 2011). For instance, the

different levels of extra-pair paternity in different populations suggest an effect of both domestication and captivity on extra-pair behaviour: 2% of extra-pair young (in 6% of the broods) were found in two wild populations (Birkhead *et al.* 1990; Griffith *et al.* 2010), 28–29% of the offspring in two domesticated populations (Burley *et al.* 1996; Forstmeier *et al.* 2011), 12% of the offspring in a captive population of wild-caught birds (Tschirren & Postma 2010), and 9% of the offspring (in 18% of the broods) in our recently wild-derived population (**Chapter 3**). The potential impacts of the captive environment have therefore to be considered for the interpretation of my experiments.

Interestingly, this species shows a mating system similar to that of humans: both species form relatively stable pairs bonds, often with life-long social monogamy, but both species also exhibit some unforced divorces as well as low, but significant levels of extra-pair paternity. Although one cannot easily extrapolate findings from zebra finches to humans, those similarities may elevate the interest one can have in understanding social and extra-pair mate choice in the zebra finch.

Social and extra-pair mate choice

In socially monogamous species, individuals are believed to be not always able to express their preferences during social mate choice, especially if all females agree on which male is most attractive, and that is why extra-pair behaviour has mainly been seen as a way to compensate for their current, potentially constrained, situation (Petrie & Kempenaers 1998; Griffith *et al.* 2002; Westneat & Stewart 2003; Arnqvist & Kirkpatrick 2005; Kempenaers & Schlicht 2010; but see Forstmeier *et al.* 2014). Indeed, if extra-pair behaviour was a fixed behaviour shared by all females, all of them would pay the possible costs of extra-pair mating, while only a few would potentially gain benefits, and this should favour the evolution of a context-dependency of this behaviour. Moreover, as the social partner provides parental care, and extra-pair males only transfer sperm (besides potential costly diseases), females could seek different types of benefits in the two contexts (Candolin 2003).

In the zebra finch, where most of the siring success is reached with the social partner, we expect selection to act more strongly on social mate choice than on extra-pair mate choice. The experiments presented in **Chapter 2** and **Chapter 3** focus on free social mate choice but allow females to compensate, *via* extra-pair mating, for their potential incompatibility with their social partner. In the set-ups used, where several females and several males are simultaneously choosing a partner, mutual mate choice and male-male competition could potentially influence the outcome of mate choice. A pilot study was performed in 2011 to confirm that mutual allopreening strongly predicts which pairs will end up breeding together (Silcox & Evans 1982), and further, that allopreening is a more accurate assessment of mating preferences than mating patterns *per se*: two flocks of 20 unpaired males and 20 unpaired females were first observed to identify allopreening pairs, and were then given access to nest material and nest boxes. In 90% of the cases (29 pairs

out of 32 pairs identified before breeding), allopreening predicted pairs that bred together. When nest boxes were added, three new pair bonds formed, which apparently resulted from intra-sex competition: when two females (or two males) preferred the same male (respectively, the same female), one of the two ended up breeding with a previously non-chosen individual. We therefore concluded that the assessment of mating preferences in this somewhat natural setting could be best evaluated by the occurrence of allopreening in future studies (e.g. **Chapter 3**).

Besides, in the zebra finch, as in most species, the within population variation in female extra-pair behaviour is still to be resolved (Petrie & Kempenaers 1998; Griffith *et al.* 2002; Westneat & Stewart 2003; Arnqvist & Kirkpatrick 2005; Kempenaers & Schlicht 2010; Forstmeier *et al.* 2014). Therefore, an experimental test of an adaptive explanation for female extra-pair behaviour will be presented in **Chapter 1**. Interestingly, two recent studies suggest that at least some of the variation in female promiscuity could be an intrinsic rather than a context-dependent trait (Forstmeier 2007), which could result from a genetic constraint, because promiscuity is selected for in males and because male and female promiscuity are genetically correlated (Forstmeier *et al.* 2011). Dr. Wolfgang Forstmeier currently breeds selection lines for male courtship rate, trying to confirm that female promiscuity can be selected indirectly along with male sexual activity.

Mating preferences for absolute quality

Mating preferences of female zebra finches have mainly been studied in relation to male beak colour and song production (see below). The other sexually dichromatic plumage traits have also been studied in the context of mate choice (e.g. size of cheek patch, flank patch, bread band, Price & Burley 1994) and sometimes were found to be attractive to females (e.g. symmetry of breast ornamentation, Swaddle & Cuthill 1994; cheek patch color, Roberts *et al.* 2007), but these isolated positive findings were not supported further. Therefore, most of the sexual dichromatism observed in this species might partly be the result of past rather than current sexual selection (see Holland & Rice 1998).

The redness of beaks has been shown to increase with the concentration of circulating carotenoids (Blount *et al.* 2003b). Carotenoids have to be acquired through the diet, they have antioxidant properties and are involved in immune functions (Blount *et al.* 2003b). Males zebra finches with redder beak have been found to have better reproductive success (Price & Burley 1994), and while several studies attempted to explain this observation by the existence of female preference for redder beak, the original evidence for this has been mixed (e.g. preference for redder beak: Burley & Coopersmith 1987; Houtman 1992; Blount *et al.* 2003b; no overall preferences: Burley *et al.* 1994; Collins *et al.* 1994; Roberts *et al.* 2007; Forstmeier & Birkhead 2004; preference for orange beak: Sullivan 1994). In 1996, Collins & ten Cate reviewed the ambiguous support for beak-colour preferences, and suggested that beak colour could instead be used in the context of male-male competition. Yet, this hypothesis was not verified when tested, as beak colour did not

correlate significantly with competitiveness in a following study (Bolund *et al.* 2007). Finally, a recent meta-analysis reported that overall, female zebra finches do seem to prefer males with redder beaks, but not when beak colour is experimentally manipulated (Simons & Verhulst 2011), which questions the causality of the overall effect.

Male song rate has been found to be correlated with male attractiveness in several studies (e.g. Houtman 1992; Collins *et al.* 1994; Collins 1995; Forstmeier & Birkhead 2004). However, song is to a large degree a feedback to female approach (Collins 1994; Rutstein *et al.* 2007). Therefore, when song rate is measured during mate choice trials, high song rates of the preferred males are partly the outcome rather than the cause of the preference (Collins & ten Cate 1996). When measured independently of the mate choice trial, a high song rate does nevertheless appear to be effective in attracting female attention during mate choice tests (Houtman 1992; Collins 1994; Forstmeier & Birkhead 2004), but this does not translate into a preference in the sense of preferential copulation (Forstmeier 2007).

Song characteristics *per se* have also been subject to a lot of investigations (Riebel 2009). While consensus is reached about the trivial fact that males need to court females, and do so with a natural rather than an artificially altered song, in order to have access to any copulations (Tomaszycki & Adkins-Regan 2005; Forstmeier 2007; Riebel 2009), song characteristics, such as song duration, syntax, presence of special elements, etc., do not seem to influence mate choice (Riebel *et al.* 2009). However, one recent study found a preference for syllable repertoire size and for a lower proportion of between-syllable silences (Holveck & Riebel 2007; but see Forstmeier *et al.* 2009), and another one for higher amplitude (Ritschard *et al.* 2010). These studies are now pending for replication.

Finally, 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; but see Naguib *et al.* 2008). However, other studies including those with the greatest statistical power, have failed to find effects of early-rearing conditions on male attractiveness (Blount *et al.* 2003a; Naguib *et al.* 2008; Bolund *et al.* 2010b).

Collectively, all the above positive findings indicate that females may prefer males in good condition. Nevertheless, there is no consensus on what particular trait makes males attractive to females or what constitutes a quality indicator. Therefore, another approach to study preferences for absolute quality would be to manipulate the overall genetic quality of individuals. Inbred birds, compared to outbred birds, show a much lower fitness (Forstmeier *et al.* 2012) and have altered condition-dependent phenotypic traits (Bolund *et al.* 2010a). Therefore, they appear to be of low genetic quality. Several experiments, originally conceived by Dr. Wolfgang Forstmeier, have been designed to measure to what extent female zebra finches discriminate against low quality (i.e. inbred) males in social pairing and courtships. Three of those experiments have been conducted by master students (Johannes

Schreiber and Thibaud Aronson) under my supervision, but the results will not be presented here, as they are still being compiled with results of previous experiments, and awaiting additional follow-up studies.

Mating preference for relative compatibility

In one study, Forstmeier & Birkhead (2004) conducted a large number of mate choice trials to investigate the variance in female preferences for unmanipulated males. They found low but significant consistency (repeatability of time allocation by a female tested twice with the same set of males $R=0.29$), and very low but significant between-female agreement (different females tested with the same set of males: $R=0.11$). Moreover, the generality vs female-specificity of male attractiveness has been assessed in communal breeding aviaries where the females willingness to engage in copulation when courted has been monitored extensively. Female responsiveness is then analysed with mixed effects models including the identity of the female, the male, and the combination of the interacting pair as random factors. Methods used for assessing and analysing female responsiveness in courtships are presented in more detail in Forstmeier *et al.* (2011) and **Chapter 3**. The important point here is that when females are still unpaired and in the process of freely choosing their social partner, the courting pair identity explain 6 times more variance in females responsiveness than the courting male identity alone, that is to say females express idiosyncratic rather than shared preferences regarding male attractiveness (analysis based on 7414 courtships involving 99 unpaired females with 128 males in 432 pair combinations). Similarly, when females are socially paired, the combination of the extra-pair courting male and female identities still explain more than twice as much variance in female responsiveness than the extra-pair male identity alone (analysis based on 7496 extra-pair courtships involving 142 females and 166 males in 613 pair combinations). Interestingly, the random effect estimates of the interacting pair identities are correlated across contexts (i.e. female pairing status), that is when the female is still unpaired and courted by a male, *versus* when she is later paired and involved in an extra-pair courtship with the same male ($R=0.42$, 95% confidence interval: 0.30-0.52, $N=205$ interacting pair combinations observed in both contexts). This suggests that females have individually-specific preferences that are potentially influenced by similar factors in both contexts, when looking for a social male and when interacting with extra-pair males (either seeking the same type of benefits or, for instance, consistently sensory exploited by the same males).

Thesis outline

The overall aim of this thesis was to assess whether female zebra finches base their social mate choice on compatibility (genetic and/or behavioural, **Chapters 2 and 3**), and whether they engage in extra-pair copulations as an adjustment to genetic incompatibility with their social partner (**Chapters 1 and 2**).

Specifically, the purpose of the experiment presented in **Chapter 3**, which covered the entire duration of the thesis, was to quantify the potential fitness benefits of mate choice for any type of compatibility owing to an original design that excludes quality effects as a confounding factor (Fig. 1). As we do not know what trait is assessed during mate choice or if only one dimension is evaluated, this study allowing free mate choice has the advantage of not targeting a specific trait as a potential source of (in)compatibility, but enables the multidimensionality of mate choice to occur in a more 'natural' setting. Moreover, in this study, direct vs indirect compatibility benefits (Fig. 1) could be further disentangle based on the fact that, in this system, genetic incompatibility leads to embryo mortality, while behavioural incompatibility leads to offspring mortality (conclusions drawn from analyses on a previous dataset, presented in **Chapter 3**). This study is therefore of primary importance to the field to set whether or not, in the zebra finch, mate choice for genetic and/or behavioural compatibility occur and lead to fitness differences.

The study presented in **Chapter 1** is, to our knowledge, the first experimental test of the fertility insurance hypothesis under which female would seek extra-pair copulations to compensate for the 'infertility' of their social partner. Although fertility benefits of extra-pair copulations have often been assumed trivial, a recent review specified the limited situations in which female extra-pair behaviour could readily evolve to counteract male infertility (Hasson & Stone 2009). One of these situations concerns species that repeatedly breed together, and where females could adjust their promiscuity to the hatching success reached with their social partner in the previous clutch (Rasmussen 1981). In our experiment, hatching success of pairs was manipulated and females monitored for their subsequent extra-pair behaviour. As, in the zebra finch, the relatively high rate of hatching failure mainly results from embryo mortality (analysis presented in **Chapter 1**), which presumably results from genetic incompatibility (analysis presented in **Chapter 3**), manipulating the hatching success of pairs is equivalent to manipulating the apparent genetic incompatibility between partners. Therefore, in this study, we fundamentally test whether female zebra finches seek compatible gene benefits by engaging in extra-pair copulations.

Finally, the experiment presented in **Chapter 2** aimed at assessing whether social and extra-pair mate choice in zebra finches is affected by genetic incompatibility associated with inbreeding depression. Studies on this question in this species have been numerous but the evidence has been particularly mixed. Therefore, we first present a meta-analysis of previous studies before performing the still lacking experiment.

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), this experimental standardisation helped avoiding possible biases that could arise from condition-dependent mate choice.

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Chapter 1 Does hatching failure breed infidelity?

Abstract

*In socially monogamous species, the reasons for female infidelity are still controversial. It has been suggested that females could seek extra-pair copulations as an insurance against hatching failure caused by male infertility or incompatibility. In species where couples breed repeatedly, females could use previous hatching success as a cue to assess their partner's infertility (or incompatibility). Hence, it has been predicted that females should increase their infidelity after experiencing hatching failures, but to our knowledge this hypothesis has never been tested experimentally. We manipulated hatching success of pairs in a captive population of zebra finches (*Taeniopygia guttata*), a species that forms lifelong pair-bonds, and measured female willingness to engage in extra-pair copulation. By experimentally cross-fostering fertile and infertile eggs, couples either experienced 100% or about 35% hatching success in each of three consecutive clutches. Contrary to our prediction, females that experienced repeated hatching failure did not increase their responsiveness towards extra-pair males relative to those females with 100% hatching success. Moreover, there was no difference in female calling rate for the partner after male removal and no occurrence of divorce when the opportunity was given. These findings seem to contradict the common view that reproductive failure weakens the pair bond. Furthermore, a critical review of the literature suggests that there is no convincing evidence supporting this hypothesis in other species either. We therefore highlight that this fundamental area of behavioural ecology research is still much in need of specific experimental work that controls for confounding factors.*

Original Article

Does hatching failure breed infidelity?

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In socially monogamous species, the reasons for female infidelity are still controversial. It has been suggested that females could seek extra-pair copulations as an insurance against hatching failure caused by male infertility or incompatibility. In species where couples breed repeatedly, females could use previous hatching success as a cue to assess their partner's infertility (or incompatibility). Hence, it has been predicted that females should increase their infidelity after experiencing hatching failures, but to our knowledge this hypothesis has never been tested experimentally. We manipulated hatching success of pairs in a captive population of zebra finches (*Taeniopygia guttata*), a species that forms lifelong pair bonds, and measured female willingness to engage in extra-pair copulation. By experimentally cross-fostering fertile and infertile eggs, couples either experienced 100% or about 35% hatching success in each of three consecutive clutches. Contrary to our prediction, females that experienced repeated hatching failure did not increase their responsiveness toward extra-pair males relative to those females with 100% hatching success. Moreover, there was no difference in female calling rate for the partner after male removal and no occurrence of divorce when the opportunity was given. These findings seem to contradict the common view that reproductive failure weakens the pair bond. Furthermore, a critical review of the literature suggests that there is no convincing evidence supporting this hypothesis in other species either. We therefore highlight that this fundamental area of behavioral ecology research is still much in need of specific experimental work that controls for confounding factors. **Key words:** divorce, extra-pair mating, fertility insurance, hatching failure, pair bond, zebra finch [*Behav Ecol*]

INTRODUCTION

Many studies have been aimed at understanding the adaptive function of female mating with multiple males (Tregenza and Wedell 1998; Jennions and Petrie 2000; Stockley 2003; Avise and Liu 2011; Slatyer et al. 2012), especially in socially monogamous species (Petrie and Kempenaers 1998; Griffith et al. 2002; Westneat and Stewart 2003; Arnqvist and Kirkpatrick 2005; Cohas et al. 2006; Liebgold et al. 2006; Bryja et al. 2008; Sefc et al. 2008; Uller and Olsson 2008). A popular hypothesis is that extra-pair copulations act as a mechanism that gives direct benefits to females in terms of fertility (reviewed in Hasson and Stone 2009). In other words, females would engage in extra-pair copulations as insurance against the infertility of their social partner or against genetic incompatibility with their social partner (Hasson and Stone 2009). Indeed, substantial rates of hatching failure are remarkably common across species. On average, about 15% of the eggs do not hatch (Koenig 1982; Eberhard 1996; Morrow et al. 2002), often because eggs do not get fertilized or because of embryo mortality (e.g., Birkhead et al. 2008; Forstmeier and Ellegren 2010).

There are two ways in which the benefits of fertility insurance could favor promiscuous behavior.

1. If there is between-female genetic variation in promiscuity (Forstmeier et al. 2011; Reid et al. 2011), the benefits of fertility insurance might favor promiscuous individuals. However, as explained in the recent review by Hasson and Stone (2009), intrinsically promiscuous females can increase fertility by mating multiply and randomly only if two conditions are met simultaneously: There must not be fertile sperm precedence within ejaculates and there has to be fertile sperm precedence among ejaculates. These conditions

are met when some males in the population do not produce any sperm (or very few) or only immobile sperm (Hasson and Stone 2009). In contrast, many other common types of male infertility would not favor such promiscuous behavior (Hasson and Stone 2009). Empirical support for this scenario where promiscuous females obtain fertility benefits has been found, for instance, in bluethroats, *Luscinia svecica*. In this species, azoospermia occurs (i.e., some males do not produce any sperm) (Lifjeld et al. 2007), and almost all females engage in extra-pair copulation (Fossøy et al. 2006). The occurrence of some broods where all eggs are fertilized by an extra-pair male (Lifjeld et al. 1993; Kroken et al. 1998) at least suggests that females may obtain benefits in terms of fertility.

2. If there is within-individual flexibility in promiscuous behavior, individuals mated to an infertile or incompatible partner might benefit from seeking extra-pair copulations (adaptive phenotypic flexibility).

Observational studies have found all sorts of association (positive, null, and negative) between hatching success and levels of extra-pair paternity (e.g., Wetton and Parkin 1991; Kempenaers et al. 1999). Positive associations have been interpreted as the females succeeding in reducing hatching failure (Kempenaers et al. 1999), whereas negative associations have been interpreted as the females unsuccessfully trying to reduce hatching failure (Wetton and Parkin 1991; Cordero et al. 1999). Comparative studies that related a species' average hatching success to its level of extra-pair paternity have found no association (Morrow et al. 2002), but this does not rule out the existence of fertility insurance benefits at the individual level. The question whether females adjust their promiscuity to the perceived risk of encountering hatching failure (scenario 2 above) requires an experimental approach, but to our knowledge such experiments have not been conducted.

For females to adjust their promiscuity to the risk of hatching failure requires prior knowledge of male fertility or compatibility. The phenotype-linked fertility hypothesis suggests

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that females might be able to assess male fertility before copulation (Sheldon 1994), but it is unclear whether a general phenotypic correlate of fertility (e.g., ornamentation) exists (Birkhead and Fletcher 1995; Birkhead et al. 1997; Pizzari et al. 2004; but see Peters et al. 2004). To the contrary, ornamentation and fertility may be negatively correlated due to pleiotropic antagonism (see e.g., Bilde et al. 2009; Simmons et al. 2010; Preston et al. 2011). Hence, it seems unlikely that females can predict fertility from male phenotypes. However, in species where individuals breed repeatedly with the same partner, previous hatching success could be the most reliable cue to male fertility (or genetic compatibility). Females that experience low hatching success with their social partner, that is, those that have a greater than average probability to be mated to an infertile (or incompatible) male, can gain in fertility by engaging in extra-pair copulation(s) during the next breeding attempts (Hasson and Stone 2009). We then expect that females increase their infidelity after experiencing hatching failure, but remain faithful when all their eggs hatch. This hypothesis was already proposed by Rasmussen (1981) long before the invention of molecular parentage assignment techniques, and highlighted again by Hasson and Stone (2009), who noticed the lack of empirical tests.

We aimed to test this hypothesis by experimentally manipulating the hatching success of breeding pairs of captive zebra finches (*Taeniopygia guttata*) and by measuring the resulting change in female extra-pair behavior. Rasmussen (1981) convincingly argued that it would be adaptive if reproductive failure would weaken the pair bond and would thereby induce females to either seek extra-pair copulations or to divorce their partner. Therefore, we also recorded the females' attachment to their social partner (contact calls uttered when the male is removed) and whether they divorced or not when given an opportunity.

Zebra finches form social pair bonds that typically last for a lifetime (Zann 1996), so females can assess the hatching success they reach with their social partner over consecutive clutches. Hatching failure is not uncommon in wild zebra finches (around 16% of eggs, Zann 1996; Griffith et al. 2008) and higher rates of hatching failure have been reported from some captive populations (e.g., 35%, Forstmeier and Ellegren 2010). In our domesticated population, when eggs are cross-fostered individually among clutches within 24 h of laying (see Forstmeier 2005) the identity of the genetic pair explains 4.8 times as much variance in hatching success than foster-pair identity (based on 1914 eggs, 283 genetic pairs, 264 foster pairs; ΔAIC for foster pairs = 8.0, ΔAIC for genetic pair = 112.6; our unpublished data). Therefore, incubation problems do not seem to be the main cause of hatching failure. Moreover, when females are allowed to breed with different partners in succession, rates of hatching failure vary dramatically within females, across pairs. Specifically, in a mixed-effect model with three random factors, pair identity accounts for 51%, male identity for 30%, and female identity for 19% of the total variance in hatching success that can be explained (based on 4386 eggs, 367 pairs; our unpublished data). Therefore, in this system, females could clearly benefit from avoiding an incompatible or infertile partner.

Although zebra finches have relatively low rates of extra-pair paternity in the wild (2% of the offspring were extra-pair young and 6% of the broods contained extra-pair young, Birkhead et al. 1990; Griffith et al. 2010), they have been used extensively as a model for the study of extra-pair mating behavior. Frequent extra-pair courtship behavior has been described both in the wild (Birkhead et al. 1988) and in captivity (Burley et al. 1994; Forstmeier et al. 2011). Wild birds that were brought into captivity showed elevated levels of extra-pair paternity (12% of the offspring, Tschirren et al.

2012), and even higher rates of extra-pair paternity were observed in domesticated populations (28–29% of the offspring, Burley et al. 1996; Forstmeier et al. 2011). In the current study, we used a population derived from the wild only 20 years ago, and we tentatively expected lower levels of extra-pair mating compared with the much better studied domesticated populations (Houtman 1992; Burley et al. 1994, 1996; Forstmeier 2007; Rutstein et al. 2007; Forstmeier et al. 2011).

Studies on domesticated zebra finches showed that females are in control of extra-pair copulations (Forstmeier 2004) and that they seek copulations from specific individuals with which they had spent more time in previous mate choice tests (Houtman 1992; Forstmeier 2007). However, these experiments did not rule out the possibility that these apparent extra-pair mating preferences resulted from simple effects of familiarity. Indeed, spending time near a male could increase the familiarity between the two individuals and therefore, potentially, the inclination of the female to respond positively to this male later on. We therefore use the current experiment also to clarify whether females preferentially have extra-pair copulations with males to which they are familiar through passive exposure (rather than through active choice as assessed in a four-way choice chamber).

METHODS

Subjects and housing

The study subjects belong to a population of zebra finches maintained at the Max Planck Institute for Ornithology in Seewiesen, Germany. The population goes back to wild-caught birds whose captive-bred offspring were imported from Australia to the University of Bielefeld (Germany) in 1992. This population (population #4 in Forstmeier et al. 2007b) is genetically still closely related to the wild Australian population, but has lost some of its genetic variability (heterozygosity at 10 microsatellite markers is 83% compared with 93% in the wild; Forstmeier et al. 2007b). Individuals included in the present study consisted of a parental generation (29 males and 17 females born in 2008) and their offspring (23 males and 21 females born in 2009). According to our four-generation pedigree, all the 90 individuals have an inbreeding coefficient of zero. Since their arrival at Seewiesen in August 2009, the birds were maintained on a diet of millet seed mixture, cuttlefish, grit, and water *ad libitum*. Water and food were changed daily, and once a week birds received salad and a vitamin supplement. They experienced natural ambient light in addition to a full spectrum artificial fluorescent light set to a 14:10 h light-dark cycle, and a temperature ranging from 5 to 35 °C. During the time of the experiment, between 1 of April 2011 and mid November 2011, breeding pairs were kept separate from one another in 1 of 36 semi-outdoor aviaries (each measuring 2 m × 5 m and 2.5 m high) under similar light and temperature conditions. Subjects received additional egg food whenever they had chicks.

Experimental design

Assignment of social mate and extra-pair males

We paired 36 females (15 from the first generation that had one previous breeding experience, and 21 inexperienced from the second generation) by putting them in individual aviaries with a male from the same generation (never with the previous partner). Using our four-generation pedigree, partners were chosen so that the inbreeding coefficient of their offspring would be either 0 (24 pairs of unrelated individuals) or 0.25 (12 pairs of siblings that grew up together).

This difference in inbreeding served the purpose of another analysis (not shown) and did not affect the behavior we are interested in here (see “data analysis”). In total, eight pairs did not breed because of the death of one member ($n = 5$) or because the female did not lay eggs ($n = 3$). Two of the females that died without having laid eggs were replaced in early June; two widowed females were re-paired, before or after their first clutch, each with a male whose partner had never laid eggs. Thus, in total, 32 pairs (36 minus 8 plus 4) received the experimental treatment (see next section). Including or excluding the additional four females in the analyses or adding the date of pairing as a covariate did not change qualitatively any of the results.

We allocated eight extra-pair males from a total pool of 16 to each female so that the inbreeding coefficient of every potential extra-pair offspring would be zero. Two weeks before the start of the experiment, every female (except for the two replacement females) was familiarized with half of their extra-pair males. To do that, each female was put in an individual cage separated by mesh from one extra-pair male, which was exchanged after 2 h by another one. On a second day (within the following 5 days), two further extra-pair males were introduced to the female in the same way. This procedure was used to mimic the familiarity that may arise from choice chamber tests (Houtman 1992; Forstmeier 2007).

Hatching success manipulation

During the time the pairs needed to complete four clutches, they had access to nest material (coco fibers, cotton fibers, and horsehairs), and to four nest boxes. Nests were checked every morning. On the day a nest was completed and on the day the first egg was laid, two extra-pair trials were executed (see next section). Once a nest was chosen for laying, the other three nests were removed from the aviary and only put back when the first chick was 15 days old (just before the time of fledging), allowing pairs to start a new clutch and be tested again for their response to an extra-pair male. Used nests were replaced with a fresh nest box when all young had fledged. Juveniles were removed from the natal aviary at 45 days of age. On the day of the first egg of the first clutch, the pairs were assigned (in alternating order) to one of two treatment groups (16 pairs in each). Pairs stayed in their treatment group throughout the entire experiment (treatments were not switched).

The treatment consisted of manipulating the hatching success of three successive clutches, so that half of the couples experienced 100% hatching success, whereas the other half experienced $\leq 50\%$ hatching success (median = 35% hatching success). For the reduced hatching success group, we ensured that at least one egg hatched in order to mimic the most common natural situation of hatching failure, that is, partial failure (Mariette and Griffith 2012). Moreover, this provided a signal to the parents that the end of the incubation period (which typically lasts 12–13 days) was reached. To manipulate hatching success, we labeled every egg on the day of laying, and checked its status (alive or dead) every day from the fifth day onwards by using a digital egg monitor (Buddy, Avian Biotech, Cornwall, United Kingdom). The original clutch size was not altered by egg cross-fostering, with the exception of eight clutches in the 100% treatment group, which received one additional hatching egg that we had in surplus. Most of the eggs (82.2%, $n = 549$ eggs) had been cross-fostered with eggs of the same age (± 3 days maximum) to randomize, among the parents and treatment groups, the genetic quality of the chicks. The cross-fostering was done at approximately 10 days of incubation to allow potential parent–embryo communication before hatching (Evans 1992), even for those pairs that had originally only dead embryos or infertile eggs

in their own clutch. Every day, adjustments were made if necessary to obtain the desired treatment, that is, in case of embryo mortality occurring during the last days of incubation. This was achieved by having an additional four breeding pairs in small cages, which ensured the availability of live or infertile eggs (or eggs that contain embryos that died of natural causes). The rate of natural infertility or embryo mortality was high enough to fulfill the treatment criteria without having to kill any embryos. The focal 32 females laid a total of 682 eggs, of which 133 broke or disappeared due to thin shells. Of the remaining 549, 78 (14.2%) were judged as infertile (no visible embryo development, i.e., they might also have died at the start of development), 122 (22.3%) died during embryo development, and 348 (63.5%) hatched.

Extra-pair trials

Before females started laying a clutch, their responsiveness toward two extra-pair males was assessed twice: once during the presumed fertile period (at nest completion) and once on the day the first egg was laid (day 0). Female zebra finches are technically fertile from day –11 to +3 (Birkhead et al. 1989) but are the most responsive from day –6 to –2 (Birkhead et al. 1989, this study). As the onset of egg-laying cannot be predicted in advance, the realized extra-pair trials took place over a wider range of days (days –12 to +2; 80% between days –6 and 0). For data analysis, the period of testing was categorized as either “peak fertility” when the test took place between days –6 and –2 or as “early fertility” and “late fertility” otherwise. The social male was caught and replaced successively by one of two extra-pair males (the order did not matter, see below), one familiar and one unfamiliar, for 5 min each. On removal of the social partner, females often uttered distance calls, which function to stay in acoustic contact with the partner (Zann 1996). As a tentative measure of the strength of the pair bond, we recorded the number of distance calls uttered by the female during each extra-pair trial. Because females either did not call at all (68.4% of the 494 trials) or called rather frequently (mean \pm s.d.: 45 ± 50 calls) we modeled this behavior as a binomial trait (absent or present). As a measure of sexual interest in extra-pair males, we scored the responsiveness of females on a five point scale following Forstmeier (2004, 2007), varying from a clear rejection of the male involving aggression (–1) to a clear solicitation of copulation involving tail quivering (+1). Intermediate scores (–0.5, 0, +0.5) were given if both positive (approaching, ritualized hopping, beak wiping) and negative (threat display, beak fencing, fleeing) cues occurred or if either positive or negative cues were only weakly expressed. Moreover, we measured the male song rate with a stopwatch as the total number of seconds of song directed toward the female. This parameter was used as a covariate in the analysis of extra-pair responsiveness, and for that purpose it was square-root transformed and then centered and scaled.

Not all of the 32 females completed all the 16 tests: two females became widowed after 10 and 12 tests, and two others stopped laying after 14 tests. Trials without any cues of female responsiveness ($n = 195$), mainly because the male did not start any courtship (95.5% of these tests), were treated as missing values. Giving these trials a responsiveness score of zero did not change qualitatively any of the results. Thus, responsiveness was scored on 299 trials (60% of the 494 trials performed). We also recorded for every trial the occurrence of successful and unsuccessful copulations (see Forstmeier 2007). We assessed the outcome in terms of extra-pair paternity using 10 highly polymorphic microsatellite markers (Forstmeier et al. 2007a). The latter two parameters (occurrence of extra-pair copulation and extra-pair offspring) seem less useful than our responsiveness scores for the

measurement of female willingness to copulate, firstly because they are binomial variables (yes/no) with low frequency of “yes,” and secondly because the outcome of extra-pair copulations also depends on male behavior and sperm competition (Fossøy et al. 2006; Forstmeier et al. 2011). The treatment did not significantly influence the frequencies of either extra-pair copulations ($P = 0.39$) or extra-pair offspring ($P = 0.60$, details not shown for brevity).

Divorce opportunity

For 24 couples (11 and 13 from the 35% and 100% treatment group, respectively), the manipulation of hatching success was extended to include the fourth clutch. After the young of this clutch fledged, these couples were released together in one of the aviaries (each individual with a unique combination of color rings) in order to give them the opportunity to change their social mate. After 6 months, we recorded during 1 day all the pairs of individuals that engaged in clumping and allopreening, which is indicative of pair bonds (Silcox and Evans 1982).

Data analysis

We analyzed the responsiveness scores of the females and whether they called for their partner with linear mixed-effect models using the lmer function from the lme4 package (Bates et al. 2011) in R 2.14.0 (R Development Core Team 2011). For the test of specific hypotheses, we calculated two-tailed P values from the z -statistic of parameter estimates. With this approach, parameter estimates that are more than 1.96 standard errors away from zero ($|z| > 1.96$) are regarded as significant (with α set to 0.05). Residuals from the models were explored graphically to check for normality. Male and female identities were included as random factors to control for pseudoreplication. The 95% confidence interval (CI) of the percentage of variance explained by these random effects was obtained by parametric bootstrapping (with 1000 simulations) using the function rpt.remLMM.adj of the rptR package (Schielzeth and Nakagawa 2011). The level of inbreeding of pairs (see above) was excluded from the final model, because it had no main effect (females from inbred pairs were not more responsive (trend against the expectation): estimate = -0.093 ± 0.103 s.e., $z = -0.92$, $P = 0.36$) and no two or three-way interaction with the number of clutches manipulated and the treatment was significant (all $P > 0.54$). Similarly, the presentation order of the extra-pair males was excluded from the final model (first male tended to be preferred; $z = -1.43$, $P = 0.15$).

RESULTS

In 291 out of 494 extra-pair trials (59%) males courted females, and in 88 trials (18%) males attempted to copulate. In 33 trials, females did not resist these attempts but in only 18 of them copulation attempts actually resulted in cloacal contact. In nine trials, the females actively solicited a successful extra-pair copulation, whereas in one trial female solicitation did not lead to copulation. Fifteen out of the 16 extra-pair males (94%) courted at least 1 female, and 13 males made at least one copulation attempt. Seventeen out of the 32 females (53%) engaged in one or more extra-pair copulation attempts, but for only 11 females this led to cloacal contact. Overall, 20 out of 449 offspring (4.5%; including dead embryos and chicks) were sired by extra-pair males, and 6 out of 32 females (19%) had at least one extra-pair offspring. In the model of female responsiveness to extra-pair males, female identity explained 17.5% (95% CI: 7–28%) of

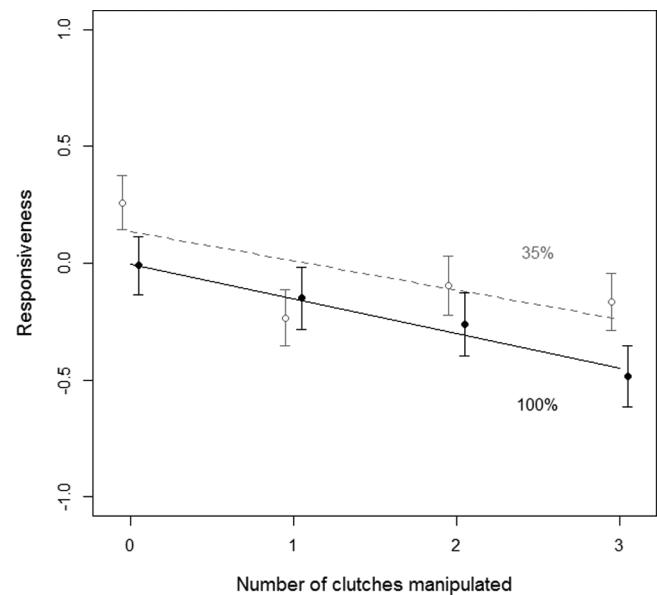


Figure 1

Female responsiveness toward extra-pair males (parameter estimates \pm s.e.) over repeated hatching success manipulations. The first estimate (0 clutches manipulated) reflects the females' baseline extra-pair responsiveness before any breeding experience with their partner. The following estimates refer to extra-pair behavior after 1, 2, or 3 experiences of either about 35% (open circles) or 100% (filled circles) hatching success. Regression lines for the two treatment groups are shown (dashed and solid line for the group of 35% and 100% hatching success, respectively). Responsiveness scores vary from -1 (aggression) to $+1$ (solicitation). Data were obtained from 32 females (16 in each treatment group) courted by extra-pair males before each clutch (299 observations). Estimates refer to the key fertile period (day -6 to -2); the responsiveness was lower when females were tested before or after that period (see Table 1).

the total variance, whereas male identity explained only 4.0% (95% CI: 0.3–10%). In other words, females were not highly consistent over time and did not agree on the overall attractiveness of particular extra-pair males.

Contrary to our main prediction, females that experienced repeated hatching failure did not increase their responsiveness toward extra-pair males (Figure 1). Instead, their responsiveness declined over consecutive clutches in the same way as in females that experienced 100% hatching success. Accordingly, the interaction between the number of clutches manipulated and the treatment group was not significant ($P = 0.67$; Table 1; Figure 1). If we removed the females that engaged in extra-pair copulations when starting their first clutch (i.e., females that were not able to judge their partner's infertility), these results did not change qualitatively (interaction: $P = 0.40$). The overall decline in female responsiveness over consecutive clutches was highly significant (after the removal of the interaction term: estimate = -0.13 ± 0.03 s.e., $z = -5.1$, $P < 0.0001$; Table 1). Allocation of females to treatment groups was done randomly when the first egg was laid, but the females of the two treatment groups differed (nonsignificantly) in their extra-pair responsiveness when tested for the first time (estimate = 0.32 ± 0.16 s.e., $z = 1.9$, $P = 0.063$; Figure 1). This initial difference was more or less maintained over the course of the entire experiment (Figure 1). Females were not more responsive toward extra-pair males they had been familiarized with ($P = 0.94$; Table 1), but their responsiveness significantly increased with extra-pair male song rate ($P = 0.004$; Table 1).

Table 1

Parameter estimates, standard errors (SE), and z -values ($z = \text{estimate}/\text{SE}$) of fixed effects on extra-pair responsiveness

Fixed effects on responsiveness score	Reference	Estimate	SE	z -value
Intercept		0.137	0.112	1.22
Clutches manipulated (C) ^a	Per clutch	-0.126	0.035	-3.59
Song (C) ^b	Per SD ^c	0.108	0.044	2.46
Familiarity yes (F)	Familiarity no	-0.008	0.060	-0.13
Treatment 100% (F)	Treatment 35%	-0.143	0.123	-1.16
Late fertility (F)	Peak fertility	-0.345	0.074	-4.69
Early fertility (F)	Peak fertility	-0.616	0.166	-3.71
Clutches manipulated * Treatment 100%		-0.023	0.053	-0.43

For continuous predictors (C), we give the slope estimate as the change in responsiveness per unit of the predictor. For factors (F), we give estimates for each level relative to the first level. Values are obtained from a mixed-effect model performed in R (lmer). Bold print highlights the test of the main hypothesis (the interaction term). Underlined z -values are further discussed in the main text.

^aNumber of clutches manipulated (0, 1, 2, or 3).

^bSeconds of courtship song, square-root transformed and z -transformed.

^cPer unit of standard deviation in the z -transformed predictor.

The attachment to the social partner, measured as the presence or absence of female calls when the partner was removed during the extra-pair trials, generally increased over the consecutive clutches ($P < 0.0001$, after removal of the interaction term; Table 2; Figure 2), and did so in a similar way for females that either experienced repeated hatching failure or success (the interaction between the number of clutches manipulated and the treatment group was not significant, $P = 0.13$; Table 2). A post hoc analysis of the data (details not shown) revealed that there was a peak of calling probability on the day before the first egg was laid (day -1). Moreover, females were more likely to call for their partner

Table 2

Parameter estimates, standard errors (SE), and z -values ($z = \text{estimate}/\text{SE}$) of fixed effects on the probability of females calling for their partner (binomial model)

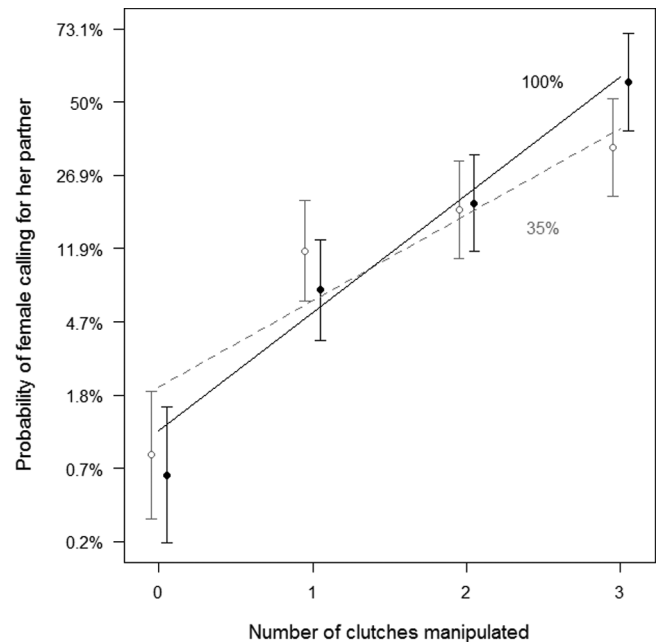
Fixed effects on female calls	Reference	Estimate	SE	z -value
Intercept		-3.892	0.711	-5.47
Clutches manipulated (C) ^a	Per clutch	1.176	0.197	5.96
Song (C) ^b	Per SD ^c	1.066	0.167	6.38
Familiarity yes (F)	Familiarity no	-0.270	0.281	-0.96
Treatment 100% (F)	Treatment 35%	-0.601	0.873	-0.69
Late fertility (F)	Peak fertility	1.033	0.359	2.87
Early fertility (F)	Peak fertility	1.949	0.819	2.38
Clutches manipulated * Treatment 100%		0.439	0.291	1.51

For continuous predictors (C), we give the slope estimate as the change in female calls per unit of the predictor. For factors (F), we give estimates for each level relative to the first level. Values are obtained from a mixed-effect model performed in R (lmer). Bold print highlights the test of the main hypothesis (the interaction term). Underlined z -values are further discussed in the main text.

^aNumber of clutches manipulated (0, 1, 2, or 3).

^bSeconds of courtship song, square-root transformed and z -transformed.

^cPer unit of standard deviation in the z -transformed predictor.


Figure 2

Probability of the female calling for her partner when he is experimentally removed during the extra-pair trials (parameter estimates \pm s.e.) over four consecutive clutches. The first estimate (0 clutches manipulated) reflects the female's behavior before any breeding experience. The following estimates refer to her behavior after 1, 2, or 3 experiences of either about 35% (open circles) or 100% (filled circles) hatching success. Regression lines for the two treatment groups are shown (dashed and solid lines for the group of 35% and 100% hatching success, respectively). Data were obtained from 32 females (16 in each treatment group) deprived of their social male before each of four successive clutches (494 observations). The y-axis is on a logit scale.

with increasing song rate of the extra-pair male ($P < 0.0001$; Table 2).

After being put in a communal aviary for 6 months, all 24 females engaged in allopreening only with their former social partner, suggesting that divorce did not occur.

DISCUSSION

Our empirical data (Figure 1) clearly refute the hypothesis that females increase their extra-pair mating behavior in response to increased hatching failure, at least for the given population and testing conditions. Firstly, the highly significant decline in extra-pair responsiveness over successive breeding attempts shows that our zebra finch females were most willing to engage in extra-pair copulation during their first breeding attempt with their social partner. Such behavior would not allow females to assess their partner's fertility or compatibility, because hatching success would also depend on fertility of or compatibility with the extra-pair males. Secondly, repeated experiences of hatching failure and success, respectively, did not induce any differences in extra-pair mating behavior. The statistical power of our experiment should be large enough (16 females in each group) to discount a biologically relevant effect size, especially when considering that in a natural situation breeding pairs would rarely experience such consistently discrepant hatching success.

It can be argued that in wild zebra finches (in contrast to our domesticated population) hatching failure may be

mostly due to incubation problems rather than to genetic incompatibility or infertility. In that case, engaging in extra-pair copulations would not increase egg hatchability, so that selection favoring the flexible promiscuous behavior that we predicted might have been absent. Therefore, future studies should clarify whether poor incubation can account for the high frequency of partial hatching failure in the wild (see [Mariette and Griffith 2012](#)), or whether these cases are better explained by genetic incompatibility, as seems to be the case in our domesticated population.

At the proximate level, hatching failure could have reduced the strength of the pair bond which might have led, if not to an elevated interest in extra-pair mates, to a higher probability of divorce [as suggested by [Rasmussen \(1981\)](#)]. Nevertheless, the repeated experience of hatching failure did not seem to alter females' attachment to their partner (in terms of calling for the partner when he is experimentally removed). Rather the data suggest that the strength of the pair bond increased with pair bond duration, regardless of the hatching success treatment ([Figure 2](#)). Moreover, females called more frequently when the extra-pair male courted them more intensively (effect of song rate in [Table 2](#)), suggesting a response to increased male harassment and an overall unwillingness to engage in extra-pair copulation. Given this apparent lack of effect of hatching failure on female fidelity in our study (no increased interest in extra-pair male or new partner), we briefly review the related literature on the effects of reproductive failure on the pair bond strength in other species.

Is there a link between reproductive failure and strength of the pair bond in other species?

It has been argued that divorce and extra-pair mating are related phenomena, because they both might represent extended mate choice, where the current breeding situation is assessed in comparison to potentially better options ([Cézilly and Nager 1995](#)). Divorce and extra-pair mating might therefore share proximate mechanisms, that is, psychological states of satisfaction with the partner or the strength of the pair bond ([Rasmussen 1981](#); [Spoon et al. 2007](#)).

Observational studies have found a small but robust effect that divorce is most likely to occur after reproductive failure ([Choudhury 1995](#); [Dubois and Cézilly 2002](#)). However, such observational studies can be misleading in several ways. First, confounding factors such as variation in age may cause or inflate the association between reproductive failure and divorce, because first-time breeders could have simultaneously a lower reproductive success and a higher probability of divorce ([Ens et al. 1996](#); [McNamara and Forslund 1996](#); [Mills et al. 1996](#)). Such confounding factors were not taken into account in the meta-analysis that showed a weak but significant association ([Dubois and Cézilly 2002](#); effect size $r = 0.11$). Second, even when reproductive failure and divorce are associated in a well-controlled analysis (e.g., [Naves et al. 2007](#)), the underlying mechanism remains obscure. In migratory species, reproductive failure may alter the birds' schedule of departure and arrival, which may result in divorce as a by-product (e.g., [Olsson 1998](#)). In species with marked site-tenacity, reproductive failure may trigger the search for a better territory or nesting site, which also might result in divorce. Indeed, in a well-studied species, the kittiwake (*Rissa tridactyla*), it appears that reproductive failure triggers dispersal rather than divorce *per se* (see [Table 6](#) in [Fairweather and Coulson 1995](#)). This pathway was further confirmed in the recent study of [Bai and Severinghaus \(2012\)](#) aiming specifically at disentangling site from mate fidelity in the Lany scops owl (*Otus elegans botelensis*). Finally, in some study

systems, lower quality individuals might both have reduced breeding success and a higher probability of being evicted from the territory ([Valcu and Kempenaers 2008](#)). These mechanisms could work independently of any effect of reproductive success on pair-bond strength.

There have been remarkably few experimental studies on divorce. Several studies on tits (Paridae) tested the effect of brood-size manipulation on the probability of divorce ([Lindén 1991](#); [Orell et al. 1994](#); [Blondel et al. 2000](#)). The findings of these studies have been mixed, and when pooling the sample sizes across the three studies, divorce rates were 21% for pairs with enlarged broods ($n = 71$) and 22% for pairs with reduced broods ($n = 68$; Fischer's exact test, $P = 1$). This might indicate that, among successful breeders, the actual number of offspring fledged is not a factor that would directly influence the probability of divorce in these species. Two experimental studies have examined how complete reproductive failure (egg removal or replacement with infertile eggs) affected a female's interest in a potential new partner (using choice chamber tests in the laboratory). Both studies produced nonsignificant trends in the expected direction, yet both studies focus on bird species in which divorce does not seem to occur in the wild. A study on captive pigeons ([Wosegien 1997](#)) found that, after failure, 54% of the females preferred the potential new male, whereas after breeding successfully, 45% did so (Fischer's exact test, $P = 0.62$). A study on domesticated zebra finches ([Drullion and Dubois 2011](#)) found that, after a single breeding failure, female zebra finches spent more time close to a neighboring male that had bred successfully and less time close to the social partner, when compared with females that bred successfully. However, due to small sample sizes ($n = 6$ females per treatment group) these results did not reach statistical significance. A more extensive replication of that experiment would be needed to assess whether those results are in conflict with our findings, and whether the potential discrepancy arises from differences in experimental design or from differences in study populations (recently wild-derived vs. domesticated). In the study of [Drullion and Dubois \(2011\)](#), a treatment of 0% hatching success was used, which could mimic a complete infertility of the partner. Future studies could explore whether this treatment is more efficient in weakening the pair bond than our 35% hatching success treatment.

In summary, neither observational nor experimental studies have produced convincing evidence that reproductive failure is causally related to the occurrence of divorce. Moreover, these studies have not clarified whether divorce proximately results from avoidance of the partner or of the territory where reproductive failure occurred ([Lindén 1991](#); [Valcu and Kempenaers 2008](#)). It is conceivable that reproductive failure due to environmental factors (e.g., nest predation, food shortage, intra-specific competition) might increase the propensity of birds to disperse to another breeding area. Divorce might result from such increased dispersal tendencies without requiring any effect of reproductive failure on the strength of the pair bond ([Bai and Severinghaus 2012](#)). Therefore, there is no conclusive evidence for the hypothesis proposed by [Rasmussen \(1981\)](#) that reproductive failure reduces the strength of the pair bond and leads to infidelity.

Pair-bond strength and extra-pair behavior in the zebra finch

Zebra finches maintain strong pair bonds all year round, whereas many other study species engage in seasonal partnerships. Because of the characteristics of our model species, our negative result may not apply to other species with weaker pair bonds (e.g., [Saitou 2002](#)).

Pair-bond strength is typically considered to increase with pair-bond duration. In most species, this has been inferred from the probability of divorce decreasing with time (Ens et al. 1996; Naves et al. 2007). In zebra finches, this can also be observed in terms of increased calling for the partner (Figure 2), decreased responsiveness to extra-pair males (Figure 1, see also Forstmeier 2007; Forstmeier et al. 2011), and increased responsiveness to the partner (Forstmeier et al. 2011). Moreover, we have regularly observed that pair bonds can break up within 2–3 weeks of pair formation in communal breeding aviaries, but this rarely happens at later stages (unpublished data). Likewise, extended periods of force-pairing seem to always result in strong and lasting pair bonds (this study, Forstmeier 2007). Therefore, the strength of the pair bond in zebra finches seems to have been under selective pressures at the expense of its adaptive weakening, for instance, in response to hatching failure.

In line with our expectations, we found that our wild-derived population showed lower levels of promiscuity than the previously studied domesticated population (Forstmeier 2007). Males were less likely to court extra-pair females (59% vs. 85% of the trials) but then did so with similar intensity (mean of 32 s vs. 37 s of courtship song). Among the trials with courtship, successful extra-pair copulation was less frequent in the present study (6.2% of trials) than in the previous study of the domesticated population (21.1% of trials). Despite this lower readiness to engage in extra-pair copulations, we still observed that throughout the 494 trials of 5 min performed on the 32 females, 17 females (53%) engaged in at least one extra-pair copulation attempt (which they did not resist, but some of which failed due to the male's behavior, e.g., losing his balance).

Some females were more promiscuous than others, although the individual repeatability of extra-pair responsiveness was somewhat lower than previously found in the domesticated population (Forstmeier 2007). The ultimate reasons for the occurrence of more promiscuous individuals, however, need not lie with potential benefits (e.g., fertility insurance) arising to females. Instead it might be that alleles for increased promiscuity are favored in males and these alleles may exert pleiotropic effects on behavior in females (Forstmeier et al. 2011). More precisely, if alleles that increase sex drive in males also cause females to engage more actively in extra-pair courtships, the promiscuous behavior of females carrying these alleles could be regarded simply as a genetic side-effect of these alleles having been favored in male carriers.

The present study does not support the idea that females obtain good-gene benefits from engaging in extra-pair copulations, as we did not find a substantial effect of extra-pair male identity on responsiveness. This either means that females do not seek good-gene benefits (but potentially compatible-gene benefits) or that they have difficulties assessing male genetic quality in the given experimental context (lack of social interactions, eavesdropping, mate-choice copying). It is noteworthy that stronger effects of extra-pair male identity on female responsiveness have been found in our domesticated population (Forstmeier 2007) which contradicts the idea that domestication has reduced variation in male attractiveness or reduced female choosiness or ability to discriminate (Griffith et al. 2010).

It appears that the female extra-pair mating preferences that were previously found in domesticated birds (Houtman 1992; Forstmeier 2007) reflect true sexual preferences and cannot solely be explained by effects of familiarity, because we here found no effect of experimental familiarization on extra-pair responsiveness (Table 1). Finally, it should be mentioned that this and an earlier study (Forstmeier 2007) are

fully consistent regarding the relationship between male song rate and female extra-pair responsiveness. Both studies show a positive association between male song and female responsiveness (Table 1; see also Pariser et al. 2010). This does not necessarily imply a female preference for males that sing a lot, because males might be stimulated to sing more when a female reacts positively. When aggregating the data for each male, both studies failed to find a relationship between a male's average song rate and its average received responsiveness (here $n = 16$, $r = -0.1$, $P = 0.72$).

CONCLUSION

More than three decades ago, Rasmussen (1981) comprehensively proposed how to experimentally test the hypothesis that reproductive failure leads to a weakening of the pair bond and hence to either divorce or extra-pair mating or both. Despite the plausibility of all the adaptive scenarios that link reproductive success with continued mate choice decisions (by means of divorce or extra-pair mating), there are still no convincing experimental studies supporting that view (and very few studies that seem to reject it). We therefore reiterate Rasmussen's call for well-designed experiments in this fundamental area of behavioral ecology research. These experiments should not only establish whether there is a causal relationship between reproductive failure and divorce, but they should also identify the relevant cues and proximate mechanisms. Reproductive failure might be due to partner traits (e.g., infertility, incompatibility, low parental quality) or environmental factors (e.g., predation, food shortage, competition over mates). An adaptive view on extra-pair mating and divorce might predict that the former factors should influence pair-bond strength, whereas the latter factors should influence habitat and nest-site choice.

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Chapter 2 Revisiting the evidence for inbreeding avoidance in the zebra finch

Abstract

Mating between close relatives nearly always leads to inbreeding depression, which should promote the evolution of inbreeding avoidance mechanisms, especially in sexually monogamous species. The zebra finch, a predominantly monogamous species, has been shown to suffer strongly from inbreeding depression, and this species has been used repeatedly in studies of inbreeding avoidance. However, no conclusive evidence for such avoidance has emerged. Here we summarize the previous research in a small meta-analysis showing that zebra finches appear to mate randomly with regard to relatedness (odds ratio for full-sibling mating = 0.96). Nevertheless, we notice that kin recognition by direct familiarization might have been prevented in all the previous studies, because siblings had always been experimentally separated before puberty and only re-united during adulthood. Recognizing individuals across different life stages may be cognitively demanding; therefore, in this species, it may require that siblings stay in contact throughout development. We conducted an experiment where birds were given the choice between a full sibling that stayed with them without interruption from hatching until adulthood and an unrelated bird familiar from independence (35 days of age) to adulthood. In contrast to all earlier studies we found a significant avoidance of inbreeding (odds ratio = 0.50). Although other mechanisms cannot be excluded, we suggest that zebra finches avoid inbreeding only if birds can keep track of their kin, and we discuss implications for the design of follow-up studies.

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Original Article

Revisiting the evidence for inbreeding avoidance in zebra finches

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Mating between close relatives nearly always leads to inbreeding depression, which should promote the evolution of inbreeding avoidance mechanisms, especially in sexually monogamous species. The zebra finch, a predominantly monogamous species, has been shown to suffer strongly from inbreeding depression, and this species has been used repeatedly in studies of inbreeding avoidance. However, no conclusive evidence for such avoidance has emerged. Here, we summarize the previous research in a small meta-analysis showing that zebra finches appear to mate randomly with regard to relatedness (odds ratio for full-sibling mating = 0.96). Nevertheless, we notice that kin recognition by direct familiarization might have been prevented in all the previous studies because siblings had always been experimentally separated before puberty and only reunited during adulthood. Recognizing individuals across different life stages may be cognitively demanding; therefore, in this species, it may require that siblings stay in contact throughout development. We conducted an experiment where birds were given the choice between a full sibling that stayed with them without interruption from hatching until adulthood and an unrelated bird familiar from independence (35 days of age) to adulthood. In contrast to all earlier studies, we found a significant avoidance of inbreeding (odds ratio = 0.50). Although other mechanisms cannot be excluded, we suggest that zebra finches avoid inbreeding only if birds can keep track of their kin, and we discuss implications for the design of follow-up studies.

Key words: inbreeding avoidance, kin recognition, mate choice, zebra finch.

INTRODUCTION

Selection should promote inbreeding avoidance behaviors because inbred individuals suffer from reduced fitness (Blouin and Blouin 1988; Pusey and Wolf 1996). Although this seems straightforward, theoretical models (e.g., Kokko and Ots 2006) that take inclusive fitness into account predict cases of inbreeding tolerance or even preference in promiscuous systems where a male does not lose other mating opportunities by mating also with kin. In contrast, close relatives should always be avoided in the context of social mate choice in monogamous species where the partner typically also sires the majority of the offspring (Waser et al. 1986; Lehmann and Perrin 2003; Kokko and Ots 2006; Szulkin et al. 2013).

The mechanisms that would allow kin recognition have received considerable attention in a wide range of species (see e.g., reviews on fish, birds and rodents, and arthropods, respectively, Ward and Hart 2003; Nakagawa and Waas 2004; Weddle et al. 2013). Two main forms of kin recognition are usually distinguished: recognition by association, when direct familiarization with the related individual is necessary for later discrimination; and “true” kin recognition, or phenotype matching, when the subject can discriminate a newly

introduced related individual based solely on its knowledge of itself (self-referent phenotype matching) or of other familiar kin (family phenotype matching). If kin recognition is due to direct association, the timing of its ontogeny and the capacity of remembering after a period of separation are then crucial (Nakagawa and Waas 2004).

In birds, kin recognition has mainly been studied in cooperatively breeding species where it has been found to work via associative learning (Komdeur and Hatchwell 1999; Nakagawa and Waas 2004). For instance, in the Seychelles warbler, *Acrocephalus sechellensis*, cross-fostered female offspring became subordinate helpers of the primary female that fed them (Komdeur 1994; Komdeur et al. 2004); whereas in the long-tailed tit, *Aegithalos caudatus*, young birds learn the vocalizations of foster kin and use these cues to discriminate potential recipients of help in the case they experience breeding failure in adulthood (Hatchwell et al. 2001; Russell and Hatchwell 2001; Sharp et al. 2005). Moreover, in birds, the onset of sibling recognition has often been assumed to occur during the dependency period in order to allow chicks to relocate their nest. This was shown for instance in the common tern (Palestis and Burger 2001a, 2001b). Therefore, dependent young have usually been used in studies of sibling recognition and only a few have studied if the

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discrimination extends beyond infancy (reviewed in Nakagawa and Waas 2004). Finally, kin recognition by phenotype matching has only been suggested in a few avian species including the zebra finch, *Taenopygia guttata*, but the evidence remains scarce and controversial (Bateson 1982; Burley and Bartels 1990; Fetherston and Burley 1990; Petrie et al. 1999; Schielzeth et al. 2008a, 2008b; Arct et al. 2010).

The zebra finch is a monogamous species with little extrapair paternity (around 2% of the young in the wild; Birkhead et al. 1990; Griffith et al. 2010) and life-long pair-bonds (Zann 1996). In this species, inbreeding leads to drastic fitness reduction (Bolund et al. 2010; Forstmeier et al. 2012; Hemmings et al. 2012). Wild zebra finches are highly nomadic and live in a very large and apparently panmictic population (Forstmeier et al. 2007b; Balakrishnan and Edwards 2009). This implies that the probability of meeting close kin after juvenile dispersal may be exceedingly small. However, some birds remain close to their natal area until they reach sexual maturity (the first breeding attempts can occur at day 80 post-hatch, Zann 1996), and recent work in a wild population shows that they can stay spatially associated with their siblings up to that age (Mariette M, unpublished data). During this period, individuals develop their adult characteristics, including adult plumage and, in males, learnt vocalizations that seem likely to function in individual recognition (Miller 1979; Zann 1984; Forstmeier et al. 2009).

The zebra finch has been repeatedly used as a model species in studies of inbreeding avoidance. However, the current evidence for kin recognition and inbreeding avoidance in this species is inconclusive. None of the mate choice experiments involving a choice between full siblings versus unrelated individuals have shown significant avoidance of siblings (Slater and Clements 1981; Schubert et al. 1989; Burley et al. 1990; Fetherston and Burley 1990; Schielzeth et al. 2008a, 2008b). Also, the validity of the various experimental setups is debatable. For instance, in most of these studies (Slater and Clements 1981; Burley et al. 1990; Schielzeth et al. 2008a, 2008b), it remains unclear whether the measured preferences are indeed of sexual nature (mate choice) or rather reflect gregariousness toward a particular individual (for instance in order to forage in a group). And, importantly, in all these earlier studies, siblings were separated for at least 50 days between independence from parents and the mate choice experiment (Slater and Clements 1981; Schubert et al. 1989; Burley et al. 1990; Fetherston and Burley 1990; Schielzeth et al. 2008a, 2008b). The separation of siblings at that age, when they are in juvenile plumage and when males are in the phase of song production learning (day 50–80; Zann 1990) in which the adult songs gradually “crystallize,” might prevent them from recognizing each other at adulthood. Indeed, recognizing an adult from memories of its juvenile features may be cognitively challenging, leading to the failure of kin recognition, as has been suggested for the Savannah sparrow (Wheelwright et al. 2006). Individual recognition by associative learning is probably much easier if siblings stay associated during puberty because the phenotypic changes happen progressively. A final criticism is that some past experimental designs have chosen siblings merely from the same parents but not necessarily from the same brood, which does not allow them to be familiar to one another from preindependence (allowing family phenotype matching but not direct familiarization). We hypothesized that, in order to discriminate each other, zebra finch siblings might need to stay in contact from hatching to mate choice, the very situation where inbreeding avoidance would be most crucial to happen for this species in the wild.

In this study, we start by summarizing earlier mate choice experiments involving siblings in the zebra finch with a formal meta-analysis. This method might reveal a weak general effect that cannot be detected by individual studies because of a lack of power. Moreover, the overview of all experimental designs and effect sizes gives us the opportunity to compare effect sizes between the different experimental approaches that allow a different set of kin recognition mechanisms to operate (direct familiarization vs. phenotype matching). Finally, we conducted a simple experiment where, in contrast to all previous studies, siblings were kept together until they were given the opportunity to breed with either their sibling or an unrelated bird to which they were familiar from independence onwards. This experimental set-up does not allow us to distinguish between the various recognition mechanisms. However, given the apparent lack of sibling avoidance in the zebra finch literature, we wanted to test whether any inbreeding avoidance can be found in the situation where it would be most needed in the wild. Specifically, this is when genetic siblings grow up together and, instead of dispersing away from all their relatives, remain in contact with siblings until pair-bonding takes place.

METHODS

All statistical analyses were performed in R 2.15.1 (R Core Team 2012).

Meta-analysis

We searched for published mate choice experiments on sibling avoidance in zebra finches via ISI Web of Science®. For each study, we calculated the observed odds of preferring (time spent in choice chamber) or mating (social pairing in aviaries) with a sibling relative to the random expectation (odds ratio). To summarize those odds, we used the R package “rmeta” version 2.16 (Lumley 2012) where we entered the logarithms of the odds (to normalize them) and their mean standard errors calculated from their asymmetrical confidence intervals (obtained from the `binom.test` function of R). Our literature search also yielded 2 experiments that studied mate choice between other types of kin, more precisely between fathers and daughters as well as between mothers and sons (Slater and Clements 1981), and between cousins (Burley et al. 1990). Therefore, for the sake of comprehensiveness of our review, we also ran another version of meta-analysis where these 2 experiments were analyzed together with the previous studies to get the odds of mating with any type of kin.

Experimental study

The study subjects belong to a recently wild-derived population of zebra finches (population #4 in Forstmeier et al. 2007b) kept at the Max Planck Institute for Ornithology in Seewiesen, Germany. The birds were maintained on a diet of millet seed mixture, cuttlefish, grit, and drinking and bathing water ad libitum. Water and food were changed daily, and once a week birds received lettuce and a vitamin supplement. During the summer 2012, birds were set up for breeding in 16 semi-outdoor aviaries (2 × 5 m and 2.5 m high). They experienced natural ambient light in addition to a full spectrum artificial fluorescent light set to a 14:10 h light:dark cycle, and natural short-term fluctuations of temperature ranging from about 10 to 45 °C. Each aviary contained 6 breeding pairs whose eggs were not cross-fostered. Chicks reared in these breeding aviaries were blood sampled (10 µL) from the brachial vein of their left wing on day 8 posthatch to determine their sex by polymerase chain

reaction amplification of the CHD-W and CHD-Z genes using the primers 3007 and 3112 (Ellegren and Fridolfsson 1997) and their parentage using 10 highly polymorphic microsatellite markers (Forstmeier et al. 2007a; Forstmeier et al. 2010).

For the experiment, we picked all 58 available brother–sister pairs (genetic $r = 0.5$) from the same brood, originating from a total of 42 different breeding pairs (30 breeding pairs produced 1 brother–sister pair, 9 produced 2, 2 produced 3, and 1 produced 4). A chi-square test for independence did not indicate that the family of origin affected the behavior (inbreeding vs. outbreeding, see Results) of the offspring (exact test for the 12 families with multiple brother–sister pairs: $P = 0.73$). We, therefore, consider all brother–sister pairs as independent data points. When offspring reached independence (day 35 posthatch), each brother–sister pair was joined with a brother–sister pair of similar age (maximally 4 days age difference), originating from a different natal aviary. The 4 birds were housed together in a “double-cage” (120 × 40 cm and 45 cm high) until day 100 posthatch, when they all had reached sexual maturity (November–December 2012). Then, the 4 birds were individually color banded and moved to 1 of 8 available indoor aviaries (2 × 2 m and 2.5 m high) furnished with 2 nest-boxes and nest material. There they experienced only the artificial light described above and a temperature stabilized at 20 ± 1 °C. The 29 groups were observed each day for 5–15 min, and the identity of individuals that showed signs of pair formation were recorded. This included clumping (i.e., sitting in body contact), allopreening, visiting a nest-box together, or incubating together. All eggs laid were collected (replaced by plastic eggs) and placed in an incubator for 5 days, when embryos were collected for parentage analysis (Forstmeier et al. 2007a). Parentage analysis was done in order to check whether females that were socially paired to their brother were more likely to produce extrapair young. Female promiscuity as a mechanism of inbreeding avoidance has been suggested in numerous studies. For instance, in the cooperatively breeding red-backed fairy wrens, females breeding with their son after the experimental removal of their partner produced more extrapair young than females breeding with an unrelated new male (Varian-Ramos and Webster 2012). In our experiment, once the social pairing was established from behavioral observations, and as soon as a few eggs were laid, the aviaries were made available (on average after 17 days, range: 8–30 days) for the next set of birds that had reached maturity.

Because the choice of 1 female within a group constrains the choice of the other female in that group, we considered the groups rather than the females as independent data points for statistical tests. The package “lme4” version 0.999999-0 (Bates et al. 2012) was used to run generalized linear mixed model (glmer) in analyses based on eggs with a binomial distribution to include the identity of the mother as random factor.

Ethical note

All described procedures are covered by the permit #311.4-si, by Landratsamt Starnberg, Germany.

RESULTS

Meta-analysis

The meta-analytic odds ratio of preferring or mating with a sibling relative to the random expectation from all published mate

choice experiments (Table 1) is 0.96 (95% confidence interval [CI]: 0.86–1.07). In other words, the number of sibling pairs observed equals quite precisely the number of sibling pairs expected under random mating. It is worth noticing that mate choice was evaluated differently in these studies: choice chamber tests were done in 5 studies on 2 different populations (meta-analytic odds ratio 0.94, 95% CI: 0.84–1.05), whereas a breeding set up similar to this study was used in 3 studies on 2 different populations (meta-analytic odds ratio 1.35, 95% CI: 0.91–2.01), although in only 2 of the latter 3 studies actual breeding was used as criteria for mate choice. Further differences in experimental design are shown in Table 1. Finally, when studies on mate choice involving kin other than siblings are added to the meta-analysis, the odds of mating with kin increases (odds ratio = 1.16, 95% CI: 0.96–1.41). However, this overall tendency toward inbreeding preference is not significant.

Experimental study

In 25 out of the 29 groups, pair identification based on behavioral observations was unambiguous. Of these, 19 pairings (76%) occurred between unrelated individuals, significantly more than expected by chance (exact binomial test against 50%, $P = 0.014$). In 3 cases, the behavioral observations showed a switch of partners over the course of the observation period: 2 cases from inbred to outbred mating and 1 case in the reverse direction (exact binomial test against 50%, $P = 0.012$, when including these 3 cases in their final mating constellation, Table 2). In a 4th ambiguous case, a female paired up first with her brother and then with the unrelated male without a complete cessation of pair bonding behavior with the initial male, leaving the final mate assignment equivocal (assigning the pairing as inbred or noninbred does not change the conclusions, the P -values equal 0.02 and 0.008, respectively).

One female died before any egg was laid in the aviary, but multiple behavioral observations had confirmed pair formation. Therefore, this case was included in the above analyses, but excluding it did not change the conclusion ($P = 0.036$). In the other 28 aviaries, eggs were found on average after 7.7 days (range: 2–15). In total, 160 eggs were collected (on average 5.6 eggs per aviary, range: 2–13 eggs), and these had apparently been laid by 50 out of 56 females (observational assignment, see Table 2). Of all eggs, 121 (76%) could be used for parentage analysis; the remaining eggs either appeared infertile (89% of 37) or did not allow embryonic development due to egg shell problems (drying out or breaking). Based on observational assignment of eggs, these eggs without embryos were not more frequent in inbreeding pairs (17% of 36 eggs) than in outbreeding pairs (28% of 118 eggs; glmer: $P = 0.47$). For the developing eggs, parentage assignment in most of the cases confirmed the identification of pairs via observations. Only 4 out of 121 embryos (3%) were sired by the other male than the social partner (Table 2). Females that chose their brother as social partner did not have more extrapair young (3 out of 30, 10%) than the females that chose the unrelated male (1 out of 85, 1%; glmer: $P = 0.82$). If the 6 inbred offspring of the female with equivocal social mate assignment are included as extrapair young or within-pair young (see Table 2), the results do not change qualitatively. Twenty-one eggs (17%) were dumped by females in another nest than the one they attended, a behavior commonly observed in this species (Schielzeth and Bolund 2010). A generalized linear model on the status (inbred or outbred) of the counts of the embryos of each group, with a quasi-binomial distribution, shows that the overall proportion of outbred embryos (72%, Table 2) was significantly

Table 1

Overview of zebra finches mate choice studies testing inbreeding avoidance among siblings. Sample sizes n1 and n2 refer to the number of different choosing individuals and the assumed number of independent mate choice decisions, respectively. Odds ratios (number of sibling pairs observed/number of sibling pairs expected) are given with their 95% confidence intervals and their *P*-values (based on n2). For each study, the main characteristics regarding sibling rearing and the potential kin avoidance mechanism(s) that were tested are indicated

Study	n1 (n2)	Odds ratio	<i>P</i>	Sibling rearing		
				Separated at puberty	From same brood only	Potential mechanism
Slater and Clements 1981 ^a	<18 (9)	1.33 (0.60–1.85)	0.5	Yes	Yes	All
Schubert et al. 1989 ^b	18 (18)	1.17 (0.52–1.93)	0.62	Yes	Yes	All
Burley et al. 1990 ^c	56 (56)	0.96 (0.69–1.24)	0.89	Yes	No	All but direct familiarization
Fetherston and Burley 1990 ^b	28 (28)	2.1 (0.59–4.81)	0.12	Yes	No	All
Schielzeth et al. 2008a ^d	90 (206)	0.9 (0.76–1.04)	0.18	Yes	Cross-fostered	Self-referent phenotype matching
Schielzeth et al. 2008a ^e	109 (109)	0.81 (0.52–1.16)	0.27	Yes	Cross-fostered	Self-referent phenotype matching
Schielzeth et al. 2008b ^{d,f}	50 (77)	0.99 (0.76–1.22)	0.38	Yes	Cross-fostered	Family phenotype matching
Schielzeth et al. 2008b ^{e,f}	63 (63)	1.21 (0.77–1.72)	1	Yes	Cross-fostered	Family phenotype matching
This study	56 (28)	0.5 (0.21–0.90)	0.01	No	Yes	All

^aAn unknown number of individuals were used several times.

^bMate choice in communal aviaries, n2 was assumed to be equal to n1.

^cTime spent in 2-ways choice chambers, males and females' tests pooled.

^dTime spent in 2-ways choice chambers, only females tested.

^eTime spent in 4-ways choice chambers, only females tested.

^fChoice for a genetic son of foster father.

Table 2

Number of groups where inbreeding or outbreeding social pairing was established in this experiment (1 case was ambiguous, see main text). The respective numbers of females with eggs are based on observational assignment, whereas the numbers of females with fertile eggs and extrapair young are based on genetic assignment. The resulting numbers of eggs (total and with DNA samples), extrapair young, and outbred embryos are also given. A range of values is shown for the case with ambiguous social pairing. Statistical tests corresponding to the indicated percentages are presented in the main text

Social pairing	Groups	Females with eggs	Females laying fertile eggs	Females with extrapair young	Eggs laid	Fertile eggs	Extrapair young	Outbred embryos
Outbreeding	21 (75%)	35	28	1	118	85 (72%)	1 (1%)	84
Inbreeding	7 (25%)	13	10	2	36	30 (83%)	3 (10%)	3
Ambiguous	1	2	1	0–1	6	6	0–6	0
Total	29	50	39	3–4	160	121	4–10	87 (72%)

higher than 50% ($P = 0.03$). Hence, extrapair fertilization neither enhanced nor blurred the inbreeding avoidance observed in terms of social pairing.

DISCUSSION

Our meta-analysis of all published mate choice experiments on sibling avoidance in zebra finches reveals no overall effect, although zebra finch studies are commonly cited as evidence for either kin avoidance (e.g., Arct et al. 2010) or kin preference (e.g., Slater and Clements 1981). In the study of Arct et al. (2010), females that were force-paired with an unknown brother were less likely to breed and invested less in egg production than those force-paired to an unrelated male, a finding that was interpreted as evidence for inbreeding avoidance (Arct et al. 2010; Szulkin et al. 2013). This result, however, is at odds with the outcome of our meta-analysis, where inbreeding avoidance via phenotype matching should have produced at least a small effect in every single study, if true

kin recognition existed in this species. Moreover, one may question whether differential allocation experiments are relevant for understanding mating preferences in a life-time monogamous species where the scope for obtaining a better partner in the future is rather low (Bolund et al. 2009). In the study of Slater and Clements (1981), mothers and sons visited nest-boxes together more frequently than unrelated birds, which was interpreted as evidence for inbreeding preference. However, this behavior might reflect filial attachment rather than sexual interest because the study did not look at actual reproduction. Zebra finches have also been regarded as being able to exert family or self-referent phenotype matching to recognize and prefer their cousins (Burley and Bartels 1990; Burley et al. 1990). The adaptive value of such preferences would be questionable because cousin mating should produce still about a quarter of the amount of inbreeding depression seen in full-sibling mating, which is quite considerable (Bittles and Neel 1994; Alvarez et al. 2009; Forstmeier et al. 2012). Also, in the same study using the same design, siblings did not discriminate between each

other (Burley et al. 1990), which clearly would be a maladaptive choice. Hence, these isolated findings of significant kin preferences (which would be maladaptive) or avoidance (based on a complex phenotype-matching recognition mechanism that does not seem to have evolved) might be interpreted as the result of many years of research combined with publication bias in favor of studies that reject the null hypothesis (Jennions and Møller 2002a, 2002b).

In our experimental study, pair formation and reproduction were significantly biased toward the avoidance of inbreeding, a result that would be predicted given the important fitness declines due to inbreeding in this species (Bolund et al. 2010; Forstmeier et al. 2012; Hemmings et al. 2012). Although the outcome of this study would need to be replicated in order to make sure this is not a false-positive result (see above), we suggest that zebra finches are able to discriminate close kin based on recognition of familiar individuals to whom they were exposed from hatching to sexual maturity, a behavior that appears adaptive in the light of their natural history. The studies presented in Table 1 that were most similar to this study in terms of 1) assessing mate choice in a breeding set up rather than in choice chamber tests and 2) allowing all potential recognition mechanisms to work (including direct familiarization before independence), but differed from this study in 3) the separation of the juveniles at puberty, produced the most contrasting odds ratios (meta-analytic odds ratio = 1.35) compared with the present experimental study (odds = 0.50). We, therefore, consider it most likely that the separation of juveniles between independence and testing is the decisive factor that explains these divergent results. In earlier studies, the separation of birds when they reached independence could have prevented mutual recognition at adulthood due to a lack of familiarity with the sibling in its adult characteristics (see Wheelwright et al. 2006). This could be especially true for females because male zebra finches develop several distinctive visual and vocal features when they reach sexual maturity. In cases where zebra finches remain in daily contact with a sibling throughout puberty and up to the time where they choose a partner, kin recognition and inbreeding avoidance might be easily accomplished. In contrast, if siblings lose track of each other for several days, not only does recognition become more challenging due to rapid phenotypic change during puberty but also the probability of encounter should be rapidly declining in this highly nomadic species with large population size. We, therefore, suggest that elaborate cognitive capacities for recognizing siblings in adulthood after having lost track of them as juveniles might not have evolved. Similarly, Jamieson et al. (2009) have suggested that a low encounter rate of siblings is the most likely explanation for the absence of kin avoidance in the New Zealand robin, *Petroica australis*, and saddleback, *Philesturnus carunculatus*.

This study shows that siblings familiar with each other from pre-independence to sexual maturity do avoid to some extent forming a pair-bond with each other. It is nonetheless noteworthy that the avoidance was not complete: sibling mating occurred in 7 out of 28 groups, a behavior that likely is maladaptive. A possible explanation for the lack of inbreeding avoidance in some individuals might be that they vary in their sensitive phase during which they form a mating aversion against associated individuals. For instance, a later joining of the 2 sibling pairs (e.g., day 40 instead of day 35) might have led to stronger inbreeding avoidance.

Although suggestive, the separation of siblings during puberty is not necessarily the relevant factor that explains the difference between this and previous studies because studies differ in many arbitrary ways. Notably, this study is also the first one conducted on a population of recently wild-derived zebra finches, whereas all previous studies in

Table 1 were conducted with more domesticated birds (Forstmeier et al. 2007b). Most importantly, this experiment varied only the pre-independence familiarity while keeping the familiarity during puberty constant. A better design would be to vary both these factors and to show that sibling discrimination happens only under the condition of familiarity during both periods. Such an experiment could also be done with cross-fostered individuals to confirm that self-referent phenotype matching is not the underlying mechanism. Testing familiar sibling versus nonfamiliar sibling has been widely done in mammals (reviewed in Nakagawa and Waas 2004), but rarely in birds (but see e.g., Pierotti et al. 1988; Hatchwell et al. 2001; Komdeur et al. 2004; Schielzeth et al. 2008a, 2008b for exceptions). Finally, it would also be interesting to assess whether the discrimination concerns nest mates specifically or extends to other individuals growing up in close association, for example, in the same natal group (aviary).

Cues involved in sibling recognition are typically discussed in the context of phenotype matching. Nevertheless, the question of how familiar individuals are recognized is also of interest. In both contexts, animals could use different sensory modalities. In their review, Nakagawa and Waas (2004) report that not only auditory but also visual cues have been shown to be used by birds, and odors by mammals, respectively. Female zebra finches might learn to recognize their brother by their contact call or by their song learned from a tutor at puberty. Moreover, they could recognize their appearance, also distinguishable for a human observer (Burley and Bartels 1990). Finally, they might also be able to distinguish individuals from their odor because dependent young zebra finches have been shown to relocate their natal nest by its smell alone (Krause et al. 2012). In a variety of species, odors seem to be involved in mate choice targeting the compatibility or the heterozygosity of the major histocompatibility complex (see e.g., Penn and Potts 1999; Zelano and Edwards 2002; Bernatchez and Landry 2003 for reviews), and it has often been assumed that individuals use self-reference for comparison (e.g., Wedekind et al. 1995). Nevertheless, chemical cues associated to a foster family can also imprint on individuals later using family phenotype matching to discriminate kin (Penn and Potts 1998; Hesse et al. 2012). If zebra finches can recognize their natal nest by its smell, it might be that they could also learn how to recognize associated siblings by their odor. However, if this would be the case, it seems that the lack of exposure to the smell of their sibling, at least during puberty, prevents them from recognizing them afterwards, either because zebra finches have a short memory of smell or because their smell changes during puberty like plumage and vocalizations.

CONCLUSION

To conclude, it is still debated whether birds have evolved any more sophisticated mechanisms of kin recognition than direct familiarization (Komdeur and Hatchwell 1999; Nakagawa and Waas 2004; this study). Alleged cases of phenotype matching would be worth following up by rigorous study designs. As to this simplest mechanism (direct familiarity), we are still far from knowing how many species might be able to recognize kin when these have undergone major phenotypic changes like in puberty and whether those changes are more drastic in males than in females (Wheelwright et al. 2006).

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Chapter 3 Fitness consequences of mate choice for compatibility in a socially monogamous species

Abstract

For many decades research on mate choice has focused on preferences for quality indicators, yet in some species mating preferences are largely idiosyncratic (with little consensus about attractiveness), suggesting that such preferences might target genetic or behavioural compatibility. Few studies have quantified the fitness consequences of allowing versus preventing such idiosyncratic mate choice. In our captive populations of zebra finches, when freshly laid eggs are individually cross-fostered for incubation and rearing, embryo mortality (before hatching) primarily depends on the identity of the genetic parents, while offspring mortality during the rearing period depends on foster-parent identity. Therefore, preventing mate choice should lead to an increase in embryo mortality if mate choice targets genetic compatibility (for embryo viability), and to an increase in offspring mortality if mate choice is aiming at behavioural compatibility (for better rearing). In communal breeding aviaries, we monitored the fitness and behaviour of recently wild-derived zebra finch pairs resulting from either free mate choice or forced pairings, using a design where variation in overall partner quality is not a confounding factor. Contrary to the genetic compatibility hypothesis, pairs from both treatments showed equal rates of embryo mortality. Yet, in line with the behavioural compatibility hypothesis, chosen pairs were better at raising offspring and overall achieved a 37% higher fitness. Further exploratory analyses reveal several interesting differences in behaviour and fitness components between such 'love' and 'arranged marriages'.

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The evolution of mate choice has received a tremendous amount of attention in the last decades, but only a few studies have attempted to measure experimentally the fitness benefits gained by choosy individuals (Petrie 1994; Sandvik *et al.* 2000; Ryan & Altmann 2001; Gowaty *et al.* 2007). Those benefits can be direct, when offspring quality or quantity is increased due to the partner's behaviour (including reproductive investment), or indirect, when offspring quality is improved by the genetic contribution of the partner. To date, the central debate has been about (i) the relative importance of direct vs indirect fitness benefits arising from the overall quality of the chosen partner (i.e. good parent vs good genes, Fig. 1 vertical black arrow) (Møller & Jennions 2001; Maklakov & Arnqvist 2009), or about (ii) the relative importance of the two types of indirect benefits, namely good genes vs compatible genes (Mays & Hill 2004; Neff & Pitcher 2005; Kotiaho & Puurtinen 2007; Cutrera *et al.* 2012, Fig. 1 horizontal black arrow). In contrast, the direct benefits arising from the compatibility between the two partners (bottom right in Fig. 1) have received only little attention (Delesalle 1986; Spoon *et al.* 2006, 2007; Schuett *et al.* 2010; Fox & Millam 2014). Synergistic advantages could indeed emerge from the combination of both parents' behaviours. Compatible partners could for instance be better at coordinating tasks, at sharing them or at complementing each other (Spoon *et al.* 2006; van de Pol *et al.* 2006; Griggio & Hoi 2011; Fox & Millam 2014; Sánchez-Macouzet *et al.* 2014), or they might simply be more effective at stimulating one another's reproductive investment (Arak & Enquist 1993; Bolund *et al.* 2012; Servedio *et al.* 2013). Mate choice for such behavioural compatibility might be especially important in species with intense bi-parental brood care and with long-lasting monogamous pair bonds, like humans or many bird species. Alternatively, idiosyncratic mate preferences may function to maximize offspring viability by bringing together compatible combinations of genes (top right in Fig. 1).

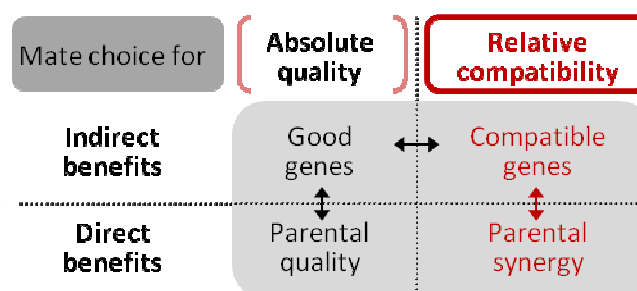


Figure 1. Schematic overview of four types of potential fitness benefits of mate choice. This study aims at separating direct from indirect benefits of mate choice for compatibility (red arrow), while experimentally controlling for effects of overall quality (red parentheses).

Previous experiments that aimed at quantifying the fitness benefits of mate choice arising from compatibility between the partners have typically compared females paired up with their preferred partner vs their non-preferred partner from a choice chamber test (Drickamer *et al.* 2000; Sandvik *et al.* 2000; Moore *et al.* 2001; Gowaty *et al.* 2003; Anderson

et al. 2007; Lehtonen & Lindstrom 2007; Raveh *et al.* 2014), or *vs* a random male (Simmons 1987; Bottoni *et al.* 1993; Massa *et al.* 1996). Problematically, with these procedures, the effects of quality and compatibility are confounded, because only force-paired pairs include males that would never have been chosen (i.e. most likely low quality males). This issue has been addressed in some studies by presenting evidence that the rejection of non-preferred partners was idiosyncratic (Ryan & Altmann 2001; Bluhm & Gowaty 2004b) or that non-preferred and preferred individuals did not differ in morphological traits (Drickamer *et al.* 2003). Yet, to clearly eliminate the effect of mate choice for quality, we propose a different experimental design, namely to compare the fitness effects of getting one's preferred partner *versus* of getting the preferred partner of someone else. Furthermore, only few studies looked at monogamous species with bi-parental care (Ryan & Altmann 2001; Spoon *et al.* 2006; Schuett *et al.* 2010; Gleason *et al.* 2012), and these studies were not able to tease apart genetic from behavioural compatibility advantages (right side in Fig. 1). The aim of this study was to quantify the benefits of mate choice that arise from the compatibility between partners, while circumventing quality as a confounding factor, in a species where genetic and behavioural compatibility can be disentangled.

The zebra finch (*Taeniopygia guttata*) is a life-time monogamous species with bi-parental care, and it is one of the species where female mate preferences are predominantly idiosyncratic (i.e. there is little consensus regarding male attractiveness) (Forstmeier & Birkhead 2004; Forstmeier *et al.* 2011), suggesting that these preferences target some kind of genetic or behavioural compatibility. The species further shows high rates of embryo and offspring mortality, and these phenomena beg explanation. In our domesticated population (population #18 in Forstmeier *et al.* 2007b), when eggs are individually cross-fostered (see Schielzeth *et al.* 2008a; b and *SI Text*), the identity of the genetic parents explains more than twice as much variance in embryo mortality than the foster-parents identity (N=1529 fertilized eggs, 28% mortality, see Table S1), while the foster-parents identity explains almost five times more variance in offspring mortality than the genetic parents identity (N=1106 offspring, 26% mortality, see Table S1). Results are qualitatively similar in our wild-derived population (#4 in Forstmeier *et al.* 2007b), where cross-fostering has been carried out at a smaller scale (see Ihle *et al.* 2013). We therefore assume that embryo mortality in zebra finches primarily reflects genetic incompatibility (like it does in other species, Dziminski *et al.* 2008; Rodríguez-Muñoz & Tregenza 2009), while offspring mortality primarily results from a failure of the parents (behavioural incompatibility). Consequently, when mate choice is prevented, we expect an increase in embryo mortality if mate choice is targeting genetic compatibility, and an increase in offspring mortality if mate choice is targeting behavioural compatibility. Alternatively, idiosyncratic mate preferences may only reflect indecision by the animal or measurement error by the experimenter (Forstmeier & Birkhead 2004). In this case, experimental treatments of allowing *versus* preventing idiosyncratic mate choice would have no fitness consequences.

We studied 160 bachelor birds from a wild-derived population of zebra finches (#4 in Forstmeier *et al.* 2007b), where each individual could freely choose a partner among 20 individuals of the opposite sex during a long non-breeding season (zebra finches are opportunistic breeders and do not reproduce if the environment is not suitable). This setup gave individuals a large number of potential partners to choose from, as likely is the case in the wild in this gregarious species. Pairs were identified by the occurrence of allopreening, a clear indicator of future breeding pairs (Silcox & Evans 1982), and sorted by their frequency of allopreening, which could be an indicator of the strength of their pair bond. Females that had chosen a partner were alternately assigned to one of two treatments: half of the females were allowed to stay with their chosen partner, while the other half of the choosy females were paired with the chosen partner of another female from the same aviary. This ensured that, on average, individuals of both treatments were of the same quality, even if assortative pairing for quality had happened due to intra-sexual competition. After this period of free choice, all pairs spent a few months in individual cages in order to enforce pair-bonding in the non-chosen pairs. Indeed, force-pairing is effective in this species if assigned mates are co-housed in a cage for long enough. Finally, pairs were given the opportunity to breed for one season (which allowed for about three successful broods) in communal aviaries, each containing three pairs from each treatment. This entire procedure was then repeated with the same birds for a second breeding season. This was planned *a priori* to get repeated measurements on individuals under different pairing conditions and a large enough sample size, to make possible the detection of weak effects. For the second year, two thirds of the pairs from the first breeding season were broken up; individuals chose a new partner and were either assigned to the same or the other treatment. The other third of the pairs were allowed to keep their partner (chosen or non-chosen) from the first breeding season. This was done to be able to better control for pair bond duration in statistical models comparing chosen and non-chosen pairs, as pair-bond formation in chosen pairs systematically started earlier (during the free choice period) than in non-chosen pairs (in cage). In total, 46 chosen pairs (C) and 38 non-chosen pairs (NC) were monitored extensively, and blindly with regard to their treatment, for both their reproductive success (paternity analyses were done for dead embryos, dead chicks, and surviving offspring), and their behaviour *via* both live observations (285 hours) and video recordings (1424 hours).

Results

When released into communal breeding aviaries, each of which contained three chosen and three arranged pairs, the majority of individuals stayed with their chosen or assigned partner (C: 46 out of 50 pairs did not divorce, NC: 38 out of 50 pairs; Fisher's exact test $P=0.05$). Only those pairs that stayed together were considered for analyses. Parameter estimates of dependent traits under the two treatments (C vs NC) are given for each general and generalized linear mixed-effect model. Other relevant statistics, as well as the structure

of the models, are given in Table S2 of the *Supplemental information* (referred to as 'TS2-test #').

Fitness differences

Relative fitness of individuals was calculated as annual reproductive success relative to the other individuals in the same aviary. The fitness proxy used was the number of genetic offspring that reached independence (35 days old). Males of chosen pairs had a higher relative fitness than males of non-chosen pairs ($C=1.16$, $NC=0.80$, $P=0.03$, $N=84$ male breeding seasons, see TS2-1 for model details, Fig.2). Females of chosen pairs also had a higher relative fitness than those in non-chosen pairs, but the difference was not significant ($C=1.09$, $NC=0.84$, $P=0.12$, $N=84$, TS2-2, Fig.2). The difference between the sexes was not significant (interaction between treatment and sex, $P=0.36$), and arises from extra-pair young sired during the experiment (discussed below). On average, individuals from the chosen pairs had a 37% higher fitness. This effect was not due to differential investment by the females of the two treatments in terms of the total number of eggs laid ($C=13.5$, $NC=14.4$, $P=0.56$, $N=84$, TS2-3).

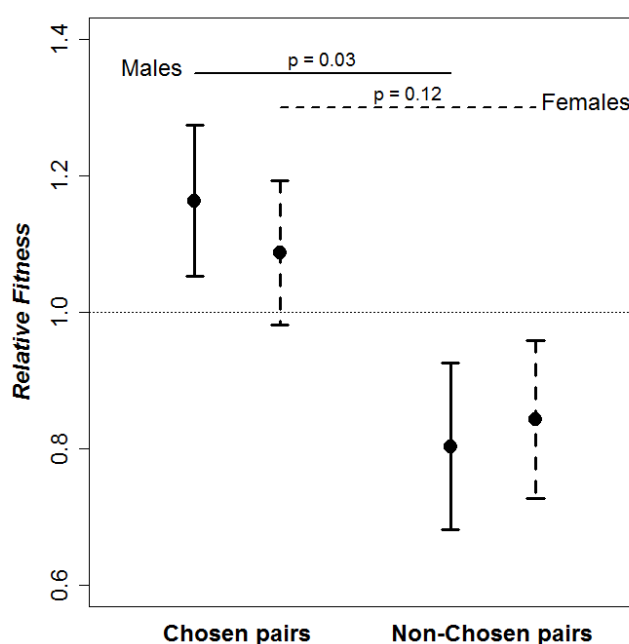


Figure 2. Relative fitness estimates (\pm SEM) of males ($N=84$) and females ($N=84$) from chosen and non-chosen pairs, as given by the general linear mixed-effect models TS2-1 and TS2-2.

Hypothesis testing: Genetic vs behavioural compatibility

To test the genetic incompatibility hypothesis, the proportions of dead embryos from all fertilized eggs were analyzed. Data only include the genetic eggs of each pair, that is, it does not include extra-pair young (9% of the eggs), but does include eggs of a pair that were

dumped into others nests (in this experiment egg-dumping accounted for 13% of the genotyped eggs that were laid). Moreover, it only includes eggs that were fully incubated, and not those that were buried before incubation was completed. There was no significant difference in embryo mortality rate between the chosen and non-chosen pairs ($C=20\%$, $NC=22\%$, $P=0.68$, $N=707$ fertilized eggs, TS2-5, Fig.3A).

To test the behavioural compatibility hypothesis, the proportions of dead offspring from all hatched eggs were analyzed. In this analysis, all offspring were assigned to the owner of the nest in which they hatched. Offspring mortality was significantly higher when chicks were reared by non-chosen pairs ($C=32\%$, $NC=52\%$, $P=0.03$, $N=594$ hatched eggs, TS2-6, Fig.3B).

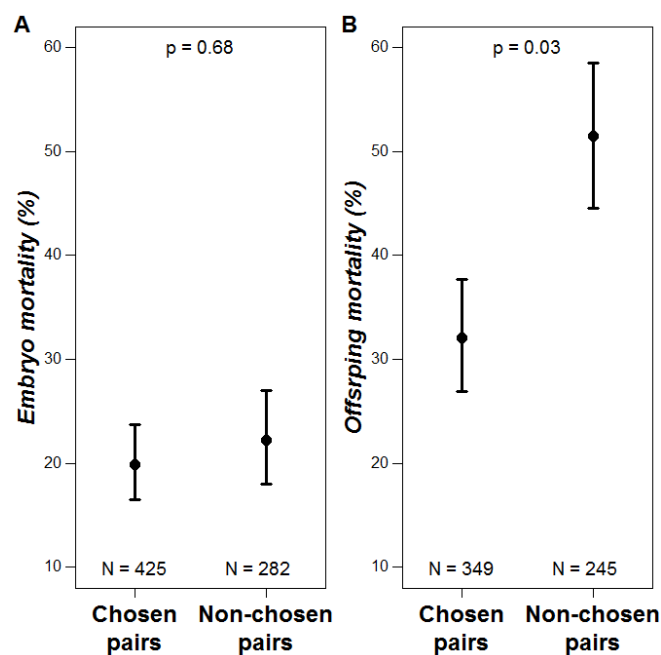


Figure 3. Embryo (A) and offspring (B) mortality rates (parameter estimates (\pm SEM)) in chosen and non-chosen pairs, as given by the generalized linear mixed-effect models TS2-5 and TS2-6 conducted on $N=707$ fertilized eggs and $N=594$ hatched eggs, respectively.

Exploratory analyses: ‘individual commitment’ and ‘pair harmony’

Within-pair courtship behaviour: Females were significantly less responsive to their partner during courtship if they were paired to a male they had not chosen themselves ($C=0.20$, $NC=0.05$, $P=0.01$, $N=2503$ courtships scored, TS2-15). This is interesting because it might explain why infertile eggs were more often present in nests attended by non-chosen pairs ($C=8\%$, $NC=23\%$, $P=0.01$, $N=216$ clutches, TS2-8). However, males from both treatments courted their partner equally often ($C=0.48$, $NC=0.52$ courtships per hour, $P=0.46$, $N=84$ male breeding seasons, TS2-20), and although within-pair courtships in non-chosen pairs

were less likely to lead to copulation, this was not significant (C=20%, NC=16%, $P=0.28$, N=2555 courtships, TS2-17).

Extra-pair courtship behaviour: Females of both treatments were courted by extra-pair males equally often (C=0.20, NC=0.25 courtships per hour, $P=0.36$, N=84, TS2-21). Moreover, female responsiveness towards a courting extra-pair male was mostly negative, independent of the treatment group (C=-0.53, NC=-0.51, $P=0.39$, N=2752 extra-pair courtships, TS2-16). However, non-chosen pairs were significantly more likely to have clutches that contained eggs sired by an extra-pair male (C=6%, NC=17%, $P=0.04$, N=245 clutches, TS2-9). While this could be due to the lower within-pair responsiveness of females in non-chosen pairs (see above), it could also be explained by the (non-significant) tendency that females of non-chosen pairs were more likely to engage in extra-pair copulations (C=6%, NC=16%, $P=0.13$, N=84 females, TS2-18).

Nest attendance: Once per day we recorded the identity of individuals attending nests as well as the fate of eggs and offspring. Male nest attendance showed a pronounced peak when eggs hatched (Fig. S1). Most of the offspring that died (58%), died within 24-48h after hatching (i.e. maximally seen once alive on daily nest checks). Thus, we considered nest attendance during the entire offspring rearing period, and also specifically on those days during which one or more offspring hatched in a given nest. Males of non-chosen pairs attended their nest less than those of chosen pairs during the days of hatching (C=42%, NC=30%, $P=0.04$, N=556 days of hatching, TS2-14), but not during the entire offspring rearing period (C=22%, NC=18%, $P=0.24$, N=2081 days with nestlings, TS2-13).

'Pair harmony': Every pair was observed daily during their first week in the communal aviaries, and before they received nest boxes and nest material, following a protocol inspired by a study on cockatiels, *Nymphicus hollandicus* (Spoon *et al.* 2007, see *Methods*). The occurrences of affiliative vs agonistic behaviour, the propensity of individuals to follow their partner, and the distance and synchrony in activity between the members of a pair were recorded. All those measures, as well as courtship rates and female responsiveness to courtships, were entered into a principal component analysis (PCA). Chosen and non-chosen pairs differed significantly in their PC1 score (C=0.24, NC=-0.29, $P=0.01$, N=84, Table S4), with individuals of chosen pairs mainly staying closer together and behaving more synchronously than those of non-chosen pairs (Table S3 and S4). This score of 'pair harmony' only weakly predicted pair fitness, though the trend was in the expected direction ($r=0.18$, $P=0.11$, Table S5). Observations were also carried out during the entire breeding season (every other day in 2012 and every day in 2013), but the PCA scores obtained for each pair during this period were neither significantly affected by treatment nor related to fitness. More details are presented in Table S4 and S5.

Discussion

Only a few studies have attempted to quantify the benefits of mate choice (Simmons 1987; Bottoni *et al.* 1993; Massa *et al.* 1996; Drickamer *et al.* 2000; Sandvik *et al.* 2000; Moore *et al.* 2001; Ryan & Altmann 2001; Drickamer *et al.* 2003; Gowaty *et al.* 2003; Moore *et al.* 2003; Bluhm & Gowaty 2004b, a; Anderson *et al.* 2007; Raveh *et al.* 2014), and to our knowledge, none so far have quantified the fitness benefits of idiosyncratic mate choice while excluding quality benefits (Fig. 1). We experimentally circumvented the effect of mate quality by comparing pairings of individuals who chose each other, with pairings we forced between random individuals who did not choose each other, but had both been chosen by another individual. Pairs allowed to choose their partner had a 37% higher fitness than pairs where mate choice was prevented experimentally (Fig. 2). This suggests that the between-individual disagreement about mate attractiveness does not just reflect indecision or measurement error, but rather reflects true preferences that lead to significant differences in fitness between ‘arranged’ and ‘love marriages’.

Our study system further allowed us to disentangle direct (behavioural) benefits of mate choice from indirect (genetic) benefits (Fig. 1, see *Introduction* and *SI Text*). Chosen pairs, compared to arranged ones, had an equal rate of embryo mortality (Fig. 3A), but a 38% lower rate of offspring mortality (Fig. 3B). These observations support the hypothesis of mate choice for behavioural compatibility, but reject the idea of mate choice for genetic compatibility to reduce embryo mortality due to inbreeding or incompatibility loci.

Previous experimental studies did not differentiate between genetic quality or compatibility effects, or favoured the genetic compatibility hypothesis based on the observation that preferred matings led to higher offspring viability than non-preferred matings (Drickamer *et al.* 2000; Drickamer *et al.* 2003; Gowaty *et al.* 2003; Bluhm & Gowaty 2004b, a; Anderson *et al.* 2007; Raveh *et al.* 2014). However, in most of these experiments females were forced to mate with random males, some of which may not have been preferred by any female because they were of low absolute quality. Hence, the previously observed effects may be explained by both genetic quality and incompatibility. Furthermore, one could argue that mate choice for genetic compatibility cannot easily evolve because it requires that the incompatibility causing loci are tightly linked (e.g. *via* pleiotropy) to a detectable phenotype, as well as a mechanism ensuring the appropriate assortative or disassortative preference (Tregenza & Wedell 2000). At least in zebra finches, such a complex mechanism might not have evolved. For instance, inbreeding avoidance in this species is not expressed if it can only be based on genetic similarity (Schielzeth *et al.* 2008a), but does take place when siblings are familiar with each other (Ihle & Forstmeier 2013).

In contrast, direct compatibility benefits of mate choice could come about through different mechanisms: the emerging behaviours of a pair in terms of coordination or

complementarity (Spoon *et al.* 2006; van de Pol *et al.* 2006; Griggio & Hoi 2011; Fox & Millam 2014; Sánchez-Macouzet *et al.* 2014), and/or the idiosyncratic stimulation of a partner's sensory system leading to a greater investment in reproduction (Arak & Enquist 1993; Bolund *et al.* 2012; Servedio *et al.* 2013). In the following we discuss our exploratory analyses on fitness components and behaviours, to tentatively favour one mechanism over another.

We found that non-chosen pairs (1) more often had clutches with infertile eggs, (2) had more offspring dying, presumably from starvation, and (3) tended to have more eggs that disappeared, supposedly due to poorer care and nest defence (see *SI Text*). Furthermore, (4) males from non-chosen pairs were more likely to lose paternity, reducing their (but not their mate's) fitness, which explains the larger effect on male fitness (Fig. 2). These effects on components of fitness may be explained by differences in behaviour of chosen and non-chosen pairs. The most prominent behavioural differences were that (a) females with assigned partners responded less positively to within-pair courtship and they tended to copulate less frequently with their partner, and (b) males with assigned partners showed poorer nest attendance during the egg hatching period.

It seems plausible that the reduced tendency of the female with a non-chosen partner to actively participate in within-pair courtship and copulation is responsible for the observed increased incidence of infertile eggs and the higher probability of having extra-pair young. The latter may also be due to force-paired females performing more extra-pair copulations, although this effect was not significant. Likewise, the lower nest attendance by the male during hatching could indicate a reduced motivation to care for the young when in a forced partnership, leading to greater offspring mortality and egg loss.

Our scores of 'pair harmony', which included interactive behaviours like affiliative and sexual behaviours, but also behavioural synchrony and the tendency to get reunited, did not significantly correlate with pair fitness. Therefore, it remains unclear what specific behaviours emerging from the pair would translate into fitness differences. In the literature, the evidence supporting the idea that pair coordination could give a selective advantage to certain pairs is indirect, and mainly comes from studies showing an increase in breeding success with pair bond duration (Ens *et al.* 1996; Black 2001; van de Pol *et al.* 2006; Griggio & Hoi 2011; Sánchez-Macouzet *et al.* 2014; but see Naves *et al.* 2007). We specifically designed our experiment with some pairs staying together for two seasons to be better able to control for possible effects of pair-bond duration. Unexpectedly, this covariate did not have an effect on fitness components (mostly showing non-significant trends opposite to expectation) and was therefore removed from those models. Moreover, in the second breeding season, pairs already established from the first year (N=14) did not have a higher fitness than newly formed pairs (N=28, $P > 0.80$ for both sexes). This suggests that behavioural compatibility (with synergistic effects on fitness) did not increase with pair bond

duration, but rather might have been determined by factors already present at the time of mating.

To summarize, although an effect of lack of coordination between pair members cannot be excluded, our exploratory analyses suggest a reduced commitment in individuals of arranged pairs. This could be explained by a mismatch in sensory systems and stimuli leading to a lower investment in reproduction. Previous experimental work on zebra finches shows that the amount of stimulation from the partner (which may be higher for a preferred mating) affects egg quality (Bolund *et al.* 2012). In our study, the viability of the offspring could have been enhanced by a stronger maternal (or paternal, in this species with bi-parental care) investment, mechanistically triggered by some form of stimulation (e.g. see Erickson & Lehrman 1964; Arak & Enquist 1993; Wachtmeister 2001; Bolund *et al.* 2012; Servedio *et al.* 2013). Similarly, earlier studies favouring the compatible gene hypothesis cannot rule out, even in species without parental care, that the type of pairing (preferred vs non-preferred) affected maternal investment (e.g. into the quality of eggs) with potential effects on offspring viability (Drickamer *et al.* 2000; Drickamer *et al.* 2003; Gowaty *et al.* 2003; Bluhm & Gowaty 2004b; Anderson *et al.* 2007). There, artificial insemination would be needed to experimentally demonstrate that higher offspring viability arises from genetic compatibility and not simply from maternal (e.g. egg nutrients) or paternal effects (e.g. sperm allocation) following greater stimulation by the partner.

If arranged pairs show a reduced investment in breeding, as our analyses suggest, the question is whether this behaviour is adaptive. Reduced investment in current reproduction could be adaptive, if it saves resources for future reproduction with a more preferred partner. This explanation seems unlikely for a species such as the zebra finch, because life-long monogamy largely precludes breeding with a different partner in the future (Zann 1996). Moreover, in a follow-up experiment consisting of a third breeding season where free choice was fully allowed, individuals could not compensate for the lower fitness previously obtained with a non-chosen partner (see *SI Text*). Therefore, the reduced commitment in non-chosen pairs could be maladaptive, either because it never or rarely occurs under natural conditions (for example, because suitable, i.e. compatible, partners are always available) or because a strong selective pressure on choosiness constrains the physiology and psychology of the animals.

To conclude, chosen pairs, compared to arranged ones, had a higher fitness presumably due to behavioural rather than genetic compatibility. Behavioural compatibility in terms of willingness or ability to cooperate with certain individuals and in terms of coordination between partners both need to be further investigated, specifically in a provisioning context. In humans, some evidence suggests that individuals are more satisfied, more committed and less likely to engage in domestic violence, when involved in a love-based rather than an arranged marriage (Xu & Whyte 1990; Sahin *et al.* 2010; but see Regan *et al.* 2012). The challenge there is also to comprehend whether stable and happy marriages

result from idiosyncratic motivation to cooperate (and to identify what stimulates such feelings, see Honekopp 2006; Todd *et al.* 2007; Asendorpf *et al.* 2011; Meltzer & McNulty 2014), or, for instance, from congruence in terms of partners' intrinsic behavioural types (Rammstedt *et al.* 2013).

Methods

Design

All birds of the experiment hatched in the summer of 2011 in large semi-outdoor aviaries. The origin of the birds, and rearing and housing conditions have been described in detail elsewhere (Ihle *et al.* 2013). Shortly after independence (when birds were 45 days old), they were put into 8 mixed-sex peer-groups of 10 males and 10 females. As soon as birds reached sexual maturity (100 days old) they were colour-banded, and peer-groups were joined two by two (yielding four groups, each allowing for 20 possible pairs to form). Sixty-six pairs were identified during *ad libitum* watches in the winter of 2011/2012. In April 2012, after assignment to treatments, pairs (half force-paired, half chosen) were put into separate cages for two months and allowed to lay one clutch. On the 21st of May, three pairs of each treatment (chosen randomly but by making sure the initial chosen partners of individuals of non-chosen pairs were not included) were put into a breeding aviary (10 replicates, 60 pairs in total). Both members of each pair had been previously colour-banded on both legs with one random color out of six (dark blue, light blue, black, yellow, orange, white), so that a pair would be unmistakably identifiable in its aviary. Forty-five out of 60 pairs (26 C, 19 NC) did not divorce and were considered for the analyses. Pairs that divorced might represent the most incompatible ones, and therefore, results obtained from the 19 forced pairs that did not divorce may underestimate the effects of incompatibility. After one week of conducting intensive focal pair observations, nest material was introduced, and nests were checked daily until 21 August (see next section). After this date, the experiment was stopped and newly laid eggs were replaced by dummy eggs. In October 2012, once all offspring had reached independence, all adults were joined to form two big flocks. Each group contained 20 newly single males (i.e. with their former breeding partner being in the other group), 20 newly single females, and 8 pairs that were allowed to stay together. Each newly single female could choose a new partner among a set of males that did not include her previous breeding partner, and in half of the cases not her initial chosen mate (for females coming from non-chosen pairs). In December 2012, after pair identification and treatment assignment (without regard to previous treatment), pairs were put into cages for six months and allowed to lay two clutches. The elongation of the period of force-pairing in cages resulted in a much lower rate of divorce than in the previous season (only one pair of each treatment divorced). On 21 May 2013, pairs were put again into breeding aviaries and allowed to breed as in the previous year. Only 42 pairs (21 of each treatment, across 7 aviaries) could be used for the second breeding season out of the 52 pairs identified in the winter groups, because 12 birds died accidentally (food dispensers were blocked for two

days in early March 2013). The selection of the best quality individuals, both by the design itself and by this accidental food shortage, does not induce a bias in one or the other treatment, but can result in an underestimation of the real fitness benefits of mate choice. Finally, one member of a pair died (and its partner was removed) within the first week of each breeding season (1 C in 2012 and 1 NC in 2013), and these two pairs were excluded from the analyses.

Breeding monitoring

Each aviary was furnished with 7 nest boxes. Every morning, nests were checked, the individual(s) attending the nest identified, and the fate of each egg and each offspring noted. Unhatched eggs were opened when neglected by the parents (for instance after offspring had fledged) and embryos were collected for parentage analysis performed with 11 microsatellite markers following (Forstmeier *et al.* 2007a) and (Forstmeier *et al.* 2010). For the same purpose, we took small (~10µl) blood samples from offspring on day 8-10 post-hatch or tissue samples if they died earlier. Eggs that could not be assigned by parentage analysis (28% of 1434 eggs laid by all birds including divorcees) were assigned to the social pair that attended the nest. These eggs comprise apparently infertile eggs (5.6% of all eggs) and those that were buried in the nest and did not develop (typically after a nest take-over by another pair) or disappeared (presumably they broke and were eaten by the birds) (22.4%). The relative fitness of an individual was calculated as the annual number of its genetic offspring that reached independence (day 35 post hatch) divided by the average number of offspring produced by all same-sex individuals of the same aviary that did not divorce.

Video surveillance and live observations

Each aviary was equipped with a dome camera set to record an artificial tree on which 69% of all courtships took place (calculated from the live observations described below). On some days of the week, in a regular and predefined manner, we also recorded a set of perches on which individuals like to allopreen, and the nest boxes. We analyzed the first hour of each day, when copulations are most frequent (Forstmeier *et al.* 2011). In pairs considered for the analyses (those that did not divorce), we recorded 1942 within-pair (WP) courtships, and 2999 extra-pair (EP) courtships (in the latter, a divorced female or male may have been the extra-pair partner). For each courtship, we scored female responsiveness (threat or aggression toward the male (-1), flying away (-0.5), mixed or ambiguous signs (0), courtship hopping and beak wiping (+0.5), and copulation solicitation (+1)) and noted whether it led to a successful copulation.

We also conducted focal-pair watches by monitoring a pair for three minutes, while standing behind a one-way glass built into aviary doors. During these watches we observed 613 WP and 800 EP courtships. We noted their location and whether they led to a successful copulation. For a subset of 561 WP and 782 EP courtships we also scored female responsiveness, as described above. During focal-pair watches, we also recorded whether

within-pair allopreening or aggression occurred during the 3 min period ('yes' or 'no'). Every 30 seconds, we recorded the distance between the partners and their synchrony in activity. Distance was averaged for each 3 min focal pair watch. Activities were split into 9 categories: feeding, cleaning, nesting or parental behaviour (nest building or attendance, and feeding of fledglings), sleeping, sitting, involved in aggression, involved in courtship, flying, and 'other'. Synchrony was simply the sum of the observations where both partner engaged in the same activity (range 0-6). Finally, we counted for each pair member all occurrences of an individual flying away from or back towards (<50cm) its partner (e.g. female flying away: Faway, male flying back: Mback). From those counts, we calculated the pair tendency to get reunited: $(\sum Fback + \sum Mback) / (\sum Faway + \sum Maway)$; and a mate guarding index: $(\sum Faway - \sum Fback) - (\sum Maway - \sum Mback)$. The latter is positive in case of male mate guarding, and negative for female mate guarding. The six pairs in an aviary were watched successively in a randomized predetermined order, and the time of observation of each aviary was randomized over the course of each day. In 2012, pairs were watched 9 to 13 times (median=11) during the first week, and 37 to 39 times (median=38) during the breeding period. In 2013, 16 to 21 focal watches (median=21) per pair were performed during the first week, and 68 to 70 (median=69) during the breeding period. All measures were averaged for all focal watches of a pair that were made during the first week (before breeding), as well as during the breeding period. Male courtship rates (WP and EP courtships per hour) and best linear unbiased predictors (BLUPs) of female responsiveness (to WP and EP courtships) were also calculated (see *SI Text*) for both periods and included in a principal component analysis (PCA).

All observations, live or from video, were done blind to the treatment of the birds. All analyses of behaviours and reproductive data comprise only pairs that 'accepted' the treatment (i.e. that did not divorce).

Data analysis

All statistical tests were conducted in R (R Core Team 2013). General and generalized mixed-effect models were performed with the 'lmer' and 'glmer' function of the lme4 package (Bates 2014) and the PCAs with the 'principal' function of the psych package (Revelle 2014).

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Supplemental information

SI Text

Causes of offspring and embryo mortality in cross-fostered eggs

To understand to what extent embryo mortality and offspring mortality depend on the parents who produce the eggs *versus* the parents that care for the eggs, we analyzed the fate of eggs that had been cross-fostered individually within 24h of egg laying. Such cross-fostering had been carried out extensively in our domesticated population of zebra finches over the course of two generations (F1 and F2) as previously described (Forstmeier 2005; Schielzeth *et al.* 2008a, b). The experimental fostering design was ideal for separating genetic from rearing effects, because a genetic clutch of e.g. five eggs would get spread among five foster nests, and each foster clutch of e.g. five eggs would be composed of eggs from five different genetic pairs. From a total of 2128 eggs that were fostered between 2004 and 2006, there were 1529 eggs that had been incubated through the whole incubation period and that were not infertile, dried out or broken but rather contained at least an embryo that we genotyped for genetic parentage (Schielzeth & Bolund 2010). Those 1529 eggs originated from 280 different combinations of genetic parents and were cared for by 260 different combinations of foster parents. Of those eggs, 423 (28%) died before hatching, leading to 1106 offspring, 283 (26%) of which died during offspring rearing (i.e. before reaching independence at 35 days of age). For each egg and offspring we coded mortality as yes or no, and extracted the variance component estimates for the two random effects of genetic pair identity and foster pair identity from mixed-effect models with binomial error structure using the lme4 package (Bates 2014) from R (R Core Team 2013) and without specifying any fixed effects besides the intercept (see Table S1).

We concluded from this analysis that – at least in zebra finches – embryo mortality is primarily a trait of the genetic pair, while offspring mortality primarily depends on the rearing parents. To formally disentangle genetic and rearing effects on embryo and offspring mortality, cross-fostering eggs individually within this experiment on the benefits of mate choice would have been necessary. However, this was not done here, because we would not have been able to quantify fitness without confounding effects of the cross-fostering.

Treatment effects on other fitness-related traits

Further exploratory analyses were conducted on breeding characteristics. Details are presented in Table S2. We tested whether disappeared or buried eggs were more common in nests of non-chosen pairs, for example due to higher within-pair conflict or to lower nest defence against other pairs trying to take over their nest, resulting in breaking or burying of eggs. Similarly, we tested whether egg-dumping rates were higher in nests of non-chosen pairs potentially less well defended against conspecific parasitism. The proportion of disappeared or buried eggs tended to be higher in non-chosen pairs (C=12%, NC=19%,

$P=0.07$, $N=1172$ eggs laid, TS2-4), while both types of pairs were equally likely to attend clutches that contained dumped eggs ($C=26\%$, $NC=21\%$, $P=0.41$, $N=209$ clutches, TS2-10). The cumulative effect of having slightly more disappeared or buried eggs and dead offspring, despite starting from a similar clutch size and having a similar rate of embryo mortality, resulted in a tendency for non-chosen pairs to have a lower brood size (count of offspring that reached 8 days of age, $C=2.59$, $NC=2.03$, $P=0.07$, $N=181$ broods, TS2-11). If non-chosen pairs are behaviourally less compatible, as we suggest (see main text), then one could hypothesize that they should also have offspring of lower body mass. However, offspring of both type of pairs did not differ in mass at day 8 post-hatch ($C=6.18g$, $NC=6.00g$, $P=0.51$, $N=421$, TS2-7). This is perhaps because non-chosen pairs tended to have fewer offspring to feed (as shown above).

We also tested whether chosen pairs had a higher breeding rate, that is, a shorter interval between clutches. This might for instance be the case if the male would take care of the fledglings while the female would start incubating a new clutch. However, chosen and non-chosen pairs took equally long before starting another clutch ($C=34.2$ days, $NC=33.1$ days, $P=0.57$, $N=135$ intervals between clutches, TS2-12).

Finally, we hypothesized that males of chosen pairs could have additional time to invest in extra-pair behaviour, for instance if they needed less time for mate guarding or for solving conflict over nest care. However, males of both treatments had identical extra-pair courtship rates ($C=0.51$, $NC=0.52$ courtships per hour, $P=0.92$, $N=84$, TS2-22) and similar success in obtaining at least one extra-pair copulation ($C=17\%$, $NC=13\%$, $P=0.64$, $N=84$ courting extra-pair males, TS2-19) or siring at least one extra-pair young ($C=32\%$, $NC=17\%$, $P=0.16$, $N=84$, TS2-23).

Details on behavioural variables used in the PCA

Courtship rates were first calculated for video and live observations separately before combining them (see below). For the videos, the number of courtships (within-pair, WP, and extra-pair, EP) by a male observed from a certain camera position was divided by the number of hours of video watched from this position. The courtship rates obtained for a male were then summed across all camera positions. This avoids creating a bias for individuals with a preference for a certain courting location. Courtship rates calculated from live observations were simply the number of courtships (WP and EP) observed in the entire aviary for a male, divided by the number of hours the aviary was monitored (focal pair watches). Courtships observed both live and on video were only considered once. To reach normality, all courtship rates were square-root transformed.

Synchrony (as defined in *Methods*) was significantly lower and courtship rates were higher in the second year, possibly also due to different observers or to slightly different protocols. Hence, synchrony scores and live and video courtship rates were standardized (z-transformed) within years. The standardized live and video courtship rates were then

averaged. Because of this complexity introduced to give a fair weight to each observation, it is not possible to back-transform model estimates into meaningful courtship rates, i.e. into number of courtships per hour. This is why analyses concerning courtship rates presented in the main text (and Table S2) were on videotaped courtships only. Nevertheless, all tests were also done on courtship rates calculated as above for the PCA, which includes all courtships observed, and the results obtained were similar.

Female responsiveness to male courtship depends on time of day and on the duration of the pair bond (see (Forstmeier *et al.* 2011) and Table S2). Therefore, the random effect estimates of each female (BLUPs) were obtained for each observation period (first week and breeding period) from linear mixed-effect models consistently structured as in (Forstmeier *et al.* 2011) and Table S2 (*e.g.* TS2-15 and TS2-16). Within and extra-pair responsiveness BLUPs of each female-season were used in the PCAs.

Follow-up experiment: Opportunity to compensate later in life.

We designed a follow-up experiment to examine whether individuals of the non-chosen pairs were adaptively reducing their brood size by saving energy for potential future reproduction with a chosen partner. In order to give every individual a maximum of free choice and an opportunity to form a new pair bond, all existing pairs were split up after the second breeding season, and all birds stayed in large unisex groups for six months. After this period, all adults whose previous breeding partner was still alive (N=35 pairs, 17 C and 18 NC) were placed together in a large breeding aviary for one month. This was done to investigate how many pairs of each treatment would re-unite after the artificial 6-months separation. Birds had unique combinations of colour-bands, and pairs were identified by the occurrence of affiliative and nesting behaviour. All observations were done blind to previous pair bonds and treatments. Eggs were replaced by dummy eggs and put in an incubator for 5 days after which developing embryos (N=114) were collected for parentage analysis (using 19 microsatellite markers). Relative fitness was calculated as the number of eggs an individual had sired (male) or laid (female) divided by the average number of eggs sired or laid by individuals of the same sex. To take into account the previous treatments of each individual, a score was given to each of them as follows: 0 for individuals that had bred with a chosen partner in both breeding seasons (C-C), 1 for individuals that had bred twice with a non-chosen partner (NC-NC), and 0.5 for individuals that had undergone one of each treatment (C-NC or NC-C). One female with score 0 died after two weeks in the experiment, without having formed a pair bond.

Results: Relative fitness under free choice in the follow-up experiment was not affected by previous treatments. The regression slopes of relative fitness over previous treatment score (coded as indicated above) were not greater than zero as would have been expected under the hypothesis of compensation later in life (female slope=-0.19, $P=0.59$; male slope=-0.19, $P=0.66$). These slopes correspond to relative fitness values of C-C=1.08 and NC-NC=0.88 for females and C-C=1.10 and NC-NC=0.90 for males. In total, 26 heterosexual pairs, one

female-female pair, one polygynous trio and one polyandrous trio showed strong affiliative behaviours. Three chosen and two non-chosen pairs reunited out of 5 chosen and 6 non-chosen pairs that had been allowed to breed together for both experimental breeding seasons. In addition, 4 pairs reunited out of 11 chosen pairs from the second experimental breeding season, as well as 1 pair out of 12 non-chosen pairs. Thus, although chosen pairs were more likely to reunite, this effect is not significant (Mantel-Haenszel chi-squared test with continuity correction, $P=0.17$; controlling for status of 1 vs 2 year-lasting pair bonds). Interestingly, two females reunited with their chosen partner of the second breeding season with whom they had not been allowed to breed (out of 8 possible such cases), and were involved in one of the two trios. Finally, one female bonded with her chosen breeding partner of the first season (out of 6 possible such cases). The 18 other pairs were new combinations of partners.

To summarize, individuals in a third breeding season where free choice was fully allowed could not compensate for the lower fitness previously obtained with a non-chosen partner. This suggests that the reduction in investment by forced pairs is non-adaptive in this species. Additionally, we show that chosen pairs were not significantly more likely to reunite than non-chosen pairs.

Table S1: Causes of embryo and offspring mortality in cross-fostered eggs shown as variance component estimates (Varcomp).

Test	Unit	N	N _{yes}	Mortality	Dependent variable	N genetic pairs	N foster pairs	Varcomp genetic pair	Varcomp foster pair	% genetic	% foster
1	Egg	1529	423	28%	Dead embryo	280	260	0.6569	0.2896	69.4%	30.6%
2	Offspring	1106	283	26%	Dead offspring	243	252	0.2079	0.9966	17.3%	82.7%

Column clarification and abbreviations:

Unit: unit of analysis for which sample sizes are given.

N_{yes}: numbers of yeses for binomial dependent variable

Table S2: Model structures of tests investigating the effect of the treatment on breeding parameters and sexual behaviours.

#	Unit	N	N _{yes}	Dependent variable	P	z	C	±SEM	NC	±SEM	Fixed effects	Random effects				Level
												FID	MID	PID	CID	
1	MID-yr	84		Male relative fitness	0.03	-2.24	1.16	0.11	0.80	0.12		55	54	70		
2	FID-yr	84		Female relative fitness	0.12	-1.59	1.09	0.11	0.84	0.12		55	54	70		
3	FID-yr	84		Number of eggs laid	0.56	0.58	13.5	1.12	14.4	1.18	A	55	54	70		Ass
4	Egg	1172	320	<i>Disappeared egg</i>	<i>0.07</i>	1.83	12%	2%	19%	3%	A+B	55	54	69	222	Soc
5	Egg	707	167	Dead embryo	0.68	0.42	20%	4%	22%	4%	A+B	52	53	64	205	Gen
6	Offspring	594	245	Dead offspring	0.03	2.18	32%	5%	52%	7%	A+C²	53	54	65	181	Soc
7	Offspring	421		Mass at 8 days old	0.51	-0.68	6.18	0.13	6.00	0.16	A+D²	49	51	59	149	Soc
8	Clutch	216	39	Infertile egg	0.01	2.62	8%	3%	23%	5%	A+E	52	53	65		Ass
9	Clutch	245	44	Female EPY	0.04	2.04	6%	2%	17%	6%	A+E	53				Gen
10	Clutch	209	55	Dumped egg	0.41	-0.82	26%	5%	21%	5%	A	54				Soc
11	Brood	181		<i>Brood size</i>	<i>0.07</i>	-1.82	2.59	0.21	2.03	0.23	A	53	54	65		Soc
12	Clutch	135		Clutch interval	0.57	-0.55	34.2	1.36	33.1	1.45	A+F+G			60		Soc
13	Day	2081	532	Male attendance	0.24	-1.16	22%	2%	18%	3%	H+I	52	53	63	172	
14	Day	556	215	Male attendance	0.04	-2.11	42%	4%	30%	4%	H	52	53	63	170	
15	Courtship	2503		WP responsiveness	0.01	-2.56	0.20	0.04	0.05	0.04	A+J+K+L+M	55	54	70		
16	Courtship	2752		EP responsiveness	0.39	0.88	-0.53	0.02	-0.51	0.02	A+J+K+L+M	55	59	322		
17	Courtship	2555	492	WP copulation	0.28	-1.09	20%	3%	16%	2%	A+J+K+L+M	55	54	70		
18	FID-yr	84	11	EP copulation	0.13	1.50	6%	4%	16%	6%	A+N	55				
19	MID-yr	84	15	EP copulation	0.64	-0.47	17%	6%	13%	6%	A+N		54			
20	MID-yr	84		WP courtship rate	0.46	0.74	0.48	0.04	0.52	0.05	A		54			
21	FID-yr	84		EP courtship rate	0.36	0.93	0.20	0.04	0.25	0.04	A	55				
22	MID-yr	84		EP courtship rate	0.91	0.11	0.51	0.06	0.52	0.07	A		54			
23	MID-yr	84	25	EPY	0.16	-1.39	32%	8%	17%	7%	A		54			

Column clarification and abbreviations:

#: test number given in the main text or *SI Text* as TS2-#.

Unit: unit of analysis for which sample sizes are given. 'MID-yr' (or 'FID-yr') stands for the combination of the male (or female) unique identity number and the year (2012 or 2013).

N_{yes}: the number of yeses for binomial dependent variables.

Dependent variables: WP and EP stand for within and extra-pair, and EPY for extra-pair young. 'Disappeared eggs': eggs that were either buried in the nest material or broken before the end of incubation, or eggs that completely disappeared from the aviary ; 'Female EPY' is whether or not in a given clutch the female had any extra-pair young ; 'Brood size' is the number of offspring in a clutch that reached 8 days of age; 'Male attendance' is whether or not the male attended its nest on a given day ; 'WP or EP copulation' are behaviourally successful copulations as opposed to unsuccessful courtships or copulation attempts.

P and **z**: p-value and z-value for the treatment.

C and **NC (\pm SEM)**: parameter estimates for chosen and non-chosen pairs and their respective standard errors. For tests with binomial dependent variables, the average of the asymmetrical back-transformed standard errors is given.

Fixed effect: **A**: year ; **B**: egg number in the laying sequence ; **C**: hatching order (coded 1-8) ; **D**: the hatching order of the offspring among those that reached 8 days of age ; **E**: clutch size ; **F**: clutch number for the pair within a year (coded 1-6) ; **G**: brood size reached in the previous clutch of the same pair ; **H**: number of offspring in the nest ; **I**: a continuous variable counting up the days from the first day with offspring in that brood, reflecting the average age of the offspring ; **J**: the number of days between the courtship and the day that is three days before the start of egg laying (with values ≥ 5 coded as 5), which means that 0 stands for the peak of fertility of that female ; **K**: pair-bond duration (in days) ; **L**: the number of eggs the female laid in the last 5 days ; **M**: the number of minutes after the light went on in the aviary on that day (artificial lights were set to turn on around 40 minutes before sunrise) ; **N**: number of courtships received, for females, or performed, for males. ² indicates that a linear and a polynomial term were included using the function 'poly' in R. All fixed effects were centered.

Random effects: **FID**, **MID**, **PID** and **CID**: the number of levels of random effects; female, male, pair and clutch identity numbers (where applicable).

Level: level at which the analyses was performed, i.e. how the units were allocated to individuals. **Gen** stands for genetic parents, **Soc** for social parents, and **Ass** for assigned parents, which are genetic when parentage analysis was possible, and social parents when it was not (infertile and disappeared eggs).

Test #20, 21, and 22 are based on videotaped courtships only to obtain meaningful courtship rates as number of courtships per hour. Nevertheless, those tests were also done on all courtships observed (live and videotaped), and gave similar results (see '*Details on behavioural variables used in the PCA*').

Bold characters in the table emphasize significance ($P < 0.05$); italic characters indicate trends ($P < 0.10$).

Table S3: Loadings of behavioural variables on PC1 for the 1st week in communal aviary (A), and the breeding period (B).

A. 1st week in communal aviary

Variable	Loading
Mean distance	-0.74
EP courtship rate*	-0.48
Female EP responsiveness [†]	-0.20
Female WP responsiveness [†]	-0.17
Male aggression	-0.09
Female aggression	-0.03
Female allopreening	0.28
WP courtship rate*	0.39
Male allopreening	0.41
Mate guarding	0.48
Synchrony [‡]	0.57
Proportion of flight back [§]	0.61

B. breeding period

Variable	Loading
Mean distance	-0.86
Female WP responsiveness [†]	-0.28
Female EP responsiveness [†]	-0.09
EP courtship rate*	0.04
Female aggression	0.11
Male aggression	0.15
Proportion of flight back [§]	0.22
Mate guarding	0.36
Female allopreening	0.66
Male allopreening	0.70
WP courtship rate*	0.74
Synchrony [‡]	0.79

*Courtship rates (within-pair (WP), and extra-pair (EP)) were calculated from both videotaped courtships and courtships observed live, see *SI Text*.

[†]BLUPs of female responsiveness in WP or EP courtships, obtained from linear mixed-effect models with the same structure as TS2-15 and TS2-16, see *SI Text*.

[‡]Synchrony was z-transformed within year, see *SI Text*.

[§]Pair tendency of getting reunited, see *Methods*.

Table S4: Results of linear models investigating the effect of the treatment on PC1 scores and on each variable included in the PCA separately, for the 1st week in communal aviary (A) and the breeding period (B). For all tests, N=84 pairs.

A. 1st week in communal aviary

Dependent Variable	P	z	C	±SEM	NC	±SEM
PC1	0.01	-2.50	0.24	0.14	-0.29	0.21
Mean distance	<0.00	3.20	40	3.0	54	3.3
EP courtship rate	<0.05	2.02	-0.17	0.13	0.21	0.14
Female EP responsiveness	0.91	0.12	0.00	0.01	0.00	0.01
Female WP responsiveness	0.30	-1.04	0.02	0.02	-0.02	0.03
Male aggression	0.28	1.08	0.01	0.00	0.02	0.01
Female aggression	0.75	-0.31	0.01	0.00	0.01	0.00
Female allopreening	0.46	-0.75	0.14	0.02	0.12	0.02
WP courtship rate	0.97	0.05	0.00	0.12	0.00	0.13
Male allopreening	0.16	-1.41	0.25	0.02	0.20	0.02
Mate guarding	0.97	-0.04	0.65	0.13	0.65	0.14
Synchrony	0.02	-2.35	0.23	0.14	-0.27	0.16
Proportion of flight back	0.87	-0.16	0.89	0.01	0.88	0.02

B. breeding period

Dependent Variable	P	z	C	±SEM	NC	±SEM
PC1	0.91	-0.12	0.01	0.15	-0.01	0.22
Mean distance	0.34	0.97	120	4.1	126	4.5
Female WP responsiveness	0.14	-1.50	0.03	0.03	-0.03	0.03
Female EP responsiveness	0.35	0.94	0.00	0.01	0.00	0.01
EP courtship rate	0.41	0.83	-0.07	0.13	0.09	0.14
Female aggression	0.22	1.24	0.00	0.00	0.00	0.00
Male aggression	0.31	1.03	0.00	0.00	0.00	0.00
Proportion of flight back	0.99	0.01	0.82	0.01	0.82	0.01
Mate guarding	0.76	-0.31	0.15	0.06	0.13	0.06
Female allopreening	0.22	-1.24	0.06	0.01	0.04	0.01
Male allopreening	0.74	-0.33	0.08	0.01	0.08	0.01
WP courtship rate	0.21	1.27	-0.11	0.13	0.13	0.14
Synchrony	0.98	0.03	0.00	0.15	0.00	0.16

More information on the dependent variables is given in the footnotes of Table S3. Bold characters emphasize significance.

Table S5: Results of linear models investigating the effect of PC1, and each variable included in the PCA separately, on the relative fitness achieved by the pairs. Analyses for the 1st week in communal aviary (A) and the breeding period (B) are shown. For all tests, N=84 pairs.

A. 1st week in communal aviary

explanatory variable	P	z	r
<i>PC1</i>	<i>0.11</i>	1.62	0.18
<i>Mean distance</i>	<i>0.06</i>	-1.88	0.20
EP courtship rate	0.64	-0.47	0.05
Female EP responsiveness	0.90	-0.13	0.01
Female WP responsiveness	0.18	1.37	0.15
<i>Male aggression</i>	<i>0.06</i>	1.92	0.21
Female aggression	0.36	0.92	0.10
Female allopreening	0.60	0.53	0.06
WP courtship rate	0.32	1.00	0.11
Male allopreening	0.63	0.49	0.05
Mate guarding	0.46	0.74	0.08
Synchrony	0.84	0.20	0.02
<i>Proportion of flight back</i>	<i>0.07</i>	1.85	0.20

B. breeding period

explanatory variable	P	z	β
PC1	0.93	-0.12	0.01
Mean distance	0.43	-0.80	-0.10
Female WP responsiveness	0.59	0.54	0.05
Female EP responsiveness	0.01	-2.83	-0.27
EP courtship rate	0.18	-1.35	-0.13
Female aggression	0.02	-2.38	-0.23
<i>Male aggression</i>	<i>0.10</i>	-1.65	-0.16
Proportion of flight back	0.88	0.15	0.01
Mate guarding	0.85	-0.19	-0.02
Female allopreening	0.87	-0.17	-0.02
Male allopreening	0.87	-0.17	-0.02
WP courtship rate	0.81	-0.24	-0.03
Synchrony	0.45	0.76	0.09

More information on the explanatory variables is given in the footnotes of Table S3. The number of days the pair was actively breeding (incubating eggs or rearing offspring) during the breeding period (B) was including as covariate for all tests. If this covariate is not included, male and female allopreening, as well as synchrony, within-pair courtship rate, and the overall PC1, are significantly negatively related to pair fitness because individuals that are not engaged in breeding activities (e.g. because their brood failed) spent more time on non-reproductive activities. Hence, this highly significant covariate of fitness is always controlled for. The coefficient of correlation r , or the standardized regression coefficient β are given to estimate the correlations between the relative fitness of pairs and the explanatory variables. β was obtained with the function 'lm.beta' from the package QuantPsyc (Fletcher 2012), in R (R Core Team 2013). Bold characters emphasize significance, italic characters indicate trends.

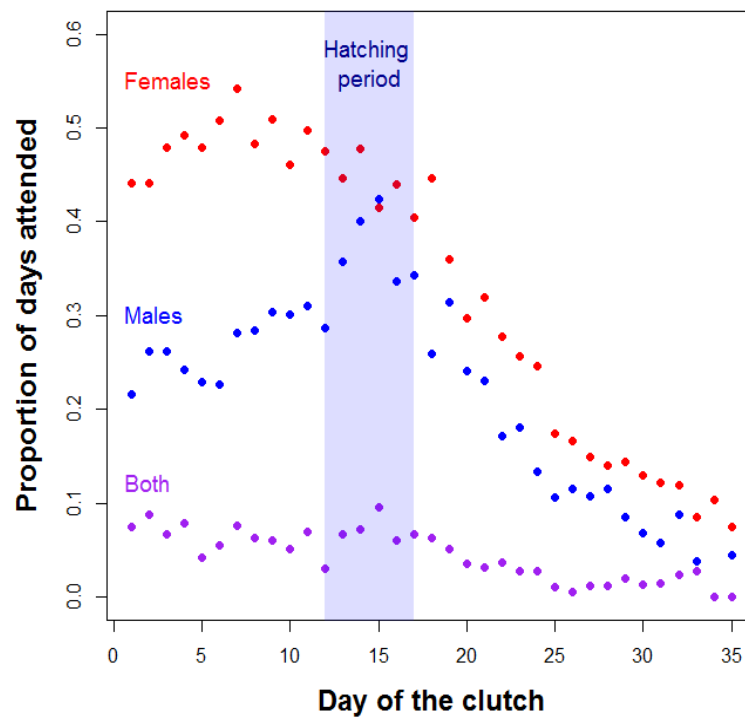


Figure S1. *Proportion of days when the female, the male, or both pair members simultaneously attended their nest while having an active clutch or a brood. The values for joint nest attendance are included in the values for females and males. Day 1 is the start of incubation. In zebra finches, incubation lasts on average 12 days, and offspring fledge 15-20 days after hatching.*

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General discussion

In this thesis, I investigated whether female zebra finches preferentially select compatible social mates and if they increase their extra-pair mating behaviour when paired to an incompatible male. In this system, apparent genetic incompatibility results in embryo mortality and therefore hatching failure (analyses presented in **Chapters 1 and 3**), while apparent behavioural incompatibility results in offspring mortality (analysis presented in **Chapter 3**). We therefore expected social mate choice for genetic and/or behavioural compatibility to optimise embryo and offspring survival, and an increase in female extra-pair behaviour to get compatible genes benefits when socially paired with a genetically incompatible male.

Lack of mate choice for genetic compatibility

Social mate choice

In the experiment presented in **Chapter 3**, females could initially choose freely a social mate among a large pool of males. They were then either allowed to stay with their chosen partner and profit from compatibility benefits, or force-paired to a male that was initially chosen by another female. In such force-paired females, the potential compatibility of their initial pair was disrupted, while, on average, the quality of their partners (initially chosen vs newly assigned male chosen by another female) was kept constant. Forced pairs from this experiment were thus deprived (only) from compatibility benefits. In this study, the hypothesis of social mate choice for genetic compatibility in order to reduce embryo mortality was not supported, as both chosen and forced-pairs obtained similar embryo mortality rates. The result of our test is surprisingly at odds with the conclusion of previous studies whose aim was also to quantify the benefits of mate choice. These studies found higher offspring viability in chosen pairings and interpreted this as evidence for the compatible gene hypothesis, although the experimental design did not control for 'good gene' effects (Drickamer *et al.* 2000; Drickamer *et al.* 2003; Gowaty *et al.* 2003; Bluhm & Gowaty 2004b, a; Anderson *et al.* 2007; Raveh *et al.* 2014). Also, these studies cannot rule out that the type of pairing (chosen vs non-chosen) affected maternal investment into the quality of eggs with potential effects on offspring viability. For instance, experimental work on zebra finches shows that the amount of stimulation from the partner (which may be higher for a preferred mating) affects egg quality (Bolund *et al.* 2012). Such synergistic effects, that in preferred pairings, the two partners might be more efficient in stimulating one another (for male sperm allocation and female investment in egg nutrients), may be an alternative explanation that was not regarded in earlier studies. Hence, to validate the conclusions of previous studies, artificial insemination would be needed to experimentally demonstrate that higher offspring viability arises from genetic compatibility and not simply from maternal or paternal effects following greater stimulation by the partner.

The meta-analysis presented in **Chapter 2** shows no support for the idea that zebra finches are able to recognize genetic similarity *per se* (for instance by self-referent phenotype matching) to avoid genetic incompatibility associated with inbreeding depression. To the contrary, the experiment presented in the same chapter suggest that inbreeding avoidance occurs in zebra finches only when the mechanisms that are experimentally allowed for are relatively simple, that is when kin can be recognized by direct familiarisation. More precisely, the discrimination seems only possible if sibling can maintain social contact from hatching to sexual maturity, and particularly without interruption during the puberty period, where the phenotypes of individuals change drastically (Zann 1996). These conclusions are nevertheless still pending for confirmation: follow-up studies should involve 1) testing the avoidance of foster-siblings, to definitively exclude mechanisms of true kin recognition, and 2) the manipulation of the period of familiarisation, to determine precisely what degree of acquaintance is necessary for avoidance to take place and during which stage of ontogeny familiarisation is effective. More generally, in birds, both these points should be further investigated, as experimental work on this subject is still lacking in avian research in comparison to the mammalian literature (Komdeur & Hatchwell 1999; Nakagawa & Waas 2004).

Extra-pair mate choice

Fertility benefits have been widely mentioned in the list of reasons why extra-pair behaviour could be advantageous to females (Petrie & Kempenaers 1998; Griffith *et al.* 2002; Arnqvist & Kirkpatrick 2005; Kempenaers & Schlicht 2010), but after careful consideration, the conditions under which female extra-pair behaviour could indeed evolve for fertility benefits are rather limited (see Hasson & Stone 2009). The experiment presented in **Chapter 1** was the first of its kind to test a specific case of the fertility insurance hypothesis under which females that repeatedly breed with the same social partner could adaptively adjust their promiscuous behaviour to the outcome of previous breeding events (Rasmussen 1981; Hasson & Stone 2009). Hatching failure, in the zebra finch, is a repeatable characteristic of the genetic parents (analysis presented in **Chapter 1**), and is mostly caused by embryo mortality (in the communal breeding aviaries in the experiment presented in **Chapter 3**, 21% of the eggs contained a dead embryo while only 9% of the eggs were categorized as infertile). In the experiment presented in **Chapter 1**, females that experienced repeated hatching failure with their social partner did not increase their responsiveness towards extra-pair males in comparison to females that obtained 100% of hatching success. Instead of compensating for an apparent genetic incompatibility with their mate, female became more and more faithful to their partner as pair-bond duration increased; and, in addition, they did not divorce when the opportunity was given. Therefore, these experimental results did not show any support for the idea that females would seek compatible gene benefits through extra-pair copulations. Nevertheless, the lack of social interaction between our experimental breeding pairs (each pair breeding in a separate aviary) could have affected the results. Potentially, a different outcome could have emerged if individuals had been

allowed to compare their own breeding success to the one of others (see e.g. Forsman *et al.* 2012 for an example of an effect of public information use on breeding decision).

In the experiment testing the existence of inbreeding avoidance in the zebra finch (**Chapter 2**), 25% of females chose their familiar brother as social partner. Potentially, in some species, pairing between siblings could be advantageous if, for instance, it eases cooperation over parental care. Nevertheless, females in such a system could still mate with unrelated individuals to avoid inbreeding depression in their offspring. In our experiment, females who choose to socially pair with their brother did not have more extra-pair young than females that paired with unrelated males, and they therefore did not compensate for the genetic incompatibility with their partner. In the zebra finch, where most of the reproductive success is reached with the social partner, inbreeding avoidance is strongly expected during social mate choice. It is therefore more likely that social pairing between siblings in this experiment resulted from assessment error, or from individual variation in the sensitive phase during which mating aversion for associated individuals is formed.

In the experiment presented in **Chapter 3**, females of arranged pairs were significantly more likely to have clutches with extra-pair young. This could be explained by their slightly lower rate of within-pair copulation but also by their slightly higher rate of extra-pair copulation. In this experiment, females did not seek compatible gene benefits during free social mate choice (see above). Therefore, the arguably higher promiscuity of force-paired females cannot be explained by a female compensation for a lack of genetic compatibility with their assigned partner. Instead, the relatively more pronounced extra-pair behaviour in females of non-chosen pairs might represent a ‘by-product’ of their social mating preferences, which are not satisfied with their non-chosen partner. Indeed, in free choice experiments, female-specific preferences seem to target the same particular males irrespective of whether the females are still socially unpaired (i.e. searching for a partner) or already paired (i.e. judging extra-pair males that court them; see **General Introduction**). As those idiosyncratic social mating preferences did not aim at optimising genetic compatibility (see above), these can be based on criteria related to behavioural compatibility, such as individually specific sensory exploitation (see below).

It is often suggested that females seek direct benefits during social mate choice and indirect benefits during extra-pair mate choice (Jennions & Petrie 1997, 2000; Candolin 2003; Kempenaers 2007). In this scenario, females could for instance recognise genetically compatible males but would preferentially mate with them in the extra-pair context (similar to the phenotype-linked fertility hypothesis, Sheldon 1994). This does not seem highly likely since no effect of genetic similarity *per se* was found in the context of inbreeding avoidance (meta-analysis in **Chapter 2**). Alternatively, females could perceive their genetic incompatibility with their social partner and consequently engage in extra-pair copulation, although such effect was not found in the experiment presented in **Chapter 1**. Finally, all females could randomly engage in extra-pair copulation if a post-copulatory mechanism

allows the discrimination of genetically incompatible sperm. This mechanism has been often suggested (e.g. see Tregenza & Wedell 2002; Foerster *et al.* 2003) but, to my knowledge, only confirmed in a single study on guppies so far (Gasparini & Pilastro 2011). In any case, provided that, in this system, embryo mortality is the main consequence of genetic incompatibility, if promiscuous females gain compatible genes benefits, extra-pair young should be less likely to die during embryo development than their respective within-pair maternal half-sib. This was not the case with the data collected in communal breeding aviaries for the experiment presented in **Chapter 3** (embryo mortality rate of within-pair young=19±3%, *versus* extra-pair young=20±6%, $P=0.83$, $N=842$ genotyped eggs, from 68 social pairs, 240 clutches, 56 genetic mothers and fathers; model otherwise built as TS2#5 in **Chapter 3**). Nevertheless, this test has a limited power (with only 19 extra-pair dead embryos) and could be confounded with several other factors such as good gene effects or female differential investment (although those factors are often thought to lead to an over- rather than under-estimation of the effect of compatible genes). To directly test the existence of a post-copulatory mechanism allowing the selection of compatible sperm (i.e. female cryptic choice), artificial insemination of a mix of sperm from related and unrelated male should be performed (see for instance Denk *et al.* 2005). This was done for the first time in the mallard, a duck species where females are not in control over copulations, and where therefore such post-copulatory mechanism could have evolved (Denk *et al.* 2005). Nevertheless, no fertilization bias toward the unrelated male was found (Denk *et al.* 2005). In the zebra finch, sperm competition is rather limited (with females being rarely promiscuous, and where no forced-copulation occurs), and therefore such post-copulatory mechanism seem even less likely to have evolved.

On the same note, if we consider that life-long social monogamy is the default in the zebra finch (although, it is still not understood why they exhibit such a mating system), and that the strength of social pair-bonding may share proximate mechanisms with the phenomenon of high sexual fidelity (Rasmussen 1981), then there is possibly not much scope for extra-pair behaviour to evolve as an adaptive response to a certain context. Such psychological constraint might also explain the negative correlation between pair tenacity (which is likely to be higher in long-lived species) and extra-pair paternity rate across species (Wink & Dyrce 1999). Nevertheless, species showing both low divorce rate and high extra-pair paternity rate also exist (e.g. European nuthatch (*Sitta europaea*), Segelbacher *et al.* 2005; and wandering albatross (*Diomedea exulans*), although in this species, extra-pair behaviour has apparently no benefits for females, Jouventin *et al.* 2007). In the zebra finch, one could still have thought that the high rate of embryo mortality occurring specifically in certain pairs could have been a strong selective pressure favouring adaptive promiscuous behaviour, even if only resulting in the low rate of extra-pair paternity observed in the wild.

To recapitulate, in the zebra finch, there is so far no evidence that females gain indirect genetic benefits from extra-pair copulations, while there is some evidence that this behaviour is partly intrinsic (Forstmeier 2007), and heritable, potentially resulting from an indirect selection on alleles that increase male promiscuity (Forstmeier *et al.* 2011). Moreover, the repeatability of female-specific preferences in both the context of social and extra-pair mate choice suggest that female extra-pair mate choice could simply be a by-product of social mate choice (**General Introduction**) rather than an adaptation.

Overall support for the hypothesis of mate choice for genetic compatibility

To summarize, this thesis comprises three studies specifically testing whether female zebra finches would choose mates according to compatible gene benefits, and this hypothesis was three times not supported (**Chapter 1**, meta-analysis of **Chapter 2**, **Chapter 3**). The hypothesis only held in the context of inbreeding avoidance between siblings when complete knowledge of kinship was made possible (experiment of **Chapter 2**). This suggests that, most likely, zebra finches have not evolved any ability to judge genetic compatibility of any kind, neither able to assess genetic similarity *per se*, nor able to assess other specific loci that are thought to cause embryo mortality. As this hypothesis has been suggested repeatedly (e.g. Jennions & Petrie 2000; Johnsen *et al.* 2000; Tregenza & Wedell 2000; Foerster *et al.* 2003; Neff & Pitcher 2005; Kempenaers 2007; Puurtinen *et al.* 2009), the present experimental work constitutes a clear progress in the field.

If zebra finches did not evolve mate choice for genetic compatibility, more experimental work should still be carried out on species where fertilization bias against unfamiliar siblings or in favour of MHC-dissimilar males has already been found. In such species, like the field mouse (Lindholm *et al.* 2013), the field cricket (*Gryllus bimaculatus*, Bretman *et al.* 2009), the red junglefowl (Pizzari *et al.* 2004; Løvlie *et al.* 2013), and the guppy (Gasparini & Pilastro 2011; but see Evans *et al.* 2008), where female choice for genetically compatible partner seems to occur without requiring familiarity as a cue to kinship, careful experiments and/or replicates should be performed. Subsequent follow-up studies should then investigate meticulously the proximate mechanism allowing such astonishing adaptive behaviour.

For instance, in the field cricket and red junglefowl, differential retention of sperm has been found following controlled natural copulations with males of different relatedness (Pizzari *et al.* 2004; Bretman *et al.* 2009), while no fertilization bias was found in the guppy, mallard, and red junglefowl following artificial insemination (Denk *et al.* 2005; Evans *et al.* 2008; Løvlie *et al.* 2013; but see Gasparini & Pilastro 2011). This suggests that contact with males might be necessary for females to discriminate against related ones. Interestingly, in an experiment on the Australian field cricket (*Teleogryllus oceanicus*), where females were shortly mated with a male of known relatedness, and subsequently guarded for a longer period by a related or unrelated males, it was found that the extent of sperm storage by the female was influenced by the relatedness of the guarding male rather than the mating male

(Tuni *et al.* 2013). Some lines of evidence indeed suggest that, in crickets, the mechanism of kin recognition could be based on the detection of cuticular pheromones following physical contact (e.g. see Simmons 1990), and this could lead to higher discrimination the longer the encounter. Similarly, the manipulation of female perception of which male she is mating with could maybe be implemented in species where artificial insemination can also be performed. For instance, males, both related and unrelated to focal females, could be sterilized, and providing that this does not alter their sexual behaviour, could be allowed to mate with females which subsequently could be artificially inseminated with sperm of a related or an unrelated male. This type of design could help quantifying the relative importance of pre- and post-copulatory processes. The guppy could potentially be an adequate species to carry out such an experiment, as the evidence for both pre- and post-copulatory mate choice regarding relatedness have been mixed (see Evans *et al.* 2008; Gasparini & Pilastro 2011 and references therein).

If discrimination against unfamiliar siblings is repeatedly confirmed in a species, and shown to be based either on a pre-copulatory mate choice mechanism (e.g. involving cuticular pheromones) or a post-copulatory mechanism (e.g. involving the interaction between the ovarian fluid and the sperm (Gasparini & Pilastro 2011)), the crucial and exciting research will then be to unravel the precise nature of this mechanism. Indeed, to be evolutionary stable, this mechanism would need to be sophisticated enough to prevent males (who are expected to be in sexual conflict with females) from overriding the system, and to allow females to both identify and discriminate against genetically closely related males without leading to a preference for heterospecific males. This particular question of mate choice for genetic compatibility highlights the importance of integrating the mechanistic and evolutionary fields of research. Robust evidence of an adaptive behaviour can set the arena for a mechanism to be discovered; and, reciprocally, comprehending the mechanisms and the constraints they can impose on evolutionary pathways could help distinguishing what constitutes a true adaptation from what is arguably more likely to be a false-positive result (Ioannidis 2005; Simmons *et al.* 2011).

Signs of mate choice for behavioural compatibility

In the experiment presented in **Chapter 3**, chosen pairs, who could benefit from compatibility advantages, achieved higher offspring survival than forced pairs (whose pair members were on average of same quality as in chosen pairs). As variance in nestling mortality in cross-fostered eggs is mainly explained by the rearing pair identity rather than the genetic pair identity (analyses presented in **Chapter 3**), higher offspring survival in compatible pairs supports the hypothesis of mate choice for behavioural rather than genetic compatibility. This study not only considered the largely neglected direct benefits of mate choice for compatibility, but also constitutes the first empirical evidence of fitness consequences of mate choice for behavioural compatibility.

First of all, to confirm our results, one might consider repeating the experiment presented in **Chapter 3** while cross-fostering the eggs before measuring the rearing success of chosen and non-chosen pairs, arguably under standardized conditions (e.g. same brood size, with offspring of a same genetic diversity, etc). If such mate choice for behavioural compatibility is found to be replicable, the criteria for mate choice and the actual male by female phenotypic interactions leading to fitness differences should then be carefully investigated. The benefits associated to behavioural compatibility could come from a range of different phenomena. In our study, we did not find evidence that pair bond duration or pair harmony was correlated with fitness, as it was found elsewhere (e.g. Spoon *et al.* 2006; Sánchez-Macouzet *et al.* 2014). For instance, in a study on cockatiels, coordination during incubation was important in determining hatching success (Spoon *et al.* 2006). In zebra finches, hatching success is more strongly dependent on the genetic parents rather than the foster parents (analyses presented in **Chapter 1**) and therefore, coordination during incubation seems not to be as crucial, or at least do not seem to vary much between pairs. Nevertheless, coordination in provisioning as observed in wild zebra finches (Mariette & Griffith 2012) could potentially be of importance if it ensures an equal distribution of food among siblings and thereby limits sibling competition (Shen *et al.* 2010; Gilby *et al.* 2011); but this specific type of coordination was not examined in our study. More details on provisioning behaviour could be obtained in further studies, notably by integrating cameras inside nest boxes, to be able to count the number of seeds delivered to specific offspring. Indeed, other measurements such as visit rate or visit duration are poor indicators of feeding rates (Gilby *et al.* 2011). Measures of food delivery per chick would give an indication on whether certain offspring reared by non-chosen pairs die from starvation because they do not get fed regularly enough due to a lack of complementarity in male and female parental care.

In addition, it has been suggested that certain combination of personality types could reduce conflict over parental care and that two individuals of similar personality could make better parents (Both *et al.* 2005; Schuett *et al.* 2011; but see Schielzeth *et al.* 2011). We did not measure any personality traits of individuals prior to the experiment, notably because we did not have strong *a priori* predictions on what the specific advantages of being behaviourally similar (for a certain aspect) would be. As the experiment was taking place in aviaries with *ad libitum* food in a known location, personality traits that relate to complementarity in foraging behaviour (e.g. exploratory tendencies) did not likely lead to the fitness differences observed. Potentially, personality traits influencing the capacity of individuals to communicate efficiently with each other could be involved, but to my knowledge, such type of between-individual variation has not been characterised so far.

Several earlier experimental studies of the benefits of mate choice found increased offspring viability in chosen pairs, and interpreted this as evidence for genetic compatibility benefits rather than for behavioural compatibility benefits because these species do not show bi-parental care (Drickamer *et al.* 2000; Drickamer *et al.* 2003; Gowaty *et al.* 2003;

Bluhm & Gowaty 2004b, a; Anderson *et al.* 2007; Raveh *et al.* 2014). However, the viability of the offspring could also have been enhanced by a stronger maternal investment mechanistically triggered by some kind of proximate stimulation (e.g. see Erickson & Lehrman 1964; Arak & Enquist 1993; Wachtmeister 2001; Bolund *et al.* 2012; Servedio *et al.* 2013). The results of our exploratory analyses on individual commitment to their relationship suggest that individuals of non-chosen pairs were indeed less motivated to breed with each other. A reduction of reproductive investment is likely maladaptive in zebra finches as they are a life-long monogamous and short-lived species (see also the follow-up experiment presented in the supplemental information of **Chapter 3**). Such lack of motivation could reflect a psychological constraint resulting, for instance, from general choosiness, irrespective of whether choosiness is directly selected for in another context, or simply not selected against. Indeed, the inherent propensity of an individual to mate more readily with certain phenotypes of the opposite sex (i.e. mating preference) leading to different ranking of males, might easily arise from inevitable variation (due to random mutation) in sensory systems leading to variation in sensory biases (Arak & Enquist 1993; Jennions & Petrie 1997; Kokko *et al.* 2006). Whether these preferences translate into mate-choice decisions depends crucially on whether there is also selection for female choosiness, as choosiness (i.e. the extent to which an individual is willing to sample mates before deciding to mate) is likely to bear some costs (Jennions & Petrie 1997; Kokko *et al.* 2006). Nevertheless, if the costs of choosiness are low, which is possibly the case in a gregarious species forming large flocks, then choosiness and variation in preferences could be maintained and lead to significant evolutionary consequences.

Testing such a hypothesis appears challenging. An original design could nevertheless be conceived from the combination of the results of this thesis: if zebra finches do avoid inbreeding only by association learning, and if there was assortative pairing for any traits that would lead to direct compatibility benefits (e.g. due to personality or sensory system matching), assortative pairing should be found among unfamiliar siblings who are more likely to share such traits. Nevertheless, with the likely low values of repeatability and heritability of those potentially important traits and preferences thereof, sample sizes for such an experiment would need to be extremely large. Such a holistic approach has nevertheless the merit of not being restricted to a putative trait of importance (e.g. exploratory behaviour) in order to confirm that mate choice for behavioural compatibility occurs based on genetically heritable traits already present at the time of mating. Alternatively, the repeatability of idiosyncratic preferences could be studied for particular traits. Song characteristics are potentially easy to evaluate by females during their first encounter with a male, and are likely candidates to the stimulation of the female's sensory system (Bolund *et al.* 2012). Although the repeatability of a female's preference for a certain song belonging to a given male stimulus within a given dyad of males has been shown across different choice devices (operant test, phonotaxis test and live male test, Holveck & Riebel 2007), the repeatability of female preferences for a certain type of song, independently of

the identity of the male, has, to my knowledge, not been studied so far. Directed song of males could be characterised for hundreds of individuals in sound boxes following the methods of Forstmeier *et al.* (2009) to get information on structural characteristics (e.g. syllable durations, number of syllables) and voice characteristics (e.g. distribution of sound amplitudes over the frequency spectrum, i.e. timbre). In male zebra finches, variation in song structure shows a low heritability because these characteristics are mostly learned from a tutor, while voice characteristics show significant heritable variation (Forstmeier *et al.* 2009). Sets of males with identical song structure (but different voices) could therefore be relatively easily formed. Females could then be assessed repeatedly for their preferences for a particular song structure in choice chamber devices where different males singing a same ‘text’ could be successively presented in pairs of stimulus males of dissimilar ‘texts’. Likewise, this could also be done to study idiosyncratic preferences for voice characteristics by using males of same families (or with similar voice characteristics) having learned their text from different tutors.

To conclude, the experiment presented in **Chapter 3** supports the hypothesis of mate choice for behavioural compatibility. It might be that chosen pairs were better at coordinating parental care and at resolving conflicts, or, alternatively, that certain combinations of partners could be better at stimulating each other to reproduce. To fully comprehend mate choice for behavioural compatibility and to be able to establish links of causality, more experimental work should be carried out in the same species, and the proximate mechanisms underlying this behaviour should be investigated.

General conclusion

Generally, this study questions the adaptiveness of social and extra-pair mate choice that is always assumed by default. Zebra finches were unable to identify partners with whom they would maximise their hatching success, and did not adjust their extra-pair mating behaviour in response to experiences in hatching success. Allowing free social mate choice did enhance pair fitness, but the adaptive significance of the underlying choosiness remains speculative. A null model consisting of random non-adaptive genetic variation in sensory systems and variation in phenotypes that are better at stimulating some sensory systems than others might well account for the observed fitness differences between chosen and non-chosen pairs.

Future avenues of research related to sexual selection theory: an integrative evolutionary behavioural ecology?

'In general, better understanding of female neurobiology and sensory capabilities is a prerequisite for any real advance in our understanding of mate choice.'

Jennions & Petrie (1997)

'The field of behavioural ecology should make better use of model organism to be able to test the underlying assumption of the hypothesis of sexual selection, quantitative genetic of the traits and preferences, and also to allow replication without which the field lack of robust scrutiny.'

Owens (2006)

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Summary

Behavioural ecologists aim at providing insights into the evolutionary and ecological processes that shape animal behaviour. Mate choice is a decision faced by most animals that can strongly affect an individual's reproductive success, an important fitness component. This behaviour has therefore the potential to show many adaptations which have been the subject of a vivid research interest over the last decades.

Studies on mate choice have typically focused on female preferences for traits that increase a male's overall attractiveness, which supposedly reflects the male's absolute quality. Preferences for such traits are expected to provide females with benefits such as good paternal care or 'good genes' for their offspring. Nevertheless, in some species, individuals actually show little consensus on what represents a high-quality partner. Such individually-specific preferences are poorly understood, but it has been hypothesized that they have evolved to optimise genetic compatibility (to increase offspring viability) or, although rarely mentioned, to optimise behavioural compatibility (to facilitate joint parental care). The zebra finch is a life-time monogamous passerine whose mating preferences for putative quality traits have been widely studied but where no such quality indicator has been repeatedly shown to influence mating decisions. In this species, preferences seem instead largely idiosyncratic.

In this thesis, I investigated whether female zebra finches choose genetically and/or behaviourally compatible social mates (**Chapters 2 and 3**). More precisely, I measured, in a large-scale breeding design, the fitness consequences of free mate choice for compatibility while experimentally controlling for effects of overall quality (**Chapter 3**). In this system, genetic incompatibility results in embryo mortality and therefore hatching failure, while behavioural incompatibility results in offspring mortality. I therefore expected social mate choice for genetic and/or behavioural compatibility to optimise embryo and offspring survival (**Chapter 3**). Moreover, I tested whether siblings who grew up together avoid choosing each other as social partner; in other words, if they avoid inbreeding depression by choosing a genetically more compatible partner (**Chapter 2**). In addition, I performed a meta-analysis of published experiments on zebra finches that allow for different mechanisms of kin discrimination to take place (**Chapter 2**). Finally, I investigated whether females enhance their extra-pair behaviour when paired to an apparently genetically incompatible male to obtain compatible genes benefits (**Chapter 1**). Indeed, extra-pair behaviour is largely hypothesized to be an adaptive response that would allow females to compensate for a potentially sub-optimal social mate choice. Specifically, I tested whether female extra-pair mating evolved as a counter strategy when females experience low hatching success with their social partner, that is to say whether female extra-pair mate choice targets fertility benefits and/or compatible genes benefits (**Chapter 1**).

Contrary to all expectations derived from optimality, zebra finches were unable to identify partners with whom they would minimise embryo mortality (**Chapter 3**), or to recognize unfamiliar kin on the basis of genetic similarity *per se* to minimize inbreeding depression (meta-analysis of **Chapter 2**), and did not adjust their extra-pair mating behaviour in response to repeated hatching failure (**Chapter 1**). This suggests that, in zebra finches, individuals have not evolved any ability (other than avoiding familiar kin, experiment of **Chapter 2**) to judge genetic compatibility of any kind, despite the adaptive value of such behaviour.

Finally, allowing free social mate choice did enhance pair fitness due to direct compatibility benefits (**Chapter 3**). This thesis provides therefore the first evidence of mate choice for behavioural compatibility. Nevertheless, the adaptive significance of the underlying choosiness remains speculative (**Chapter 3**). A null model consisting of random non-adaptive genetic variation in sensory systems and variation in phenotypes that are better at stimulating some sensory systems than others might well account for the observed fitness differences between compatible and incompatible pairs.

This study highlights that there are limits to adaptation.

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Chapter 1 Does hatching failure breed infidelity?

WF conceived the study. MI and WF designed the experiment with input from BK. MI conducted the experiment. MI analysed the data with input from WF. MI and WF wrote the manuscript with input from BK.

Chapter 2 Revisiting the evidence for inbreeding avoidance in the zebra finch

MI and WF conceived and designed the study. MI conducted the experiment and analysed the data with input from WF. MI and WF wrote the manuscript.

Chapter 3 Fitness consequences of mate choice for compatibility in a socially monogamous species

WF conceived the study. MI, BK and WF designed the experiment. MI conducted the experiment. MI analysed the data with input from WF. MI, BK and WF wrote the manuscript.

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Statutory Declaration and Statement

Ehrenwörtliche Versicherung

Ich versichere hiermit an Eides statt, dass die von mir vorgelegte Dissertation von mir selbstständig und ohne unerlaubte Hilfe angefertigt ist.

Muenchen, den

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Hiermit erkläre ich, dass die Dissertation nicht ganz oder in wesentlichen Teilen einer anderen Prüfungskommission vorgelegt worden ist. Im Weiteren erkläre ich, dass ich mich nicht anderweitig einer Doktorprüfung ohne Erfolg unterzogen habe oder ohne Erfolg versucht habe eine Dissertation einzureichen oder mich einer Doktorprüfung zu unterziehen.

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