CONDITION DEPENDENCE AND FITNESS CONSEQUENCES OF SEXUAL TRAITS IN ZEBRA FINCHES

DISSERTATION

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Summary

The focus of this thesis is on sexual selection within a behavioural and evolutionary framework. Sexual selection is concerned with the factors that determine reproductive success. I have used a laboratory population of zebra finches to address fundamental questions that remain unanswered despite decades of research on this model organism in studies of sexual selection in monogamous species. The aim of this thesis was to perform a rigorous investigation of how male zebra finches achieve reproductive success. To do this, I tried to evaluate the signalling value of candidate traits and investigate the mechanisms that lead to reproductive success through both intra- and intersexual selection.

The honesty of signals has had a special place in my work. Current theory suggests that secondary sexual signals should be costly to be able to function as honest indicators of male quality in a mate choice context. If ornaments are costly to develop or maintain, this would lead to condition dependent expression of ornaments. I tested the condition dependence of a broad range of traits so that I could compare the condition dependence of sexual and non-sexual traits and between traits in males and females (chapter 1). I found that zebra finches are remarkably resilient to stressful conditions during early development and can develop normally in most traits with very little consequences for fitness or longevity. These results indicate that not all traits that appear to be sexually selected characters will exhibit condition dependence.

To study fitness consequences, I used an aviary breeding set-up. This allowed me to study the process of sexual selection in a socially complex environment where natural selection pressures had been relaxed. Since only selection acting on the genetic component of a trait can lead to evolutionary change, I used a quantitative genetic approach to look at selection pressures. Contrary to the prevailing view, I found that beak colour and courtship song rate and song complexity were not subject to female choice and not relevant for reproductive success (chapter 3). Beak colour was also not important in male-male competition (chapter 2). These findings necessitate a re-evaluation of the function of these traits. I suggest that courtship song rate reflects male reproductive strategies, while song structure is important for individual recognition in this highly social species and the beak colour might function as a signal of breeding status.

The strongest candidate for a condition-dependent trait with consequences for fitness was body size (measured as tarsus length). Tarsus length was dependent on early condition (chapter 1), played a role in intrasexual competition (chapter 2) and was related to reproductive success in the aviaries (chapter 3). However, the quantitative genetic approach revealed that the selection was acting mainly on the environmental, not the genetic component of tarsus length, explaining why sexual size dimorphism has not evolved.

Contrary to the expectations of honest signalling theory, males with a higher undirected song rate (i.e. non-courtship song) were less successful in the aviaries (chapter 4). However, females invested more into reproduction when paired to a high undirected

song rate male, indicating that this type of song might function in reproductive stimulation of the partner and maintenance of the pair bond.

Furthermore, female reproductive investment can be influenced by male quality. In contrast to previous studies, I found that females invested more when paired to a less attractive male (chapter 5). This investment pattern fits with the breeding system of this species, since life-time monogamy and a short lifespan means that the chances of breeding with a higher quality male in the future is very low. Thus, females should do the ‘best of a bad job’ and compensate by investing more when paired to a less attractive partner.

Collectively, this work has provided several unexpected insights that call for a reinterpretation of several of the classical views of this model organism. This illustrates how novel experimental approaches can provide new insights into the process of sexual selection, also in well-studied model systems.

General introduction

"I begin to think that the pairing of birds must be as delicate and tedious an operation as the pairing of young gentlemen and ladies."

Darwin, C. R. to Weir, J. J. 18 April 1868, Letter 6128

As society changes around us, our view of the world and the way we interpret the phenomena we observe change along with it. Therefore, we are all constrained by the predominant thinking of our own time. Major scientific breakthroughs often happen when someone is able to break free from these constraints and develop new ideas. Darwin's theories of natural and sexual selection (Darwin 1859, 1871) are outstanding such examples. However, even in the revolutionary works of Darwin, one can in places trace an influence of the Victorian view of his time. For example, while he talks of males as trying to indiscriminately mate with as many females as possible, he describes females as coy and passive, conforming to the spirit of his time.

In the century and a half that has passed since then, society has gone through major changes and generations of scientists have worked to confirm, elaborate and nuance Darwin's original ideas. This work has been driven to a large extent by empirical studies, and these have stimulated theoretical research as hypotheses are supported or defied. This has lead to a more dynamic picture of sexual selection, where we for example talk about choosy males, and females that actively seek extra-pair copulations (Dewsbury 2005; Clutton-Brock 2009). During my PhD, I have used an empirical approach to address some questions that are of interest to the field today. Throughout this process, results have not always conformed to expectations. This has more than once made me take a step back and view a given finding from several perspectives and explore alternative explanations.

Darwin (1871) introduced a dichotomy between *natural selection* acting on traits that increase the survival of an individual and *sexual selection* acting on traits that increase the reproductive success of the individual. This dichotomy is, of course, mainly a semantic one, since evolutionary change is the result of differences in reproductive success among individuals. Because it is reproduction that matters, survival alone is not enough, as only genes of reproducing individuals will be represented in coming generations. Still, the distinction between traits that increase survival versus those that increase reproduction can be useful. In this thesis, my primary focus is to investigate different aspects of traits and behaviours that increase the reproductive success of a model species in studies of sexual selection, the zebra finch (*Taeniopygia guttata*). I combine a perspective that focuses on one sex, to elucidate the routes to reproductive success available to male zebra finches, and a perspective that includes both sexes, to obtain an integrative view of sexual selection in this model species.

In the remainder of this chapter, I will briefly review the current framework of sexual selection theory and introduce relevant concepts that will provide the background for the journey of exploring sexual selection in the zebra finch. This is followed by a brief description of the contents of the chapters of this thesis.

According to Darwin (1871), sexual selection arises from differences in reproductive success caused by competition over mates and heritable differences among them. Important factors that will determine the strength of sexual selection in the two sexes are non-random variance in reproductive success and uncertainty of paternity (Bateman 1948; Trivers 1972; Kokko and Jennions 2008). Both of these factors will often lead to stronger sexual selection pressures in males than in females. Sexual selection can be divided into on the one hand intrasexual selection, where members of one sex compete with one another to mate with members of the other sex, and on the other hand intersexual selection, where members of one sex are attracted by members of the other sex that carry specific traits (called ornaments). Since it is often males that are competing for access to females, and females that are choosing males, the terms male-male competition and female choice are commonly used instead of intra- and intersexual selection (Andersson 1994).

Females can choose males based on a variety of criteria. Classically, the benefits that females may accrue from choice are divided into direct (e.g. parental care, Hoelzer 1989) and indirect (e.g. good genes) benefits. The indirect benefits may be due to inheritance of genes for quality, attractiveness or genetic compatibility (good genes: Zahavi 1975; Grafen 1990a; Getty 2006, sexy sons or the runaway process: Fisher 1930, Lande 1981, compatible genes: Tregenza and Wedell 2000). Females may also prefer traits that do not confer any benefits to them, if the male exploits a pre-existing sensory bias in the female (Endler and Basolo 1998), for example for a certain colour. The models for indirect benefits have traditionally often been seen as separate and exclusive alternatives to explain the co-evolution of female preferences and male traits. However, recent theoretical models tend to instead emphasize the interaction between different processes (see e.g. Kokko et al. 2006).

The relative importance of the different kinds of benefits of female choice will vary among mating systems. During my thesis, I have focused on a mating system that is common in birds: social monogamy with some extra-pair paternity (EPP) and biparental care. Some 75 % of all socially monogamous bird species have varying levels of extra-pair paternity (Griffith et al. 2002) and about 90 % of bird species provide biparental care (Cockburn 2006). In this mating system, there is scope for all the processes of female choice outlined above to come into play. This opens the stage for several routes to achieve reproductive success for males: (1) Obtaining a partner, either by being attractive to females or by competing with other males (2) Trying to prevent the partner from having EP-copulations (3) Seeking own EP-copulations (4) Adjusting own level of parental care (5) Manipulating partner investment into reproduction.

Part of the reason why the zebra finch mating system is especially fascinating to study, are the similarities to the human mating system. The study of species with mating systems similar to our own might offer novel perspectives and insights into the biology of our own species.

Honest signalling of quality

Mate choice is built around signalling, and as outlined above, females could choose males with elaborate traits for many different reasons. During my thesis, I have been especially interested in the honest signalling perspective, which assumes that ornaments are honest signals of something of relevance for the female.

A signalling system requires reliability of signals to be maintained over evolutionary time (Searcy and Nowicki 2005) and honest signalling theory generally assumes that signalling costs ensure this reliability (Zahavi 1975; Grafen 1990a, b; Getty

2002). If traits are costly to develop or maintain, an individual in better condition can afford a more elaborate ornament. Thus, it is widely assumed that condition-dependence is a common feature of sexual traits (e.g. Johnstone 1995, Rowe and Houle 1996). Since the condition of an individual will be influenced both by its genes as well as by its past and current environment, quality indicators could signal either (1) genetic or (2) environmental quality variation, or both, among males (Iwasa and Pomiankowski 1999; Cotton et al. 2004). Females might benefit from choosing highly elaborated males either via good genes (if 1) or direct benefits (if 1 and 2). The genes and the environment can interact, for example, if high genetic quality individuals do well in all environments, while low genetic quality individuals pay disproportionately increasing marginal costs of ornament elaboration as environmental conditions deteriorate (Cotton et al. 2004). This gene-by-environment interaction could magnify the genetic differences between individuals (David et al. 2000). Thus, genetic variance in sexually selected traits is predicted to be highest in harsh environments, as these tend to reveal and amplify differences between individuals with respect to quality (David et al. 2000; Cotton et al. 2004).

There are several hypotheses that try to pinpoint when and how the cost of ornament elaboration is paid. This has lead to the division of costs into two main categories; developmental costs and maintenance costs. The developmental period is especially crucial, since some traits develop during a short time window and cannot be modified later in life. Examples in zebra finches include body size and song structure.
This lead Nowicki et al. (1998) to suggest a mechanism for the maintenance of the honesty of signals: the developmental stress hypothesis. If males with more elaborate ornaments have fared better in the face of early stress, ornaments can signal past condition of the male. If the condition dependence is genetically mediated, ornaments could signal both genetic and phenotypic quality. A female choosing a male with more elaborate ornaments will therefore get a mate in better condition. This male might provide direct benefits (e.g. parental care), and, at least under harsh environmental conditions (which might predominate in nature, Zann 1996) also indirect benefits. In this thesis, I take two approaches to investigate the condition dependence of sexual traits in the zebra finch. In chapter 1, I perform a rigorous test of the developmental stress hypothesis on a range of traits. In chapter 4, I test if song rate functions as an honest indicator of male quality, by relating male song rate to a proxy of fitness.

Measuring selection on quantitative traits

The majority of traits that are important in determining reproductive success are influenced by genes at many loci, i.e. they are quantitative traits (Falconer and Mackay 1996). The phenotype of an individual is composed of a genetic and an environmental component. Selection acts on the phenotypes of individuals, while an evolutionary response to selection requires genetic transmission to the following generations. Thus, evolution can be thought of in terms of gene frequency changes over generations. Therefore, to determine the potential for evolutionary change of a trait, it is necessary to disentangle the genetic from the environmental components of the trait. Only selection acting on the genetic component of a trait will lead to an evolutionary response (Kruuk

2004), while selection on the nonheritable environmental component of the phenotype will lead to no evolutionary response (Price et al. 1988; Rausher 1992). Recently, there has been a surge of studies looking at the strength and direction of selection and the potential for evolutionary change (Kruuk 2004; Kruuk et al. 2008). This has been made possible by the application of computationally intensive quantitative genetic methods that have been used for more than 50 years in animal breeding, where identification of genetically promising individuals is of great interest for the process of artificial selection, for example for increased milk yield in cows. Only relatively recently have these methods entered the field of evolutionary research.

These methods use pedigree information to disentangle the variation in traits into the underlying genetic and environmental components. The additive genetic component of a trait can be estimated on a population level, this is the heritability of the trait. Thus, the response to selection on a population level is given by the product of heritability and the strength of selection (the breeders equation, Falconer and Mackay 1996). However, to predict individual responses to selection, we need an estimation of the additive genetic component on an individual level. To do this, we can estimate the so-called breeding value of an individual, which is the expected phenotype of an individual relative to the population mean. If an individual is mated at random to a number of individuals in the population, the breeding value is equal to twice the mean deviation of the progeny from the population mean (Falconer and Mackay 1996, Kruuk 2004). By measuring the phenotypes of many relatives of the individual and weighting them according to relatedness, it is possible to estimate the breeding value for a trait. Hence, selection pressures acting not only on the phenotype, but also directly on the genetic and the environmental components of a trait can be quantified and thus it is possible to make predictions about evolutionary change. In this thesis, I take this approach to study patterns of selection acting on key traits in male zebra finches (chapter 3).

Measuring fitness consequences

An near ideal measure of the fitness of an individual would be the number of grandchildren produced, as this takes into account not only the lifetime reproductive success of an individual, but also the differences, due to sexual selection, in mating success of sons and fecundity of daughters (Kokko et al. 2003; Hunt et al. 2004). Since this is something that is difficult to measure directly, the common approach is to use proxies for fitness, which will correspond more or less closely to the actual fitness of the individual. In this thesis, I use genetic parentage of eggs under aviary breeding conditions as a proxy for fitness (chapters 3-4). This provides a good estimation of fitness when the focus is on indirect benefits of female choice, since direct benefits (parental care) are controlled for by random cross-fostering of eggs to a separate set of foster parents, that reared the offspring under standard cage conditions.

It might be argued that the laboratory environment is ill-suited for the study of fitness consequences, and that only studies in the wild can measure fitness consequences under realistic and relevant conditions. However, the type of question we want to answer, will decide whether it is best addressed in the lab or the wild. Questions relating to natural selection will usually be better investigated in the wild, where natural selection pressures (which are arguably the strongest selective pressures acting on populations in the wild Zann 1996) are important and fluctuating. In contrast, in the laboratory, the forces of natural selection are minimised, by standardising as many aspects of the environment as possible (food access, predation, parasitism, etc). Thus, since natural selection pressures have been largely relaxed in the lab, it is suitable for addressing questions pertaining to sexual selection. These include such issues as competition for mates, mutual mate choice, extra-pair paternity, mate guarding and sperm competition. In the wild, on the contrary, disentangling the forces of sexual selection from those of natural selection might be more difficult. Another important consideration is how one aims to answer the questions posed. The laboratory and the wild offer very different opportunities regarding reliable parentage assignment, achievable sample sizes, and the opportunity to manipulate specific aspects of the biotic and abiotic environment.

Wild and domestic zebra finches differ in one important feature in this context: the level of extra-pair paternity is much higher in the laboratory (22-34% of eggs in chapter 3-4, depending on the definition of EPP; see also Burley et al. 1996) than in the wild (2.4% of offspring: Birkhead et al. 1990). Levels of EPP are generally not highly conserved within genera, but can fluctuate considerably also among closely related species (for example in the Australian fairy wrens, Rowe and Pruett-Jones 2006). Thus, a rapid change in EPP-levels when ecological conditions change considerably (from the wild to the laboratory) might not be very surprising.

Outline of thesis

From the above introduction, it becomes clear that if one aims to obtain a relatively complete picture over how sexual selection acts in a species, it is necessary to use an integrative approach, to look at different aspects of reproductive success, to determine the signalling value of ornaments and to measure not just the phenotypes of individuals, but also estimate the underlying genetic and environmental components of traits. This provided the starting point for my thesis. The zebra finch is a model organism in studies of sexual selection in monogamous species, yet, many fundamental questions remain unanswered. The availability of a large laboratory population of zebra finches spanning several generations and the possibility of genetic paternity assignment has allowed me to rigorously test questions of key importance, not only for studies of zebra finches, but also for the field of sexual selection in general.

Figure 1 schematically illustrates the framework of this thesis, by outlining the routes to reproductive success in zebra finches. While the main focus of the diagram is on male reproductive success, some of the connections are also applicable to females (mainly the connections marked 1 and 2). Some parts of the diagram have received considerable research attention (e.g. the connection between environment, condition and ornaments, and the connection between specific ornaments and intersexual selection), while others remain virtually unaddressed. By and large, an integrative approach has been missing, and few studies have attempted to perform a rigorous investigation of how male zebra finches achieve reproductive success.

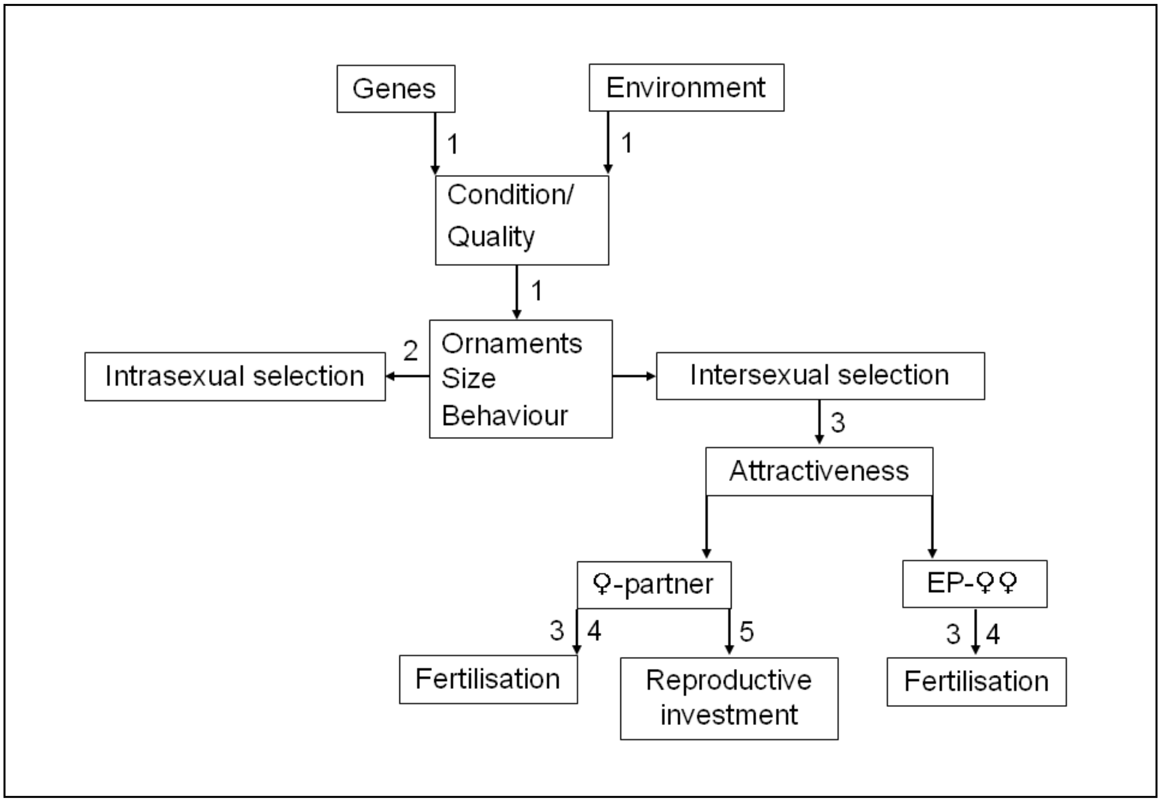

Figure 1.
A schematic overview of the routes to reproductive success in zebra finches. Numbers indicate which part of the diagram is addressed by each chapter of the thesis.

Thus, in this thesis, I set out to investigate the importance of the connections outlined in figure 1 and test the condition dependence and fitness consequences of both ornaments and behaviour.

Condition dependence
In chapter 1, I test the developmental stress hypothesis, by looking at the effect of early environmental stress on a range of traits in both males and females. Thanks to our standard protocol of cross-fostering offspring and a quantitative genetic approach, I was able to isolate the environmental, excluding the genetic, contribution to early growth and test how it affects ornaments, size and behaviour.

Intrasexual competition
Chapter 2 addresses the potential intrasexual signalling function of ornaments and body size, in both sexes, by staging short competitive encounters in a sexual context.

Determinants of reproductive success
In chapter 3 and 4, I try to elucidate the main determinants of male reproductive success. I use data from an extensive breeding experiment under aviary conditions, and relate

male genetic paternity to ornaments and behaviour. To determine the possibility for evolutionary change, I compare selection acting on the genetic versus the environmental basis of three target traits; one ornament, one type of behaviour and body size.

Female investment into reproduction

Chapter 5 changes the focus from the stage of fertilising eggs, to what happens after fertilisation, since reproductive success could also be increased if the partner invests more into reproduction. Thus, in chapter 5, I quantify within-female changes in investment in relation to changes in partner genetic and parental quality obtained from the aviary breeding experiment (chapters 3-4). In chapter 4, I take a candidate trait approach in an effort to pinpoint the proximate mechanism of female reproductive stimulation. Thus, I relate changes in male partner undirected song rate to within-female changes in reproductive investment.

Study species: the lab rat with wings

Ecology

The zebra finch is a small, colonially breeding Australian Estrildid that forms life-long socially monogamous pair bonds (Zann 1996). The breeding range extends through most of the dry interior of the continent, excluding the wetter coastal areas. The zebra finch is an opportunistic breeder with several physiological and behavioural adaptations that allow breeding to be initiated immediately when weather conditions permit, that is, when the rain comes. For example, unlike most seasonally breeding species, zebra finches do not retract their testes, and pair bonds are maintained throughout the year. Offspring care is biparental, with males providing on average about 45% of the incubation and feeding of the young (Burley 1988 and unpublished data) The social monogamy and near even division of parental care implies that the variance in male and female reproductive success could be very similar. However, the zebra finch is markedly sexually dichromatic, hinting that sexual selection might be stronger in males. As mention above, extra-pair paternity occurs, and this offers a mechanism to increase variance in male, as compared to female, reproductive success.

Appearance

While the plumage of the female zebra is drab and grey-beige, it shares some of the more conspicuous characters of the male, namely the black and white stripes under the eyes, black tail coverts with white dots, and the orange-red beak. Still, the beak colour is markedly sexually dimorphic, with some overlap between the sexes (Burley & Coopersmith 1987). The colour, which is largely due to carotenoid deposition (Zann 1996; McGraw et al. 2002), ranges from orange to dark red in males and from light orange to light red in females. Males have a number of colourful plumage ornaments, based on black eu-melanins and chestnut coloured pheo-melanins: a black breast band, black and white stripes on the throat and chestnut-coloured flanks with white spots.

Song

Song is not used in territory defence in this species, and males sing throughout the year, also in the non-breeding season, regardless of their pairing status (Zann 1996). Song rate peaks during the fertile period of the partner (Dunn and Zann 1996a; chapter 4). The zebra finch is a close-ended learner with a short motif of about 0.8 seconds that is sung repeatedly. The song is highly stereotyped within, but greatly variable among, individuals. Females have been shown to recognise their partner based on song alone (Miller 1979), and auditory contact (songs and calls) alone is enough to sustain the pair bond also during prolonged periods of visual separation (Silcox and Evans 1982). In the wild, 70% of the total amount of song occurred within 1 meter of the nest and 88% while the female was inside the nest (Dunn and Zann 1996a). A distinction is usually made depending on the context in which the song is sung, between courtship song ('directed song' i.e. song directed at the female) and song sung in any non-courtship context ('undirected song') (Zann 1996). In this thesis, chapters 1 and 3 focus on directed song while chapter 4 focuses on undirected song. Song has been suggested to function in the attraction of social mates as well as extra-pair females (Dunn and Zann 1996b, a).

General methods

The data for chapters 3 and 4 (and the background data for chapter 5) were obtained from a large scale aviary breeding experiment. With the help of molecular parentage assignment, it was possible to obtain a precise measurement of male success at siring both within and extra-pair offspring. This set-up also allowed us to manipulate the operational sex ratio by adding birds to the standard 6 males and 6 females inhabiting each aviary. The hexagonal shape of the aviaries provided each pair with its own compartment with a nest box, while they had access to a common area in the centre of the aviary for flocking activities. An artificial tree in the centre of the aviary provided a much preferred stage for courtship since it allowed birds to hop in a circle (30% of all courtships and 80 % of successful courtships (i.e. resulting in copulations) took place on the tree). This allowed us to document within- and extra-pair courtship activities in great detail with a video surveillance system.

For the studies on female reproductive investment (chapter 4-5) and the study on intrasexual competition (chapter 2), I used a standard cage set-up. Individual cages measured 60*40*45 cm and could be combined to form longer cages (chapter 2).

Chapter 1

Effects of developmental stress on adult morphology, ornaments, behaviour and fitness in the zebra finch

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ABSTRACT

The developmental stress hypothesis offers a mechanism to maintain honesty of sexually selected traits, since only high genetic quality individuals will be able to fully develop ornaments in the face of early stress. The zebra finch has become a model species in studies of developmental stress. However, results have been mixed and a unifying perspective is missing. Using a quantitative genetic approach, we study the effect of the sometimes dramatic differences in early growth conditions experienced by a total of 413 cross-fostered male and 395 female zebra finches on 20 male and 14 female traits. We find moderate effects of growth conditions on body size, mass and fat deposition (mean r = 0.22), weak effects on beak colour (mean r = 0.10) and no effect on song or plumage characters. Looking at possible life-history trade-offs, we found a weak effect on the success at fertilising eggs under aviary conditions, in males, but no effects on longevity in either sex. We conclude that zebra finches are remarkably resilient to early developmental stress. Our results do not support the hypothesis that sexually selected traits show heightened condition dependence as compared to non-sexually selected traits.

INTRODUCTION

Current models of sexual selection assume that ornamental traits should be costly to develop or maintain to serve as honest indicators of quality (Zahavi 1975; Grafen 1990a; Getty 2006). This has lead to the wide-held assumption that condition dependence is a common feature of sexual traits (e.g. Johnstone 1995, Rowe and Houle 1996). Since most traits will exhibit some degree of condition dependence, a convincing demonstration of condition dependence of a sexual ornament requires that the relative condition dependence of sexual traits are compared to that of naturally selected traits (Hamilton and Zuk 1982; Zuk et al. 1990; Cotton et al. 2004). Quality indicators could signal either (1) genetic or (2) environmental quality variation among males (Iwasa and Pomiankowski 1999; Cotton et al. 2004). Thus, sexual trait condition dependence can evolve to signal male genetic or environmental quality, or both, and females might benefit from choosing elaborated males either via good genes (if 1) or direct benefits (if 1 and 2).

Environmental conditions during early development might be especially influential in shaping the development of sexual ornaments. This lead Nowicki et al. (1998) to suggest a mechanism for the maintenance of the honesty of signals: the developmental stress hypothesis. Stress during this crucial developmental period might have strong effects on the development of sexual ornaments such that males with more elaborate ornaments will have fared better in the face of early stress. Thus, ornaments can signal past condition of the male. If the condition dependence is genetically mediated, ornaments could signal both genetic and phenotypic quality. This could come about if high genetic quality individuals do well in all environments, while low genetic quality individuals pay disproportionably increasing marginal costs of ornament elaboration as environmental conditions deteriorate (Cotton et al. 2004). Such a gene-by-environment interaction could magnify the genetic differences between individuals (David et al. 2000). Thus, genetic variance in sexually selected traits is predicted to be highest in harsh environments, as these tend to reveal and amplify differences between individuals with respect to quality (David et al. 2000; Cotton et al. 2004; Hunt et al. 2004).

Thus, ornaments will reflect past condition and a female choosing a male with an elaborate ornament might receive direct benefits (e.g. parental care), and, at least under harsh environmental conditions (which might predominate in nature, Zann 1996) also indirect benefits. The ease with which environmental quality can be manipulated in the laboratory has lead to a surge of studies that have tested this hypothesis in a range of taxa and found effects on sexual ornaments (e.g. David et al. 2000; Nowicki et al. 2002; Ohlsson et al. 2002; Scheuber et al. 2003).

However, there also exists a line of evidence showing that individuals that grow up under poor conditions are often able to compensate for this when conditions improve (Metcalfe and Monaghan 2001). To be adaptive, this compensatory growth should bring potential fitness benefits that outweigh the potential long-term costs (Metcalfe and Monaghan 2001). For example, it might be beneficial to trade-off investment in favour of a sexual ornament if this leads to short-term fitness benefits (reproductive success) that outweigh long-term costs due to the reduced somatic maintenance (e.g. shortened life-span, Lindström et al. 2005).

Thus, studies are needed that look at life-time effects of early condition, not only on subsequent growth and development of secondary sexual characters, but also on life-history consequences.

The zebra finch has become a model species to study the effects of developmental stress. Typically, two or three treatment groups are created by manipulating the early environment (e.g. brood size or food access or quality) and then traits of interest are compared between the two groups in adulthood (see Table 1). Results are rarely consistent among studies (Table 1). Still, studies in the lab have found clear condition dependence of many traits, despite the relatively mild treatments that have been applied (mainly due to ethical considerations). Typically, the mean mass at the age of 8 days is about 10-15% less in the food stress treatment than in the control group (Spencer et al. 2003; Arnold et al. 2007). Zann and Cash (2007) reports an effect size of d = 1.06 for the difference between the treatment and control groups in mass at day 12 (explaining 22% of the total variation). This approach, however, does not make use of the variation within treatment groups, which is considerably larger than the treatment effect. As pointed out by Cotton et al. (2004), a range of stress levels is more likely to reflect biologically relevant conditions. In our population, where all offspring are cross-fostered at the egg stage within 1 day of laying, variation in growth is very substantial (Schielzeth et al. 2008). Offspring mass at day 8 varied between 1.9 and 12 grams (7.6 ± 1.7, mean ± SD, N = 808). Obviously, an approach that can make use of this considerable variation is preferable.

Here, we test the effects of this substantial variation in early growth conditions on a range of traits in a large sample (N = 808) of birds. The full cross-fostering and available pedigree information allows us to obtain a comprehensive measure of early growth that reflects only environmental and not genetic variation in growth. We measure both presumably naturally and presumably sexually selected traits in both sexes, so that the condition dependence of sexually selected traits can be compared to that of non-sexually selected traits, or the homologous trait in females (Cotton et al. 2004). To identify any life-history trade offs, we also look at reproductive success and longevity.

In this way, we hope to obtain a relatively complete picture of the potential for traits to honestly indicate quality (phenotypic and/or genetic) by reflecting past condition in the zebra finch. While we might expect most traits to exhibit some degree of condition dependence, the developmental stress hypothesis predicts that sexually selected traits (e.g. song rate, beak colour, plumage ornaments) should show heightened condition dependence in comparison to naturally selected traits. Further, if compensation occurs and is costly, we would expect effects on reproductive success and/or longevity. We expect the strongest effects on traits that develop during the actual period of stress (e.g. tarsus length, metabolic patterns), while we expect weaker effects on traits that develop after the period of stress has ended.

METHODS

Subjects and housing

We used 395 females and 413 male zebra finches from two consecutive generations (referred to as F1 and F2). Sample sizes for some measurements are reduced due to deaths. The F1 generation was held at the Max Planck Institute for Ornithology in Seewiesen, Germany, since October 2004. For details of rearing conditions for these birds, see Forstmeier (2005). The F2 generation was produced during the aviary experiments described below. For details of general housing conditions, see Bolund et al. (2007). Birds were randomly and individually cross-fostered between nests within 24 hours of egg-laying, ensuring that foster-siblings were unrelated to each other and to their foster parents. Whenever possible, the size of the foster clutch was matched to the size of the original clutch, and clutch sizes were kept within the natural range (2-6 eggs).

Measurements

The mass of all chicks was measured at day 8 after hatching to the nearest 0.1g (massD8). This measure formed the basis for our estimation of early growth conditions. However, we need to account for the genetic relatedness among individuals, which makes pseudoreplication an issue in the study design. Thus, to randomise genetic and parental effects, we used full cross-fostering. Hatch-order was randomised and hatch order effects on early growth statistically controlled for in any analyses. This design randomises the environmental influence on early growth and controls for genetic influences except, importantly, the influence of the individual's own genotype. To deal with this, we employ the 'animal model', which uses pedigree information to disentangle the genetic from the environmental contribution to mass at day 8 for each individual (Falconer and Mackay 1996; Lynch and Walsh 1998). The heritability of mass at day 8 was 15%, with the remaining 85% of the variation due to environmental influences. While the additive genetic component of an individual's phenotype cannot be estimated explicitly, it is possible to express the expected effect of the genes that an individual passes on to its offspring, the so-called breeding value of the individual (i.e. including both good-gene effects and selectively neutral alleles). The breeding value can thus be used to look for good gene effects on for example fitness. The residuals from a regression of the phenotypic mass at day 8 over the breeding value represent only the environmental contribution to mass at day8. This provides us with a comprehensive measure of the early growth conditions.

At nutritional independence (day 35), young were weighed again and transferred to peer groups of approximately same-aged birds. Peer groups differed in group size (2-75 birds) and sexes were either separated (F1 and F2), or mixed (F2). At approximately 100 days of age (106.5 ± 12.1 mean ± SD), we took a range of measurements on all birds:
1) Mass (to the nearest 0.1g)
2) Tarsus length (to the nearest 0.1 mm)
3) Wing length (to the nearest 0.5 mm)
4) Fat score (clavicular and abdominal fat, scored on a scale from 0-5, with 0.5 point increments)
5) Width of the breast band (in males, on a scale from 0-5, with 0.5 point increments)
6) Beak colour (scored according to the Munsell colour chip system, see Forstmeier and Birkhead 2004). In the F1 and F2 generations, beak colour was also scored with spectrophotometry to capture the UV-part of the spectrum. From the beak colour spectrograms, we extracted two measures that were used for further analyses: a PC1 of 6 spectral characteristics (described in Bolund et al. 2007) and the point were the reflectance curve reaches half its maximum height in the red part of the spectrum ("nmredhalf"). This measure reflects the 'redness' of the beak and is highly correlated with the Munsell scores (measures from the day 100 measuring sessions: $r = 0.97$, $p < 0.00001$, $N = 654$). All beak colour measures range from orange females-like (low values) to red male-like (high values) beaks.
7) Cheek patch colour, which ranges from orange to brown, was also measured with spectrophotometry (in males of the F2 generation only). To obtain one value for cheek colour, we used the percent reflectance at every 20nm over the relevant part of the spectrum (310-690 nm) and entered these into a principal component analysis. PC1 reflects mainly brightness and explained 78 % of the total variation. All entered points had strong positive loadings, and higher values of PC1 correspond to cheeks that were visually scored as more orange. PC2 explained a further 19 % of the variation. Points from the lower half of the spectrum loaded negatively on PC2, while points from the upper half of the spectrum loaded positively.
8) Song: we measured courtship song rate (also termed 'directed' song rate, Zann 1996) under standardised conditions as described in Forstmeier (2004). Female responsiveness to the male was measured in the same trials (Forstmeier 2004). The song of all males was recorded in a soundproof chamber (the male was presented with a female and the directed song recorded). We used sound analysis Pro version 2.063 (Tchernichovski et al. 2004) to extract three parameters that have frequently been used in zebra finch song research: the

length of the motif, the number of different elements in the motif, and the syllable rate (for details, see Forstmeier et al. in press.)

9) Male attractiveness and female choice behaviour: Male attractiveness was measured in a choice chamber set-up as described in Forstmeier and Birkhead (2004). We measured two aspects of female choice behaviour in the choice chamber trials: total number of hops, and a measure of the strength of the preference for a particular male (the deviation from a random time allocation among males) (for details of these measures, see Forstmeier 2005).

10) In the F1-generation, we measured survival over the first 1500 days of life (at this point, 140 out of 307 birds had died). We compared birds that died before this day versus birds that survived longer with a t-test. In the group that had died, we looked at the relationship between mass at day 8 and longevity.

11) To measure the fluctuating asymmetry (FA) of the tarsus, both tarsi were measured twice and the FA was estimated according to the mixed regression method described in Van Dongen et al. (1999).

Several of the measures were taken repeatedly over an individual's life in different 'measuring batches'. To obtain one measure per trait per individual, we used the random effect estimates from linear mixed effect models controlling for measuring batch as a fixed effect. Mean ± SD of number of measures for traits that were measured repeatedly: spectrometric beak colour measures during nonbreeding conditions (zebra finch beak colour in both sexes substantially declines over the breeding cycle, Burley et al. 1992): 1.8 ± 1.1, song rate 5.8 ± 2.0, female responsiveness 5.3 ± 2.6, male attractiveness in the choice chamber 4.1 ± 0.8, for female choice behaviour in the choice chamber, we used the first trial of a female's life only. Adult mass was measured on average 2.5 ± 2.3 times over the lifetime. Using only the mass at day 100 (and body condition calculated based on this mass) did not alter the results. Since birds were between 74 and 140 days old at the day 100 measures (106 ± 12, mean ± SD), and beak colour and cheek patch colour continue to change during this period, we also accounted for the precise age at measure for the day 100 measures by including age as a fixed effect in the model for these traits. Remaining traits measured at day 100 showed no influence of the precise age at measure, thus no age correction was applied.

As a measure of body condition, we used the residuals from a multiple regression of linearized adult mass (cube-root transformed) over tarsus and wing length.

12) Fitness consequences: groups of zebra finches bred in aviaries. Birds from the F1-generation were allowed to breed for periods of three months, in nine aviaries during two breeding rounds (2005 and 2006). Aviaries contained six males and six females, but three aviaries held an additional three females (sex ratio 0.4), and another three held an additional three males (sex ratio 0.6). Analyses control for this sex-ratio treatment. Pairs were exchanged among aviaries and sex-ratio treatments between years. We replaced dead birds so that in total 139 birds from the F1 generation were used. Birds from the F2-generation were allowed to breed in six aviaries in 2007 (for details, see Martin 2008). These aviaries had an even sex ratio of six males and sex females and 74 birds were used in total. The paternity of eggs or offspring in both studies was determined using 10 microsatellite loci (Forstmeier et al. 2007), and assigned to parents as described

elsewhere (Forstmeier et al. manuscript). Calculations are based on 2087 eggs. We measured male success as the total number of eggs fertilised and female success as the total number of eggs laid. We relate these measures of fitness to early growth (the environmental component and the breeding value of mass at day 8) with linear mixed effect models. Male attractiveness to females was measured by scoring female responsiveness to males in extra-pair courtships (N = 100 males, 2120 EP-courtships). This was based on 280h of early morning observation (using video surveillance) from the 2006 breeding season and 1827h of observation (video coverage of the complete first three weeks) from the 2007 season.

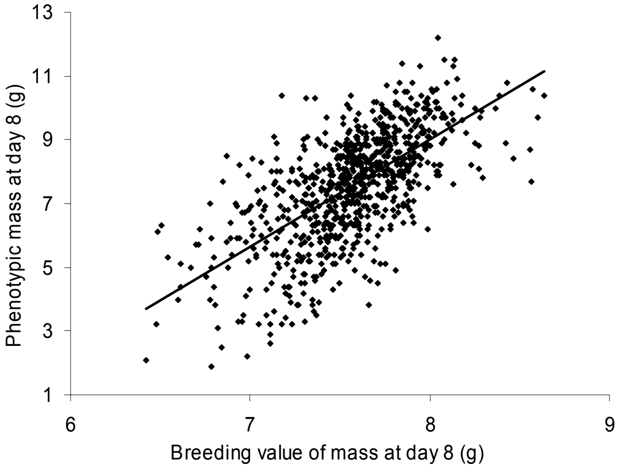

Figure 1.
Regression of the phenotypic value of mass at 8 days (mass8) on the breeding value of mass8. The breeding value is expressed in g by adding the population mean phenotypic value of mass at day 8 (7.6 g) to each value. Thus, the x-axis illustrates the genetically determined mass at day 8 that would be achieved under a standard environmental condition, while the y-axis illustrates the actual phenotypic mass at day 8 that was achieved under the realised environmental conditions of the individual. The residuals from the regression represent the environmental contribution to mass8 for each individual (env. mass8) and are used for analyses throughout.

Quantitative genetics

To obtain one value representing the environmental component of mass at day 8, excluding the genetic contribution, we took an animal model approach. For quantitative genetic analyses we used REML VCE 4.2.5 (Groeneveld 1998) to separate the phenotypic variance in mass at day 8 into additive genetic, maternal and foster environment effects. We used a pedigree including all genetic relationships of our focal birds and their direct ancestors; $N = 1221$ individuals. Phenotypic data of mass at day 8 was available for $N = 821$ individuals ($V_{\text{additive}} = 0.14 \pm 0.036$), divided into 355 clutches ($V_{\text{maternal}} = 0.12 \pm 0.03$) and raised in 300 broods ($V_{\text{foster}} = 0.21 \pm 0.028$). To account for hatch order effects, we entered hatch order as a fixed effect. Hatch order explained 6.3 % of the total variation, thus, the heritability of mass at day 8 (V_{additive}: the ratio of additive genetic variance to total phenotypic variance, Lynch and Walsh 1998) calculated after accounting for hatch order effects is slightly elevated (h^2 without accounting for hatch order effects = 13.5). Note that standard errors (but not variance component estimates) are slightly underestimated in this version of VCE. With the PEST software (Groeneveld et al. 1992), we then used the REML estimates of variance components to estimate best linear unbiased predictors (BLUPs) of the breeding value (the expected effect of the genes that the individual passes on to its offspring, Falconer and Mackay 1996) of mass at day 8 for each individual. The breeding values were used to calculate an environmental deviation for each individual (the residuals from a regression of individual phenotype on the breeding value, fig. 1). This environmental deviation represents only the environmental contribution to mass at day 8, and was used for further analyses.

Statistics

We used SPSS (SPSS for Windows, Rel. 15.0.1. Chicago: SPSS Inc.) and R 2.7.0 (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria) for statistical analyses. All statistical tests are two-tailed. Traits were transformed when necessary to approach normality. To obtain random effect estimates of traits for individuals and to look at the relationship between mass at day 8 and fitness, we used the lmer function from the lme4-package in R 2.7.0 (Bates et al. 2008). To calculate effect sizes we first calculated d based on means and standard deviations (σ) for the two treatment groups as: $d = M_1 - M_2 / \sigma_{pooled}$, where $\sigma_{pooled} = \sqrt{[(\sigma_1^2 + \sigma_2^2) / 2]}$. Using the pooled standard deviation accounts for differences in N between treatment groups. We then used the following formula to convert d to r: $r = d / \sqrt{(d^2 + 4)}$.

RESULTS

The very pronounced environmental differences in early growth (the residuals from Figure 1: ‘env. mass 8’) had moderate effects on tarsus length (Figure 2a), mass and fat storage over the life-time, in both sexes (Table 1). Fluctuating asymmetry of the tarsus was significantly affected only in males. Beak colour was weakly affected by early growth in both sexes, with individuals that were environmentally heavier as nestlings having more male-coloured beaks as adults (Figure 2b). Effects on the remaining traits were extremely weak and nonsignificant, in both sexes (Table 1). Environmental mass at day 8 (env. mass8) was weakly positively correlated with our measure of fitness (total fertilisation success of eggs) under aviary conditions in males (Table 1, Figure 3a) (lmer accounting for sex ratio treatment: $b = 0.27 \pm 0.13$, $P = 0.03$, $N = 103$). In females, there was a trend in the same direction, so that env. mass8 was positively correlated with number of eggs laid (Table 1, Figure 3a, $b = 0.12 \pm 0.07$, $P = 0.08$, $N = 107$). The breeding value for mass at day 8 was not related to fitness in either sex (males: $b = 0.10 \pm 0.11$, $P = 0.34$, $N = 103$, females: $b = -0.09 \pm 0.06$, $P = 0.15$, $N = 107$).

Birds that survived longer than 1500 days did not have a higher env. mass8 than birds that died before this age (males: $t = 0.99$, $df = 163$, $P = 0.32$, females: $t = -0.13$, $df = 140$, $P = 0.90$). Within the group of birds that died before 1500 days of age, there was no correlation between env. mass8 and longevity (Table 1, Figure 3b).

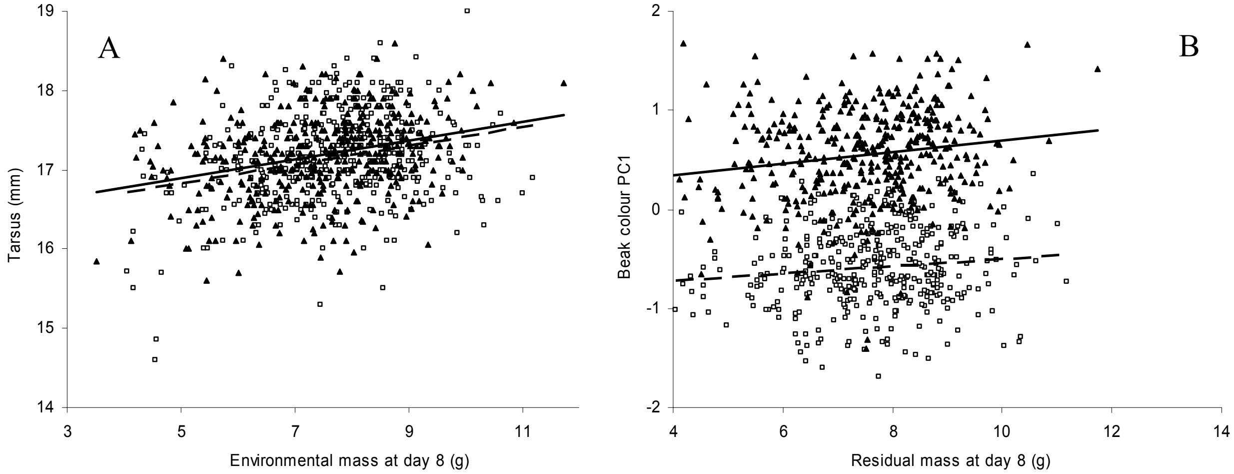

Figure 2
(a) The correlative relationship between environmental mass at day 8 (env. mass8, expressed in g) and adult tarsus length in males (filled triangles, solid regression line) and females (open squares, dashed regression line). (b) The relationship between env. mass8 and adult beak colour in males and females. Beak colour was measured with photospectrometry. The env. mass8 is expressed in grams by adding the population mean phenotypic value of mass at day 8 (7.6g) to each value.

Table 1.
Correlations between the environmental component of mass at day 8 (env. mass8) and a range of traits in male and female zebra finches. For details of traits investigated, see text.

	Males			Females		
Trait	*r*	*p*	*N*	*r*	*p*	*N*
Adult mass	0.31	<0.0001	409	0.27	<0.0001	392
Adult tarsus	0.3	<0.0001	413	0.26	<0.0001	395
Adult wing	0.16	0.001	413	0.13	0.012	395
Adult condition	0.2	<0.0001	409	0.2	<0.0001	392
Adult fat score	0.18	<0.001	409	0.15	0.002	391
Fluctuating asymmetry tarsus	0.14	0.0045	383	0.081	0.12	370
Beak colour, PC1	0.14	0.0047	408	0.11	0.030	388
Beak colour, red reflectance	0.098	0.048	408	0.10	0.041	388
Beak colour, Munsell score	0.10	0.044	409	0.054	0.29	392
Cheek colour, PC1	-0.039	0.55	241			
Cheek colour, PC2	0.024	0.71	241			
Breast band size	-0.0083	0.87	408			
Song rate	0.019	0.71	400			
Responsiveness to males				-0.082	0.11	389
Repertoire size	0.017	0.75	358			
Motif duration	-0.027	0.62	358			
Syllable rate	-0.072	0.17	358			
Extra-pair attractiveness	-0.031	0.76	99			
Choice chamber attractiveness	0.014	0.79	393			
Choice chamber hops				-0.013	0.8	374
Choice chamber pref. strength				-0.017	0.74	374
Longevity	-0.12	0.33	67	0.042	0.72	79

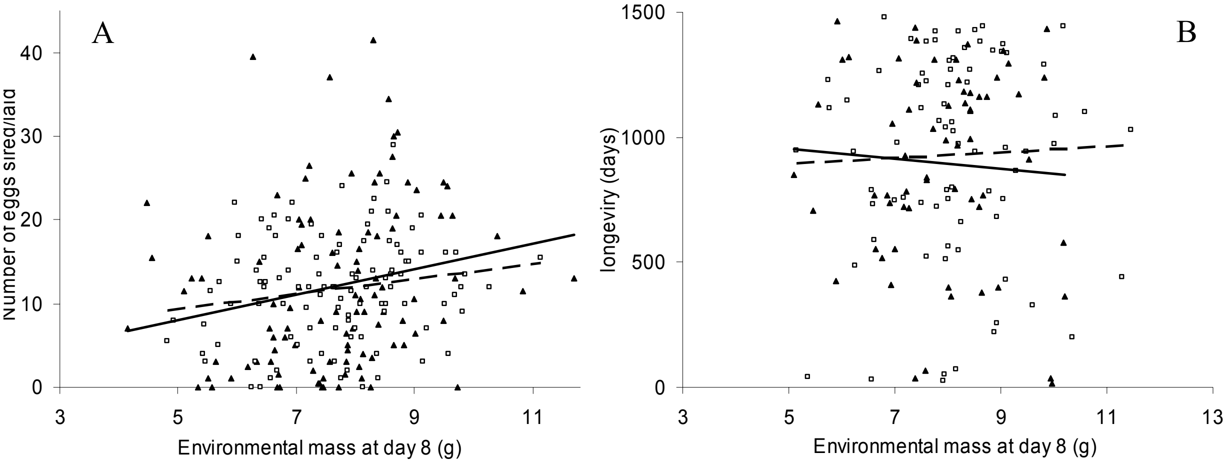

Figure 3.
The correlative relationship between environmental mass at day 8 (env. mass8, expressed in g) and 'fitness' (a) and longevity (b). Fitness under aviary conditions was significantly related to env. mass8 in males (number of eggs fertilised, filled triangles, solid regression line), but not quite significantly in females (number of eggs laid, open squares, dashed regression line). Raw counts of egg numbers are shown, counts for individuals that participated in two breeding rounds (F1-generation) are represented by one average value. Longevity during the first 1500 days after hatch was not related to env. mass8 in either sex.

DISCUSSION

Previous studies on developmental stress in zebra finches have found mixed effects of manipulated early growth conditions on adult traits. These studies have all used a relatively benign treatment. We instead made use of the considerable variation in early growth present in our population, where some of the birds are severely undernourished (Figure 1), but still survive to adulthood. Remarkably, these extreme differences in early growth had very modest lasting effects on a broad range of traits and behaviours in adulthood. We also found no evidence of life-history trade-offs, thus, it seems zebra finches can compensate for stressful early growth conditions without carrying long-term costs. In this discussion, we will explore our results in relation to previous studies and suggest alternative functions of the non-condition dependent traits.

Effects of developmental stress

We found a moderate effect on size, mass and fat deposition with a mean *r* of 0.22. Since zebra finches, like most altricial bird species, grow to full structural size in the nest before fledging (Ricklefs 1969), this could explain these lasting effects, while traits that are developed mainly after independence (e.g. song structure, plumage ornaments), can develop normally when environmental conditions are more favourable.

There was a weak effect on fertilisation success under free-flying aviary conditions, primarily in males. It seems possible that this effect is mediated by the size differences between birds, since we have indications that larger males are more successful under these conditions (Bolund et al. 2007; Bolund et al. in prep.)

Interestingly, the beak colour showed lasting effects in both sexes. The effect was similar in males and females, thus, it did not show heightened condition dependence in males as compared to females. This finding contrasts with an earlier study which found opposing selection for beak colour in the two sexes, with redder males, but more yellow females, being more successful in aviaries (Price and Burley 1994). Our results do not indicate such a sexually antagonistic selection, since females with redder (not more yellow) beaks were in better condition. The beak colour is a dynamic trait that is dependent on continuous new input of carotenoids into the beak. Hence, our results indicate that the carotenoid metabolism may be primed by early growth experiences. Carotenoids are transported in the plasma exclusively by lipoproteins (Parker 1996). Thus, it seems possible that the decreased beak colour is connected to the effects on fat deposition that we found. Studies on zebra finches have shown that testosterone is involved in the regulation of lipoprotein levels in the bloodstream (McGraw et al. 2006), thus providing a possible mechanism that could regulate both fat and carotenoid metabolism.

We found no effect of developmental stress neither on other traits that are commonly considered as ornaments, namely plumage traits, song structure and song rate, nor on male attractiveness to females and on female mate choice behaviours. Male attractiveness was measured both in a choice chamber set-up and in a socially complex setting in aviaries. This strongly indicates that stressful early growth conditions do not affect traits that are important for female choice.

A survey of previous studies on zebra finches shows that effects on morphology often persist into adulthood, while effects on other traits are rarely consistent among studies (Table 2). Importantly, only traits that were also investigated in the current study are included in the table. Hence, several of the studies looked at additional traits not shown here. Still, few studies have looked simultaneously at a broad range of traits. For example, a number of studies do not report if the treatment effect on size persists into adulthood, despite the ease with which this can be measured. This indicates a possible publication bias in favour of positive effects, which could be part of the reason why our results contrast with those of earlier studies. We note that comparisons between studies would be greatly facilitated if effect sizes would be routinely reported (see Nakagawa and Cuthill 2007, this was done only in 1 of the 15 studies in Table 2).

Table 2.
Overview of zebra finch studies relating early growth conditions to the same adult traits that are investigated in the present study. When possible, effect sizes (r-values) are given. Effect sizes were either obtained directly or, in most cases, estimated from figures in the original publications. Significant effects are marked in bold and the direction of the effect is indicated with + and – . For studies where an estimation of effect sizes was not possible, the effect is indicated only with + and -, cases where the direction of the effect was not indicated in the original study, is denoted by 0.

Trait	Direction and strength of effects											
Mass	+	.	**+**	.	0	**+0.24**	**+0.44**	**+0.45**	+	+	+0.43	**+0.29**
Tarsus		.	**+**	**+0.42**	.	**+0.24**[5]	**+0.57**	**+0.45**	+	**+0.37**	.	**+0.28**
Wing	.	.	**+**	.	**+0.66**[6]	.	**+0.47**	.	+	**+0.58**[1]	.	**+0.15**
Condition	.	.	**+**	.	.	.	.	**+0.12**	.	.	.	**+0.2**
Beak colour	.	.	.	.	.	0	.	**+0.33**	-	+	-	+0.077
Attractiveness	**+**	.	+	.	.	.	.	**+**	+	.	.	+0.01
Song rate	0	.	0	.	.	**-0.32**	.	**+0.32**	.	.	-	+0.02
Syllable rate	.	**+0.4**	.	**-**	.	.	.	.	.	.	.	-0.07
Repertoire size	+	0	0	+	0	.	.	.	.	.	.	+0.02
Motif duration	**+0.24**	**-0.36**	0	+	0	.	.	.	.	.	.	-0.03
Longevity	.	.	.	.	.	.	.	+[2]	.	.	**+**[3]	-0.04
Type of stress	Food access	Food access	Brood size	Brood size	Food access	Brood size	Food quality	Brood size	Food quality	Food quality	Food quality	Natural variation
Approximate duration	D 5-30	D 0-30	D 2-35	D 0-35	D 5-30	D 0-35	D 0-100	D 0-50	D 0-15	D 0-15	D 0-35	D 0-35
Study	Spencer et al. 2003; Spencer et al. 2005	Zann and Cash 2007	Naguib et al. 2004; Gil et al. 2006; Naguib et al. 2008	Holveck and Riebel 2007	Brumm et al. 2009	Tschirren et al. 2009[4]	Boag 1987	de Kogel and Prijs 1996; de Kogel 1997	Blount et al. 2003	Arnold et al. 2007	Birkhead et al. 1999	This study

Footnotes:

1: wing: effect in females only
2: survival: effect only in females, Beak colour: measured in males only
3: survival: measured in males only
4: effects averaged for wild and domesticated birds
5: body size PC1
6: wing: effect in males only

A life-history perspective

Poor early environmental conditions can be compensated for by accelerated growth when conditions improve (reviewed in Arendt 1997). In our, as in most laboratory studies, food was available ad libitum after independence from the parents at day 35 (Table 2). Compensatory growth could be costly, leading to life history trade-offs (Metcalfe and Monaghan 2001; Lindström et al. 2005). We would then expect to find effects on longevity. However, longevity was not affected in this study. Considered together with the low effect on fitness, this indicates that individuals seem to be able to compensate for poor growth conditions without paying long-term costs. Thus, it seems possible that the lab environment might offer ample opportunities to fully compensate in the development of most traits. We can only speculate about the effects of longer periods of stress. It seems likely that extended nutritional stress during the entire period until sexual maturity would have more pronounced effects on development.

Of the two previous studies that have looked at effects on longevity in zebra finches (Table 2), one study found a strong effect on survival until day 500 in males (Birkhead et al. 1999) while the other found a weak effect on survival during the first year of life in females, but not in males (de Kogel 1997). Both these studies also found a significant effect on tarsus in adulthood. Thus, it is possible that the effect on longevity might be mediated by differences in size, if size affects dominance relationships (Bolund et al. 2007) or food access under relatively crowded aviary conditions. In the wild situation, where crowding is not a concern, this effect might be absent. In general, predictions about the costs and benefits of relative size in the wild are difficult to make and would be an interesting venue for future studies.

Studies on developmental stress measure the effects of the treatment on a subset of individuals, namely those that survive to adulthood. Thus, a non-random mortality during the nestling phase would bias the results. For example, in a nest with little food, low genetic quality individuals with a low metabolic efficiency are likely to starve to death, while high genetic quality individuals with a high metabolic efficiency will survive despite the food shortage. Due to the bad conditions, the surviving offspring would still have a low mass at day 8. At adulthood, the high genetic quality individuals might have a dark beak due to their efficient metabolism. This will weaken the relationship between early mass and adult beak colour, since the high genetic quality of the surviving offspring obscure the effect of the early growth conditions. In a well-fed nest, both high and low genetic quality offspring can survive and reach a high mass at day 8. This, again, would obscure the relationship between early growth conditions and adult traits. While we cannot account for the effects of biased mortality in the current study, the effects of an individual's own genes on the growth rate is accounted for by using only the environmental component of mass at day 8. This approach also allowed us to look at only the genetic component (the breeding value) of mass at day 8. This was not related to fitness. Hence, individuals carrying genes for a high mass at day 8 were not more successful, i.e. genes for fast growth do not equate good genes. In general, the genetic basis of condition dependence remains poorly understood and is a promising venue for future studies (see Cotton et al. 2004).

Exploring the alternatives

Our results fit well with a recent review that found that there is surprisingly little evidence for heightened condition dependence of sexually selected traits (Cotton et al. 2004). This review emphasised the importance of comparing sexually selected traits to non-sexually selected traits, since sexually selected traits are expected to show heightened condition dependence. While we found that the environmental conditions during growth had effects on traits commonly used as indices of condition (body size, mass, fat deposition), we found very low or no effects on presumably sexually selected traits, such as song, beak colour and plumage ornaments. This suggests that these might not function as honest signals of male quality. In the following, we suggest some alternative functions of these traits.

Burley and Symanski (1998) found that female zebra finches have a latent sensory bias for a novel ornament in the form of a white feather on the head. It seems plausible that the cheek patches, breast band and chestnut flanks could have evolved due to such a sensory bias. To test this, a promising approach would be to imprint female offspring on parents of colour strains lacking specific plumage ornaments and then reintroduce the ornament in a mate choice context in adulthood.

In another study, Burley et al. (1992) found that beak colour changes dramatically over the breeding cycle in both sexes. It thus seems likely that the beak colour could function as a signal of breeding status in this colonially breeding species.

The developmental stress hypothesis was originally proposed as a mechanism to maintain the honesty of bird song a signal of quality (Nowicki et al. 1998). While previous studies on developmental stress in zebra finches have assumed that there is selection for increased song complexity, results have been mixed (Table 2) and our study indicates that song does not reflect past condition. The low complexity of the zebra finch song (the length of one motif, which includes the full repertoire, is approximately 0.8 s) compared to many other song bird species, indicates that song structure is unlikely to reflect developmental or production constraints. Miller (1979) found that females can recognize their partner based on song alone. It therefore seems likely that individual recognition is an important function of song.

While the structure of the song is fixed for life after song crystallisation at around 90 days of age (Zann 1996), the rate is flexible and could potentially reflect current, rather than past, condition. However, we have no indications from our population that this is the case (data not shown). Importantly, the song rate reported here, and in other studies on developmental stress in the zebra finch (Table 2) refers to the song sung during courtship to a female ('directed song', Zann 1996). This amounts to about 8 minutes of song per day (in the aviary experiment described in the methods, data not shown). Thus, an energy constraint is hard to imagine. We therefore suggest that the rate of directed song likely reflects differences in male strategy rather than in male quality (Forstmeier 2004; Forstmeier 2007; Bolund et al. in prep.).

Conclusions

In conclusion, our results indicate that zebra finches are remarkably resilient to early developmental stress. We found lasting effects mainly on traits that develop during the actual period of stress, while traits developing later were not affected, with the exception of a very small effect on beak colour. Thus, it seems that zebra finches are largely able to compensate for early environmental stress, with very little costs in terms of fitness consequences or longevity. Our results indicate that the developmental stress hypothesis will not apply to all traits that appear to be sexually selected characters. Further, they illustrate the necessity of comparing the condition dependence among different traits and between the sexes.

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Chapter 2

Intrasexual competition in zebra finches, the role of beak colour and body size

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ABSTRACT

Colourful ornaments are typically thought to be selected through female choice (intersexual selection), but often they also function as signals of dominance in intrasexual competition. The zebra finch (*Taeniopygia guttata*) is a well-studied model species for the evolution of colourful ornaments through female choice, and most research interest has focused on the red beak of males. However, results have been mixed, and in our population, we have found no female preference for males with redder beaks. To investigate whether beak colour is instead used as a signal in intrasexual competition, we studied aggressive behaviour of 160 male and also of 133 female zebra finches in experimentally staged encounters. Beak coloration was sexually dimorphic in the red and orange, as well as the ultraviolet (UV) part of the spectrum, where females showed a more pronounced UV-peak. There was a trend for females with less male-like UV-reflectance to be more aggressive, but otherwise, beak colour explained little, if any, of the variation in aggressiveness within sexes during competition for a potential partner. Hence beak colour is unlikely to be a signal of dominance, at least under our experimental conditions. Body size often influences the outcome of competition, and we found a weak but significant correlation between body size and aggressiveness that differed between the sexes. Large males were more aggressive than small males whereas small females were more aggressive than large females. Generally, the level of aggressiveness was highly repeatable within individuals, but beak colour and body size explained only little of the between-individual variation.

INTRODUCTION

Colourful male ornaments are generally thought to evolve through sexual selection. Female choice for males with more elaborate ornaments is usually invoked as the most plausible evolutionary mechanism, but intrasexual competition between males represents an, often overlooked, alternative mechanism (Andersson 1994).

Zebra finches are regarded as a prime example of the evolution of colourful ornamentation in monogamous birds. Male zebra finches have several colourful plumage ornaments, such as the orange cheek patches and the chestnut-coloured flanks, but the primary focus of research interest has been on the colouration of the beak (Collins and ten Cate 1996; Zann 1996). Zebra finch beak colour is sexually dimorphic, with some overlap between the sexes (Burley and Coopersmith 1987). The colour, which is largely due to carotenoid deposition (Zann 1996; McGraw et al. 2002), ranges from orange to dark red in males and from light orange to light red in females. Most research on zebra finch beak colour has focused on female mate choice (reviewed in Collins and ten Cate 1996; Blount et al. 2003). However, results have been mixed, and several studies, including our own, have found no female preference for males with redder beaks (Collins et al. 1994; Sullivan 1994; Weisman et al. 1994; Vos 1995; Balzer and Williams 1998; Forstmeier and Birkhead 2004).

Reviewing the literature on female mating preferences in relation to male beak colour, Collins and ten Cate (1996) suggested that beak colour might also be important in male-male interactions. However, this idea has received scant attention (Zann 1996), and has never been tested rigorously in zebra finches. This lack of information is problematic, since male-male competition represents an alternative mechanism to female choice for the evolution of elaborate ornaments. Indeed, there are several examples where colour signals are likely to have evolved through male-male competition, over access to females or resources necessary to attract females, rather than through female choice (e. g. Møller 1987; Mateos and Carranza 1997; Pärt and Qvarnström 1997; Senar 1999; Pryke et al. 2001; Gonzalez et al. 2002; Hein et al. 2003; Pryke and Andersson 2003. Shawcross and Slater (1984), studied red-billed queleas, *Quelea quelea*, which, like zebra finches, have a variable carotenoid-dependent red beak, and found that males with redder beaks were dominant over males with less red beaks.

The few studies focusing on same-sex interactions in zebra finches have not found any clear patterns. When males could choose to feed close to either of two caged males, they showed no aversion to feeding in the proximity of a male with a beak painted red compared to a male with a beak painted orange with nail varnish (Etman et al. 2001). In this experiment males were separated by mesh, which prevented any physical interactions. Similarly, Burley and Coopersmith (1987) found no preference of individuals to associate with either red- or orange-beaked individuals of the same sex in a comparable choice situation. Cuthill et al. (1997) found that, with red leg rings, males were dominant over light-green-ringed males when competing for access to a single food source. However, this dominance pattern was only in terms of displacement from the food source; there was no difference in overall feeding time or in aggressive pecking behaviour between red- and green-ringed males.

Ornamentation is only one of several characteristics that can be important in determining the outcome of aggressive interactions. Body size is usually thought to affect dominance (Parker 1974; Archer 1988). Larger individuals typically have a higher resource holding potential (RHP, Parker 1974) and therefore tend to win aggressive encounters with smaller individuals (e.g. Enquist and Leimar 1983; Petersen and Hardy 1996). However, under certain conditions smaller individuals can be expected to win. This can happen if individuals differ in how valuable a resource is to them, or in other words, if there is a value asymmetry (VA) between the contestants. The individual who is more motivated is then likely to win (Davies and Houston 1981; Krebs 1982; Johnsson and Forser 2002). Small individuals could be more aggressive than large individuals if the contested resource is more valuable to them, for example if they have fewer alternative options to obtain the scarce resource (the “desperado effect”, Grafen 1987). According to a recent model by Morrell et al. (2005), a similar outcome can be reached when resources are relatively abundant and hence competition is relaxed. In this situation, large individuals may benefit from avoiding a fight, while small individuals may be more desperate to secure the resources. Both the effects of RHP and VA are expected to act at the same time, but their relative importance may vary with the overall intensity of competition such that RHP is expected to be more important when competition is intense, and VA gains in importance as the intensity of competition decreases.

In this study, we use the zebra finch to investigate the roles of beak colour and body size during competition for a potential partner. Most studies on dominance tend to focus on interactions between males only. However, since females vary in beak colour and body size to a similar extent as males, these traits might play similar roles in both sexes. Since females most likely are the choosier sex in zebra finches (Clayton 1990; Zann 1996), we expect competition to be more intense in males than in females. This difference in the intensity of competition could influence the relative importance of differences in RHP vs. VA for the outcome of competition.

Zebra finches do not form stable dominance hierarchies (Evans 1970), so we stage short encounters where two same-sex individuals competed for access to the opposite sex. We assumed that dominance during these 5-min periods reflected general aggressiveness and hence ability to monopolize access to the opposite sex. The above-mentioned studies on intrasexual interactions in zebra finches were not in the context of competition for a partner. We therefore test, for the first time, whether short-term competition for a potential partner is related to the beak colour or body size in zebra finches.

Most previous studies have rated zebra finch beak colour with the use of colour charts (Burley and Coopersmith 1987; Houtman 1992; Collins et al. 1994; Sullivan 1994; Weisman et al. 1994; Vos 1995; de Kogel and Prijs 1996; Balzer and Williams 1998; Birkhead et al. 1998; Forstmeier and Birkhead 2004). This method, however, captures only part of the variation in beak colour as perceived by zebra finches. Since zebra finch beaks also reflect in the ultraviolet (UV) part of the spectrum (this study), which has not been detected by earlier studies using spectrophotometry (Bennett et al. 1996; Hunt et al. 1997; Hunt et al. 2001), we here present a detailed description of the reflectance of zebra finch beaks over a wide range of wavelengths, including UV radiation. We predicted that if beak colour is used as a signal in dominance interactions, the wavelengths where males differ the most from females are likely to convey this information, that is, individuals with more male-like beak colour should be dominant

METHODS

Subjects and housing

We used birds originating from a large captive population maintained at Sheffield University. They hatched between March and June 2004, were cross-fostered at the egg stage and reared by foster parents (for details, see Forstmeier 2005). After reaching independence (at 35 days of age), birds grew up in large, same-sex aviaries. In October 2004 they were transported to, and thereafter kept at, the Max Planck Institute for Ornithology in Seewiesen, Germany. For the current experiment we used the entire F1-generation of our population, consisting of 160 males and 133 females. Two months prior to the experiment, the birds were housed unisexually in groups of four, and during the experiment in unisexual groups of two, in cages measuring 60 × 40 cm and 45 cm high.

Rooms were maintained at a constant temperature of 24 ± 1ºC, with humidity ranging from 40 to 60%. The birds recieved a millet seed mixture, cuttlefish, grit and water ad libitum. The diet was supplemented once a week with salad and a multivitamin supplement. Rooms were illuminated by full spectrum fluorescent light (Osram Lumilux T5 FH 28W/860 Daylight) on a 14:10 h light:dark photoperiod.

Experiments were conducted during February-May 2005, and birds were a mean ± SD of 320 ± 42 days of age when they entered the experiment. For practical reasons we divided the birds into five subsets, each consisting of 32 males and 32 females. This required 27 females drawn from subsets 1 and 2 to be used again in subsets 4 and 5. We did this to keep the test conditions (number and sex ratio of birds maintained in the experimental room) constant for each of the five subsets. We decided that this double use was preferable to introducing 27 females from the parental generation into the experiment. In some of our descriptive analyses, which aim at yielding mean trait values or effect sizes rather than hypothesis testing, we treat these 27 double-used females as if they contributed 54 (2 × 27) independent data points. This pseudoreplication is relatively unproblematic when means or effect sizes are to be estimated, but might underestimate standard errors. In contrast, pseudoreplicates have to be excluded or statistically accounted for when testing hypotheses (calculating *P* values). To show both the overall effect sizes and the correct *P* values, we present tests both including and excluding the subsets where the 27 females were used for the second time.

Aggression trials

To study same-sex aggressive interactions, we conducted two separate, but analogous experiments for the two sexes. Male trials involved two focal males competing for a stimulus female and, in the female trials, two focal females competed for a stimulus male. We used three adjacent cages, forming a cage 180 cm long, initially divided by two opaque dividers. The two focal individuals were placed in the side cages and the opposite-sex stimulus bird in the middle cage. To identify individuals during trials, we randomly gave each focal bird a plastic leg ring (males: dark blue or orange, females: white or orange, rings from A. C. Hughes, Hampton Hill, Middlesex, U.K.). These colours are neutral with regard to aggressiveness for this population of birds (Forstmeier and Birkhead 2004, and unpublished data). The individual with the orange ring was always placed in the left cage. Trials, lasting 300 s, started when the opaque dividers between the three cages were removed, such that the cages were fully connected and the birds free to interact with each other. We recorded behaviours live with the Observer software (Noldus Inc., Wageningen, The Netherlands). Each trial was also recorded on video, to enable rescoring of trials when necessary. We recorded the durations of aggressive interactions between the two focal individuals using the following two categories (following Zann 1996): (1) beak fence, which is fencing with the beaks between two perched birds, and (2) chase, where one individual displaces the other, and subsequently chases, the displaced individual. Displacements not followed by a chase were scored as a chase of short duration (mean ± SD = 0.51 ± 0.22 s). In the male trials, we also scored the duration of song directed towards the female (since only males sing).

Within each subset of 32 males and 32 females, we conducted 16 male trials per day, on 4 consecutive days, so that each male was tested once per day and each female served twice (every other day) as a stimulus bird. On the fifth day, we recorded beak colour and weight (±0.1 g) of all individuals. The right tarsus of each bird had been measured (±0.1 mm) at the age of 35 days. Tarsus length provides a good estimation of body size, while the residuals from a linear regression of mass on tarsus are commonly used as a measure of condition (e.g. Ots et al. 1998). The female trials were then conducted on the following 4 days. Test order was randomised within subsets and days. We chose opponents at random, but ensured that nest mates (i.e. unrelated birds fostered into the same nest of rearing; see Forstmeier 2005), genetic full siblings and cage mates never met. Focal birds were colour-ringed the day before trials started, such that in each subset half the individuals had blue (males) or white (females) rings and the other half had orange rings. After2 days we exchanged ring colours in half the individuals to ensure opponents always had different colours. This procedure means that, within each sex, 80 focal birds had the same colour in all their four trials, 40 were always orange and 40 were always blue (males) or white (females), whereas 80 birds changed colour, 40 from orange to blue (males) or white (females) and 40 from blue (males) or white (females) to orange.

Data analysis of aggression trials

In each trial we summed the seconds of active beak fencing and the seconds of active chasing for each individual, where active means that the bird initiated the behaviour. Since chasing is considerably more aggressive than beak fencing, we chose a priori to weigh it by a factor of three relative to beak fencing, before summing the two behavioural categories, giving us a single composite measure that best seemed to reflect aggressiveness level, and hence dominance in this context. In response to comments from referees, we redid the analyses using different relative weightings of beak fence and chase. However, neither weighting both behaviours equally (1 to1) nor weighting chase by a factor of 10 (10 to 1) altered the conclusions. In this way, we obtained 640 (160 individuals, four tests each) aggressiveness scores for each sex, 262 of which (41%) were zeroes in males and 391 (61%) in females. This composite was transformed as $y' = (\ln(y + 1))^{0.5}$ to reduce deviation from normality (Kolmogorov-Smirnov test: males: $Z = 8.30$, $N = 640$, $P < 0.001$; females: $Z = 9.41$, $N = 640$, $P < 0.001$). Despite the significant result, caused by an excess of zeroes, upon graphical visual inspection, the data appeared roughly normal after transformation (Quinn and Keough 2003). However, the estimates of individual repeatability, based on these scores, should still be regarded cautiously. The individual means of the transformed aggressiveness score (averaging the four trials, which is the first four trials in double-used females) are closer to normality, especially in males (males: $Z = 1.12$ $N = 160$, $P = 0.11$; females: $Z = 2.34$, $N = 133$, $P < 0.001$). We use these individual means to test for associations of beak colour and body size with aggressiveness level. Since every individual was tested with four different opponents we feel that the influence of one individual on the total behavioural phenotype of another individual is rather small. Furthermore, since only active behaviour was counted, both individuals involved in a trial have an opportunity to obtain high scores in that trial. We therefore consider individual mean aggressiveness scores as independent data points in statistical tests.

In a different approach, we used trials, rather than individual means, as the statistical unit. In these analyses, we subtract, within every trial, the aggressiveness score of the orange-ringed individual from that of the other. Hence, a positive value means the blue-ringed (males) or white-ringed (females) individual was more aggressive, while a negative value means that the orange-ringed individual was more aggressive. Trials with no recorded aggression by any of the two focal birds were excluded, which means that the total sample size was reduced from 320 to 270 trials (84%) in males and to 206 trials (64%) in females. We used different transformations for males and females to optimise the fit to normality (Kolmogorov-Smirnov test: males, $y' = (\ln(y+1))^{1.6}$: $Z = 1.15$, $N = 270$, $P = 0.14$; females; $y' = \ln(y+1)$: $Z = 0.60$, $N = 206$, $P = 0.87$). To deal with the problem of pseudoreplication in the female trials, we used the used the first three subsets in the tests, excluding subsets 4 and 5, which contained double-used females. Thus, analyses of single test days are free of pseudoreplication in both sexes. To obtain the best estimate of the true effect size, we also pooled the 4 test days, and included all subsets in the female trials.

Colour of the leg ring did not influence individual aggressiveness scores in females. First, females that had white rings throughout did not differ from females that had orange rings throughout (Student's *t* test: $t_{68} = 0.61$, $P = 0.54$). Second, females that changed ring colour did not change their behaviour in a consistent way (repeated measures ANOVA: effect of ring colour: $F_{1,68} = 0.058$, $P = 0.81$; interaction of ring colour with order: $F_{1,68} = 0{,}03$, $P = 0.41$; analyses exclude the second tests for double-used females). However, males that wore a blue ring in all four trials were slightly (effect size of transformed values, $d = 0.47$), but significantly (*t* test: $t_{78} = 2.06$, $P = 0.042$) more aggressive than males that always wore orange. This result was not significant with a nonparametric test (Mann-Whitney *U* test: $U = 540.5$, $N_1 = N_2 = 40$, $P = 0.12$). Given the marginal *P* value and the previous evidence (Forstmeier and Birkhead 2004), the significant result might represent a type 1 error, since males who changed ring colour halfway through the experiment did not change their behaviour accordingly (repeated measures ANOVA: effect of ring colour: $F_{1,78} = 0.049$, $P = 0.53$; interaction of ring colour with order: $F_{1,78} = 0.01$, $P = 0.79$).

Spectrometric measurements

Previously, beak colour in this population has been measured with Munsell colour charts (Forstmeier and Birkhead 2004). To determine the agreement of this approach with the spectrometric measurements, all individuals were again given a beak colour score, by the same person as previously (W.F.), immediately before taking spectrometric measurements.

We used a hand-held spectrometer (Avantes, AvaSpec-2048, Eerbek, The Netherlands) with a deuterium-halogen light source (Avantes, Ava-Light-D(H)-S). The spectrometer was accidentally used with three different fibre-optic probes; however, all three probes were of the same brand (Avantes). A cylindrical plastic sheath (opening diameter 2.5 mm) was mounted on the probe to exclude ambient light and to standardize measuring distance (3 mm). The probe was held perpendicular to the surface of the beak, and five repeated measurements were taken from each individual on random parts of the upper mandible, avoiding areas infected by mites when necessary. Since reflectance varies considerably over the surface of the beak, five measurements provide a good overall representation of the beak colour. Reflectance intensity was recorded every 0.273 nm in the 260-820 nm spectral range. This amply spans the range of wavelengths visible to birds (320-700 nm). Reflectance was calculated relative to a WS-2 white standard (Avantes). Dark current and white standard reference measures were taken at regular intervals in order to minimise error associated with drift of the light source and sensor. The person holding the probe was blind to the computer screen showing the spectral curve.

Data analysis of beak colour measurements

Figure 1a shows the average reflectance curves of the beaks of the two sexes. To characterize each of the 1600 curves measured (5 × 320), we extracted from each the following eight parameters. (1) The average reflectance over the entire spectrum (brightness). (2) The wavelength where the reflectance peaked below 470 nm (Nm-UV-max). (3) The wavelength where the reflectance peaked above 470 nm (Nm-red-max). (4) The wavelength where half of the UV peak height was reached when going from the minimum of reflectance towards the local maximum (Nm-UV-half); and (5) similarly for the red peak (Nm-red-half), which has been referred to as hue or 'redness' by e. g. Pryke & Andersson (2003). We also used the reflectance at the three points of greatest sexual dimorphism (Fig. 1b). As these reflectances were highly correlated with overall brightness (range of r = 0.95-0.98), we used residuals from a regression on brightness, resulting in (6) residual reflectance at 358 nm (Res358), (7) residual reflectance at 570 nm (Res570), and (8) residual reflectance at 707 nm (Res707).

The five subsets of experimental birds differed significantly in reflectance spectra (subset explained an average of 23.7% of the variation in the eight beak colour parameters; median values of eight two-way ANOVAs with sex included as factor: $F_{4,314} = 33.0$, $P = 8*10^{-21}$). We used one fibre optic cable for subsets 1 and 2, a second one for subset 3 and a third one for subsets 4 and 5. Identity of the cable explained 93% of the above-mentioned differences between the five subsets of birds (i.e. 22.0% of the total variation; median of 8 ANOVAs: $F_{2,316} = 59.7$, $P = 2*10^{-20}$). In contrast, most colour parameters did not differ between subsets when the same cable had been used (explaining only 2.1% of the total variation; median of 16 ANOVAs, eight for cable 1 and eight for cable 3: $F_{1,125} = 3.7$, $P = 0.055$). Hence, we standardized all data by means of Z transformation to account for the unwanted discrepancy between cables: from each data point the respective cable mean was subtracted and the result divided by the standard deviation for that cable.

After this adjustment, most parameters were highly repeatable among the five measurements taken on each individual (Table 1). The five measurements were thus averaged for each individual. We obtained similar repeatabilities when we checked for consistency in the 27 females that were measured twice with a mean ± SD of 53 ± 9 days between sessions. In that case Nm-red-max and brightness were not significantly repeatable, probably because of low statistical power. We decided to exclude nm-red-max from all subsequent analyses for its low repeatability. However, despite low repeatability, we kept brightness as a parameter since it has received much attention in the literature.

Finally, to deal with the problem of type 1 error of multiple testing, we decided to extract one principal component from the six most repeatable colour parameters and to test first whether this one best descriptor of sex-related variation in beak colour was related to aggressiveness. The between-trait correlations in males were very similar to those in females ($N = 15$ correlation coefficients, $r^2 = 0.93$, Mantel Test: $P = 0.042$).
In a second step, we examined colour parameters singly in their relation to aggressiveness, with the appropriate Bonferroni correction.

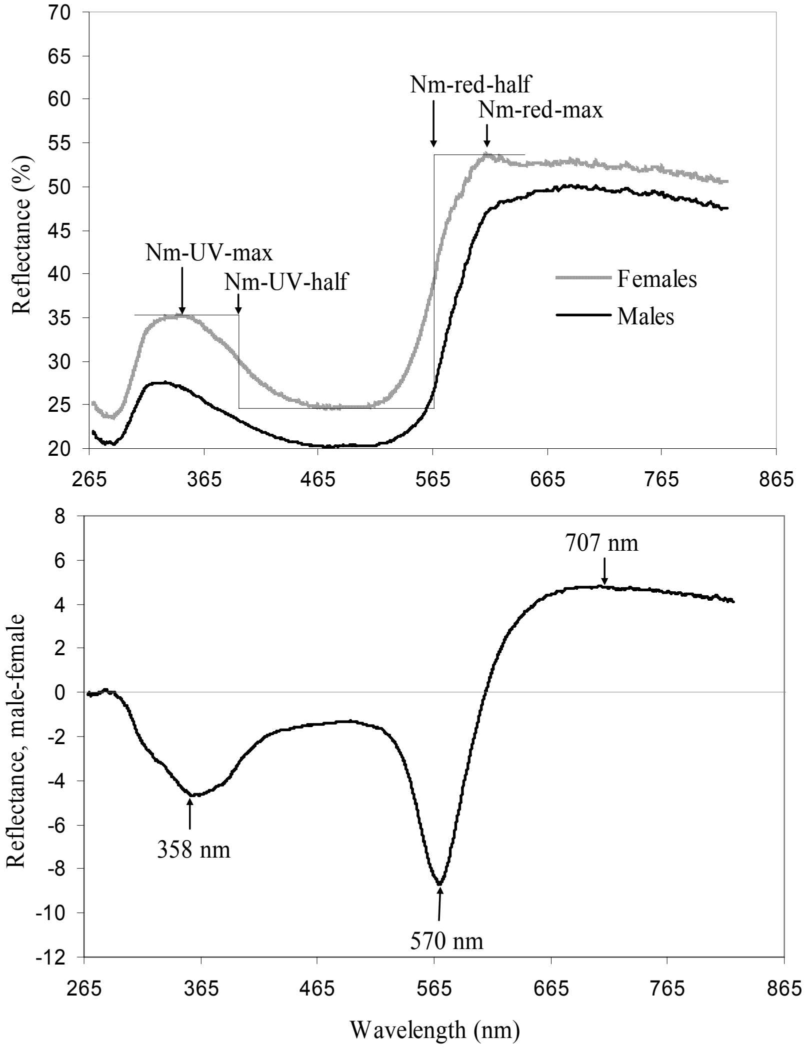

Figure 1.
(a) Average reflectance curves of the upper mandible of 160 male and 133 female zebra finches, based on 800 measurements on each sex. Curves were smoothed by averaging over a range of 10 measurements (spanning 2.73 nm of wavelength). Lines and arrows illustrate, on the example of the female curve, how the positions on the *X* axis of peaks and half peak-heights were defined (see text for definitions). (b) Sexual dimorphism in zebra finch beak colour. The *Y* axis shows the difference (male minus female) in percentage reflectance after adjusting for the difference in overall brightness. Arrows indicate the maxima in sexual dimorphism.

Ethical note

During the 5 min of a trial, aggressiveness was clearly more frequent, but not more severe, than under standard cage conditions. A male was chased on average 10 s during a male trial and a female was chased on average 1.7 s per female trial. In the worst case, one male was chased a total of 190 s during the entire experiment. This individual did not show any signs of stress other than those observed under standard cage conditions, where aggression can also occur. In general, we would have interrupted trials if the chased individual stopped fleeing or defending itself, but this never happened. Feather plucking was rare, an estimate of 10% of males and < 5% of females were plucked once during the course of the experiment. No adverse long-term effects were observed. None of the 293 focal birds died within 14 days of participating in the experiments, and 20 died during the following 12 months. This long-term mortality rate (0.068) was slightly lower than that of the previous generation for the same age period (0.077; $N = 222$).

Statistics

We used SPSS (SPSS Inc., Chicago, Il, U.S.A.) for all statistical analyses. All statistical tests are two-tailed and α was set to 0.05. All significance values are reported without Bonferroni correction unless stated otherwise. Repeatabilities were calculated following Lessells and Boag (1987). As a complement to the parametric statistics, we also used non-parametric statistics when this was possible. The results remained qualitatively unaffected for all but one test, the effect of ring colour on male aggressiveness, mentioned above.

RESULTS

Beak colour

Average reflectance curves (Fig. 1a) were trough-shaped with a minimum reflectance around 490 nm, a UV peak around 340 nm and a high plateau in the red part of the spectrum from about 600 nm. Female beaks were higher in overall brightness and were characterized by a narrower trough. The sexual dimorphism in the shape of the reflectance curves was clearer when we adjusted for the sex-difference in brightness (multiplying male values by 1.15) and subtracted female from male values (Fig. 1b). After this adjustment for brightness, it is clear that, relative to females, male beaks reflected the most in the red part of the spectrum (most extreme at 707 nm) and the least in the UV (358 nm) and orange (570 nm) parts.

Table 1.
Mean values, individual repeatability and sexual dimorphism of beak colour traits.

				Individual repeatability			
			Sexual dimorph.	Both sexes (within session)	Males (within session)	Females (within session)	Double-used females (between sessions)
Trait	Males	Females	*F*	*R*	*R*	*R*	*R*
PC1	0.77±0.53	-0.70±0.71	415.4 ***	0.899 ***	0.822***	0.710***	0.740 ***
Nm-UV-max	328.8±6.5	339.9±11.0	116.1 ***	0.633 ***	0.412***	0.594***	0.632 ***
Nm-Red-max	696.3±19.8	650.4±38.9	169.6 ***	0.462 ***	0.234***	0.345***	0.082
Nm-UV-half	384.5±6.6	393.9±7.0	119.7 ***	0.642 ***	0.441***	0.666***	0.691 ***
Nm-Red-half	581.5±5.5	565.5±5.4	627.3 ***	0.885 ***	0.626***	0.769***	0.591 ***
Res358	-0.16±0.15	0.13±0.20	202.6 ***	0.791 ***	0.549***	0.750***	0.501 **
Res570	-0.23±0.16	0.22±0.17	535.9 ***	0.876 ***	0.622***	0.776***	0.504 **
Res707	0.11±0.11	-0.09±0.11	221.0 ***	0.760 ***	0.547***	0.695***	0.378 *
Brightness	34.6±9.0	39.3±9.6	18.8 ***	0.383 ***	0.389***	0.338***	-0.232
Munsell	4.2±0.3	2.9±0.5	781.8 ***	-	-	-	0.720 ***
Df			1,291	319,1280	159,640	159,640	26,27

Means ± SD of all traits are shown for males (*N* = 160) and females (*N* = 133) separately. The degree of sexual dimorphism is measured as *F* values from ANOVAs with sex as the factor. Individual repeatability refers to the five measurements taken on each individual in quick succession. Between-session repeatability is for 27 females, five measurements averaged for each session, and with 53 ± 9 days between sessions. PC1: First principal component of the six most repeatable traits. The following four traits are the wavelengths (nm) at which the UV or Red reach their peak maximum (max) or half the peak height (half). Res358 refers to residual reflectance at 358 nm, controlling for overall brightness (Res570 and Res707 accordingly). Brightness refers to overall brightness (260-820nm). Munsell: Munsell-based beak colour score. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

The sexual dimorphism in brightness was much less pronounced (i.e. high variability within sexes) than the dimorphism in other parameters (Table 1). The six most repeatable parameters were entered into a principal component analysis. The first principal component (PC1) explained 78.2% of the total variation (factor loadings: Nm-UV-max: -0.85; Nm-UV-half: -0.85; Nm-red-half: 0.93; Res358: -0.88; Res570: -0.94; Res707: 0.85). These consistently strong factor loadings mean that PC1 is a good representative of each of the six parameters, and that all six parameters are strongly intercorrelated. The Kaiser-Meyer-Olkin Measure of sampling adequacy was 0.81 and Bartlett's test of sphericity was highly significant (approximated $\chi^2_{15} = 2308$, $P < 0.001$). Beaks with a high PC1-score reflect much red, but little UV and orange, and have a wide trough, i.e. they are male-like (Fig. 1). This PC score thus captures the variation in beak colour on an axis from female-like (negative values) to male-like (positive values) beaks. Across the two sexes, PC1 was not significantly correlated with brightness (Pearson correlation: $r_{291} = -0.09$, $P = 0.11$). However, within males, redder beaks were also brighter (males: $r_{158} = 0.21$, $P = 0.008$; females: $r_{131} = 0.11$, $P = 0.21$). PC1 was positively correlated with body mass in females but not in males (Table 2). Since females were on average 7.4% heavier than males the effect was not present across the two sexes. Correlations with tarsus length were generally weak. The Munsell-based beak colour score (Munsell) was best reflected by variation in Nm-red-half, but PC1 was an almost equally good predictor, particularly in females (Table 2).

Table 2.
Pearson correlation coefficients between pairs of traits.

	N	Tarsus: PC1	Body mass: PC1	Munsell: PC1	Munsell: Nm-red-half
Males	160	-0.071	0.101	0.435 ***	0.594 ***
Females	133	-0.012	0.382 ***	0.734 ***	0.742 ***
Both sexes	320	0.066	-0.045	0.866 ***	0.898 ***

Munsell: Munsell beak colour score; PC1: principal component of beak colour; Nm-red-half: wavelength where the red peak reaches half its height. Correlations with the two sexes pooled are based on 160 measures of each sex, to give the sexes equal weight. *** $P < 0.001$.

Intrasexual competition

The individual repeatability of the aggressiveness score of the four trials was high in both sexes (ANOVA: females: $F_{132.507} = 5.48$, $P < 0.001$, repeatability = 0.48; males: $F_{159,480} = 5.63$, $P < 0.001$, repeatability = 0.54).

Differences between the two focal individuals in beak colour and body size proved to be rather poor predictors of the outcome of pairwise encounters in both sexes (Table 3). PC1, which summarises most of the repeatable variation in beak colour, was not significantly correlated with the aggressiveness score. If we then considered the components of the beak colour separately, Nm-UV-half was the best predictor of aggressiveness (Table 3). Individuals with Nm-UV-half at longer wavelengths, i.e. with a more female-like beak in this part of the spectrum (Fig. 1), tended to be more aggressive, and this was consistent between the two sexes.

Differences in tarsus length between the two focal individuals were correlated with aggressiveness (Table 3), but differently in the two sexes. Large males were dominant to small males, whereas the opposite was found in females. Two additional measurements of body size, body mass and condition (residuals of a linear regression of mass on tarsus length), showed trends in the same direction as tarsus length.

To maximise the power of detecting any effects, we also performed correlations where the trials from all four days were included as if they were independent data points, which they are not since every individual participated in four (and some females in eight) encounters. This approach revealed that all effect sizes (of the transformed values) were smaller than $r = 0.20$ (Table 3). The separate analysis of each day of testing showed that these small effects were rarely consistent, which is expected because of the high sampling error when the sample size is limited. After Bonferroni correction for testing eight traits on each of 4 days, all correlations were nonsignificant, albeit this test is overly conservative, considering that both the tested traits and the test days are strongly intercorrelated.

Table 3.
Pearson correlation coefficients between pairwise differences in aggressiveness scores and differences in physical attributes of pairs of competing birds

	Males					Females					
Trait differences	Pooled, subsets 1-5 (270)	Day 1 (69)	Day 2 (73)	Day 3 (67)	Day 4 (61)	Pooled, subsets 1-3 (136)	Day 1 (29)	Day 2 (39)	Day 3 (34)	Day 4 (34)	Pooled, subsets 1-5 (206)
PC1	-0.05	-0.02	-0.04	-0.04	-0.11	-0.09	-0.27	-0.01	-0.08	-0.09	-0.12
Brightness	-0.03	0.22	0.00	-0.13	-0.13	0.00	0.22	-0.28	-0.12	-0.03	-0.01
Nm-UV-max	0.03	-0.11	0.02	0.00	0.19	0.09	0.38*	-0.03	0.03	0.04	0.08
Nm-UV-half	**0.13**	0.12	0.05	0.19	0.17	0.16	0.36*	0.08	0.16	0.15	**0.18**
Nm-Red-half	0.04	-0.17	0.01	0.20	0.05	-0.06	-0.24	0.09	-0.02	-0.07	-0.08
Res358	0.08	-0.04	0.04	0.19	0.12	-0.02	-0.01	-0.05	0.02	-0.02	0.05
Res570	-0.05	-0.08	0.00	-0.11	-0.01	0.07	0.20	0.01	0.07	0.07	0.10
Res707	-0.06	0.00	-0.09	-0.08	-0.06	-0.07	-0.04	-0.12	-0.08	-0.09	-0.11
Munsell	**0.13**	-0.01	0.18	0.23	0.11	-0.02	0.14	0.12	-0.03	-0.03	-0.06
Body mass	**0.12**	0.16	-0.09	0.34**	0.07	-0.06	-0.06	-0.20	-0.01	-0.15	-0.08
Tarsus length	**0.13**	0.19	0.17	0.21	-0.04	-0.15	0.07	-0.35*	-0.23	-0.24	**-0.18**
Residual mass	0.08	0.12	-0.16	0.29	0.07	-0.07	-0.09	-0.12	0.00	-0.09	-0.09

Positive values indicate that the individual with the higher trait value was dominant. Sample sizes (in parentheses) are number of trials, and these vary trials where no aggression was recorded were excluded. Each individual was tested on 4 consecutive days (days 1-4). For females, tests are based on subsets 1-3, to avoid double-used females. Pooled data for all subsets (1-5) are also showen. Abbreviations of beak colour traits are explained in Table 1. Residual mass is from a linear regression on tarsus length. For the pooled data, bold print highlights effect sizes that would be significant without Bonferroni correction if sample sizes were based on truly independent replicates. All correlations were non-significant after Bonferroni correction. Significance for single test days without Bonferroni correction: * $P < 0.05$; ** $P < 0.01$.

To deal with the problem of pseudoreplication, we used individual means of the aggressiveness scores, instead of differences between the two focal individuals in a trial. These aggressiveness scores reflect only active behaviour, i.e. they do not become negative when the focal individual is being chased. This approach yielded similar results. First, there was a considerable sex difference in the level of aggressiveness (ANOVA, $F_{1,292} = 40.63$, $P < 0.001$, Fig. 2a), with males on average being much more aggressive than females. Second, large males, as reflected by tarsus length, were more aggressive than small males (Pearson correlation: $r_{158} = 0.17$, $P = 0.03$), whereas small females were more aggressive than large females. ($r_{131} = -0.18$, $P = 0.037$; Fig. 2a). However, tarsus length explained only 2.8% of the variation in individual mean aggressiveness scores in males and 3.3% in females. Overall, there was a significant sex × tarsus length interaction, i.e. the slopes of the aggressiveness over tarsus length regressions differed significantly between the sexes (generalized linear model, GLM, $F_{1,289} = 8.8$, $P = 0.0033$).

The individual means of the aggressiveness scores were significantly correlated with Nm-UV-half in females, but not in males (males: $r_{158} = 0.067$, $P = 0.40$; females: $r_{131} = 0.18$, $P = 0.038$; Fig. 2b). Again, the position of Nm-UV-half explained only 3.2% of the variation in individual mean aggressiveness scores in females. The interaction between sex and Nm-UV-half was non-significant (GLM, $F_{1,289} = 0.37$, $P = 0.52$).

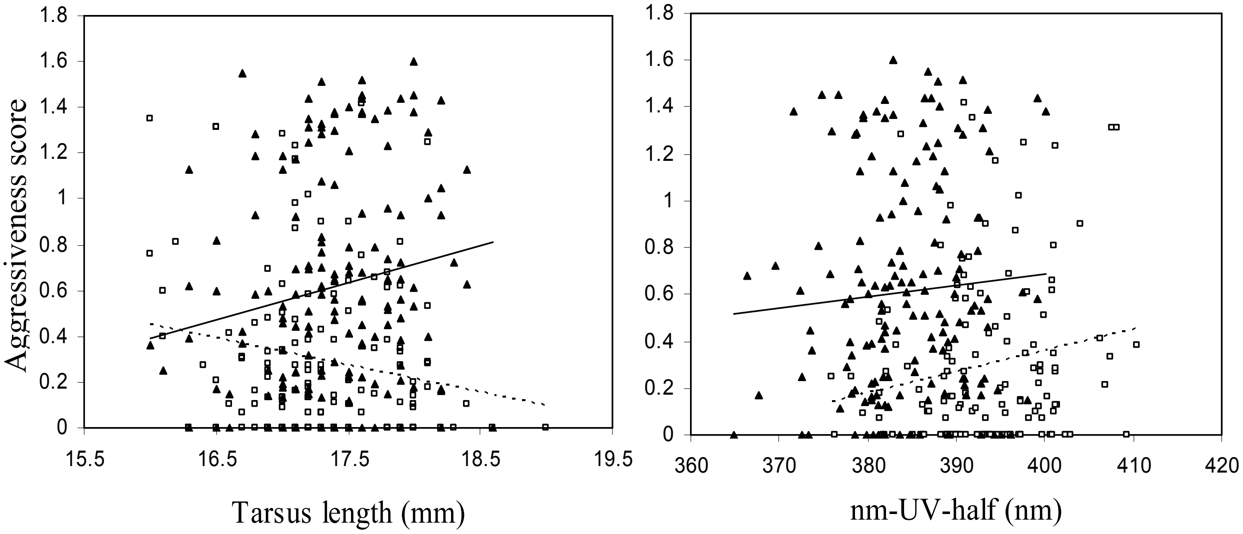

Figure 2. Individual means of aggressiveness scores in relation to individual (a) tarsus length and (b) position of the point Nm-UV-half. Separate regression lines are indicated for the two sexes (Males: black triangles and solid regression line, females: open squares and hatched regression line). A positive slope means that the (a) larger, or (b) more female-like coloured individual was more aggressive. Each data point represents one individual and only the first use of the double-used females is included. Of 160 males, 21 showed no aggression, i.e., they are zeroes in the graph, and of 133 females, 38 were zeroes.

DISCUSSION

UV reflectance of beaks

Reflectance spectra of the beaks of zebra finches in our population showed a clear peak in the UV part of the spectrum. This is in contrast to earlier studies, which have found very low UV reflectance of zebra finch beaks (Bennett et al. 1996; Hunt et al. 1997; Hunt et al. 2001). The difference in results might be because these studies measured only males and, in our population, males have, on average, lower UV peaks than females (Fig 1a), with some males showing curves similar to those in the above-mentioned studies. Generally, it is likely that the reflectance of the beak is determined largely by the interplay between the carotenoids and the underlying structural tissue (keratin; Shawkey and Hill 2005). The possible mechanisms of this interaction are not well understood, but it is plausible that a greater deposition of carotenoids in the beaks of males than in those of females is causing the reduced UV reflectance and shift of the UV peak towards shorter wavelengths in males since the involved red keto-carotenoids have their peak absorption in the range of 400-500 nm (McGraw et al. 2002; Shawkey and Hill 2005).

Beak colour and intrasexual competition

Based on both the analyses of pairwise differences and the analyses of individual means of the aggressiveness score, we can conclude that beak colour played no major role in same-sex aggressive interactions in zebra finches. Individuals with redder beaks, as perceived by humans (Munsell-based colour score), were not more aggressive than individuals with less red beaks, in either sex. The principal component, PC1, which objectively captured most of the sex-related variation in beak colour, was similarly not correlated with aggressiveness. Only the UV part of the spectrum showed some correlation with aggressiveness. Individuals with the point Nm-UV-half further towards the blue tended to be more aggressive, but this was significant only in females, and may represent a type 1 error, since the weak effect did not hold after Bonferroni correction. These more aggressive females had less male-like beaks (Fig 1a), which is contrary to the idea that individuals with more male-like features (beak colour) should show more male-like behaviour (aggression).

Body size and intrasexual competition

The analyses of pairwise differences together with the analyses of individual means also showed that body size had little influence in same-sex aggressive interactions. Tarsus length correlated significantly with aggressiveness level, but explained only a small fraction of the total variance. Larger males were more aggressive than smaller males, whereas smaller females were more aggressive than larger females. This difference between the sexes in the relationship between body size and aggression might be related to the intensity of competition. We found that competition was much more intense among males than among females (see *Y* axis of Fig. 2), as is to be expected for a species with classical sex roles. The outcome of intense male-male competition may thus more honestly reflect differences in RHP, with larger males tending to be dominant when competing for a potential partner. Females, in contrast, are the choosier sex and hence they were overall less competitive. Yet, among the females, the smaller ones adopted a more offensive strategy whereas larger ones seemed less inclined to fight over a male. This observation would make sense if males preferred large females to small females. This would create a value asymmetry between large and small females, whereby large, and hence presumably more attractive, females might behave choosy and show less interest in the male, whereas small females might be more desperate and actively compete for the male. This explanation remains speculative although, and, in our study population, we have not found any correlation between body size and measures of female attractiveness (i.e. how much males sang towards each of the two females in the experimental trials; data not shown). Nevertheless, our findings appear consistent with Morrell et al. ’s (2005) model which shows that small individuals may successfully chase away larger individuals from a contested resource if competition is not very intense. In that situation, large individuals may be better off avoiding the cost of a fight and instead search for another, uncontested resource.

It is important to keep in mind that the correlations of both differences in Nm-UV-half and differences in tarsus length with the outcome of competition were weak. The average effect size (of the transformed values), with the four test days pooled, was 0.155. To obtain significant ($\alpha = 0.05$) results without pseudoreplication, and with a power of 0.8, 321 tests would have to be conducted, which means testing 642 individuals of each sex. Our analysis of pseudoreplicated data hence seems the only practicable way of finding an estimate close to the true effect size. The analysis of individual means, which minimises the degree of non-independence, yielded similar effect sizes.

Signalling aggressiveness

There was a high individual repeatability of aggressiveness, with some individuals consistently attacking the opponent and others consistently being passive. However, we failed to explain most of this between-individual variation with the parameters measured. The current study is not a complete test of the hypothesis whether beak colour functions as a badge of status, since we did not manipulate beak colour. However, the badge of status hypothesis makes the clear prediction that individuals with naturally redder beaks should chase away less red individuals, and not vice versa. We can thus reject the hypothesis that beak colour signalled dominance under the given experimental conditions. In addition, variation in body size explained only little of the differences in aggressiveness.

Possibly, signals other than the ones we measured could serve as indicators of an individual’s readiness to compete for a potential partner. One such signal could be the breast band, which is based on melanin and fairly variable in size between males. It therefore has the potential to function as a classical badge of status, similar to the chest patch of house sparrows, *Passer domesticus* (e.g. Møller 1987; Gonzalez et al. 2002). Some females (27% in our population) also have a rudimentary breast band. All our subjects were given a breast band score at a mean ± SD of 114 ± 15 days of age (procedure following Burley and Bartels 1990). However, this measure did not correlate with aggressiveness in either sex in our experiment (E. Bolund & W. Forstmeier, unpublished data). We have no measures of the breast bands from about 300 days of age, when our experiments took place, but the size is likely to have changed relatively little over this period. Thus, even though we cannot preclude the possible importance of hitherto unmeasured visual signals in aggressive interactions in zebra finches, our results suggest that zebra finches might not rely on visual signals during the kind of same-sex competitive interactions we investigated. Individuals may rather follow different strategies, whereby they vary along a continuum, from those that always tend to behave aggressively to those that always tend to withdraw from conflict. This would be reminiscent of different personalities regarding competitive behaviour over a potential mate (see Sih et al. 2004, for a review of behavioural syndromes in animals).

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Chapter 3

Differential selection on genetic versus environmental components of beak colour, song rate and body size in zebra finches

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ABSTRACT

Selection will only result in evolutionary change if it is acting on the genetic component of a trait. The application of quantitative genetic methods that use pedigree data to disentangle the genetic ('breeding value') and the environmental components of traits has lead to a recent surge of studies looking at the potential for evolutionary change in animal populations. The zebra finch is a model species in studies of sexual selection, yet little is known about fitness consequences of presumably sexually selected traits, like beak colour, song rate and body size. Here, we investigate patterns of selection acting on these traits, by looking at genetic paternity under free-flying aviary conditions. We further disentangle the pathways leading up to fertilisation by looking at success at fertilising eggs outside the pair bond, success at keeping paternity within the pair bond, and attractiveness to females in extra-pair courtships. For each of the three traits we find significantly different selection acting on either the genetic or the environmental component. This finding illustrates the limitations of a purely phenotypic approach when one wants to draw conclusions about the current evolution of characters.

INTRODUCTION

Selection acts on the phenotypes of individuals, while an evolutionary response to selection requires genetic transmission to following generations. Thus, only selection acting on the genetic component of a trait will lead to an evolutionary response (Kruuk 2004), while selection on the nonheritable environmental component of the phenotype will lead to no evolutionary response (Price et al. 1988; Rausher 1992). Recently, there has been a surge of studies looking at the strength and direction of selection and the potential for evolutionary change (Kruuk 2004; Kruuk et al. 2008). This has been made possible by the application of computationally intensive quantitative genetic methods that can use pedigree information to disentangle the variation in traits into the underlying genetic and environmental components. These methods have been used for more than 50 years in animal breeding, where identification of genetically promising individuals is of great interest for the artificial selection on relevant traits, but have only relatively recently entered the field of evolutionary ecology.

While the additive genetic component of an individual's phenotype cannot be estimated explicitly for each individual, it is possible to estimate the expected effect of the genes that the individual passes on to its offspring. This is called the breeding value (BV) of the trait (Falconer and Mackay 1996, Kruuk 2004). Since the BV represents the genetic contribution to the trait, while the phenotype represents both the genetic and the environmental contribution, the residuals from a regression of individual phenotype on the BV will represent the environmental deviation for each individual, i.e. the environmental component of the trait. Hence, selection pressures acting not only on the phenotype, but also directly on the genetic and the environmental components of a trait can be quantified and thus it is possible to make predictions about evolutionary change.

A limitation to this method was pointed out by Postma (2006), since BV estimation is based not only on the phenotypes of relatives but also of the focal individual, the estimated BV will include some amount of environmental variation in addition to the genetic variation, while the residuals will represent only environmental variation (see figure 1). Thus, the breeding value is biased towards the phenotypic value of the focal individual. This problem becomes more pronounced as the heritability of the trait decreases, since the phenotype then to a larger extent represents environmental variation. The problem is also exacerbated by small sample sizes (e.g. poorly linked pedigrees, or traits with a sex limited expression), because as the pedigree becomes smaller, the influence of an individual's own phenotype for estimating its BV increases. To remedy this problem, Postma (2006) suggested that the phenotypes of the target individuals could be removed from the pedigree while calculating their BVs. An aim of this study was to implement a version of this suggestion, and remove the phenotype of the individual in question from the pedigree data file when calculating its BV. This BV, calculated only from the phenotypes of relatives, will bee free from environmental variation (there is no shared environmental effects with relatives except early maternal effects), but will also represent only part of the genetic variation (Figure 2). Hence, we can compute two types of BVs, one reflecting the entire genetic, but also some environmental, variation, and one reflecting only, but not the entire, genetic variation. As far as we know, this approach has never been taken.

We address this issue by looking at patterns of selection in a model species in studies of sexual selection, the zebra finch. The zebra finch is socially monogamous with some extra-pair paternity (Zann 1996). A plethora of studies have focused on female preferences for male beak colour and courtship song rate (traditionally termed 'directed song' rate in estrildids (Zann 1996), henceforth 'song rate'), either in choice chamber type set-ups (reviewed in Collins and ten Cate 1996; Forstmeier and Birkhead 2004), or, in a few studies, under more realistic aviary conditions (Burley 1985, 1986; Burley et al. 1994; Burley et al. 1996; Burley and Calkins 1999). A majority of the studies have found a preference for redder beaks or higher song rates. However, no study so far has used genetic paternity assignment to relate male traits to success at both gaining and keeping paternity, neither has any study decomposed the phenotypic variation into its genetic and environmental components. Looking at actual paternity patterns is especially important given previous findings from our population, since we have found no female preference for males with redder beaks in choice chamber trials (Forstmeier and Birkhead 2004) or for males with higher song rates in extra-pair copulation trails (Forstmeier 2007). Since previous evidence from our population showed that body size is of some importance in aggressive interactions (Bolund et al. 2007) and body size is often controlled for in choice chamber studies (e.g. Burley et al. 1982, Burley and Coopersmith 1987; Burley and Foster 2006), it might be important for male success under aviary conditions. Thus, in the current study, we relate song rate, beak colour and tarsus length to an important fitness component: success at fertilizing eggs under free-flying aviary conditions, and decompose the selection acting on the genetic and environmental components of the traits.

A further aim of the study was to elucidate the patterns of selection on overall success and disentangle possible contributing factors. Two main routes to achieve fertilisation success are female choice and male-male competition. In an attempt to separate these routes, we take several approaches, 1) influence the opportunity for sexual selection and the relative importance of female choice and male-male competition by means of sex ratio manipulation, 2) decomposing total success into extra-pair paternity (EPP) success and within-pair (WP) success, and 3) pinpointing the female choice component of EPP-success by looking at how positively females respond to the extra pair courtships of each male in a large sample of courtships obtained with a video surveillance system. We measure female preferences in the socially complex environment of the aviaries, thus, preference patterns might differ from those found in choice chamber experiments because of competition or other constraints. We expect that traits that are not important in female choice should also not be related to male attractiveness in EP-courtships, but might still be positively related to EPP and WP-success if competition effects are of importance, we would thus expect effects of the sex ratio treatment for such traits.

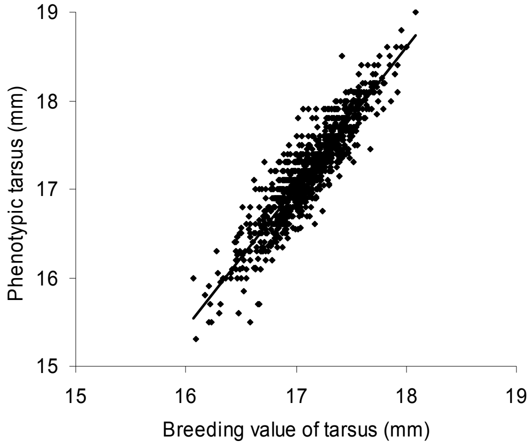

Figure 1.
Regression of the phenotypic value of tarsus on the breeding value (here BVincl) of tarsus. The breeding value is expressed in mm by adding the population mean of tarsus to each value. Thus, the x-axis illustrates the genetically determined tarsus length that would be achieved under a standard environmental condition, while the y-axis illustrates the actual phenotypic tarsus length that was achieved under the realised environmental conditions of the individual. The residuals from the regression represent the purely environmental contribution (RESincl) to tarsus for each individual.

METHODS

Measurements of traits

To measure directed song rate, we performed 5 minute male-female encounters involving socially unpaired males and females. To maximise statistical power, we included both pair-wise encounters (Forstmeier 2004) and encounters in trios (Bolund et al. 2007). In each trial we measured the total duration (in seconds) of male song directed towards the female. Song rate was measured repeatedly over the life-time in this way (in separate 'trial batches'), males participated in 7.16 ± 3.93 trials (mean ± SD, N = 586 males). Each male received a life-time score of directed song rate by taking the random effect estimates of song rate from a linear mixed effect model, controlling for trial batch and cohort effects.

Beak colour was measured with spectrophotometry to capture the variation over the full bird visible spectrum (320-700 nm), including the UV-part. Beak colour was measured at several occasions over the life time for all birds, always under nonbreeding conditions (1.8 ± 1.1, mean ± SD, N = 1019 birds). The males in the present experiment were measured at 100 days of age, at the start of each of the two breeding rounds in the aviaries, and, for a subset of 32 males, also at two later non-breeding occasions. From the beak colour spectrograms, we extracted 6 spectral characteristics, which were corrected for measuring batch and precise age at measure by taking random effect estimates from linear mixed effect models. We then extracted a PC1 of the 6 spectral characteristics as described in Bolund et al. 2007). The pronounced sexual dimorphism in beak colour measured in this way was removed from the PC1 because male and female beak colour is strongly genetically correlated ($r_g = 0.951 \pm 0.204$, unpublished data, see also Price and Burley 1993: $r_g = 0.91 \pm 0.12$). Removing the sex effect allowed us to include female beak colour when running quantitative genetic analyses to improve the estimates of breeding values of beak colour. The PC1 mainly captures beak colour variation on a female to male axis, with lower scores reflecting beaks that are more orange (as opposed to red) and have a higher reflectance peak in the UV.

Tarsus length was measured to the nearest 0.1 mm at 35-367 days of age (mean = 1.44 measures, range 1-2, N = 1049 birds)

Aviary experiment

All subjects were kept at the Max Planck Institute for Ornithology in Seewiesen, Germany, since October 2004. For details of rearing conditions for these birds, see Forstmeier (2005). For details of housing conditions, see Bolund et al. (2007).

Six females and six males in each of nine aviaries were allowed to breed for three months in September–November 2005. As part of a sex-ratio treatment, three aviaries had an additional three females, three aviaries an additional three males, while the remaining three had no additional birds added, giving sex ratios of 0.4, 05 and 0.6, respectively (Schielzeth et al. manuscript). The sex ratio manipulation was successful in manipulation the variance in reproductive success in males and thus the opportunity for sexual selection (Schielzeth et al. manuscript). After a non-breeding period without nesting opportunities, breeding pairs were exchanged among aviaries and sex-ratio treatments, such that each pair faced five new unfamiliar pairs, and allowed to breed for another three months in April-June 2006. In total, due to the replacement of deaths, 71 females and 68 males were used. The paternity of eggs or offspring was determined using 10 microsatellite loci (Forstmeier et al. 2007), and assigned to parents as described elsewhere (Forstmeier et al. manuscript.) Calculations are based on 1727 eggs.

For each male, we calculated overall success at fertilising eggs as a proxy of fitness. This includes eggs laid by the partner, by extra-pair females and by unpaired females. This is a fairly precise proxy of fitness under the selective regime in these aviary conditions, since all eggs were cross-fostered and raised by foster parents. To look specifically at extra-pair paternity (EPP) and success at keeping paternity within the pair-bond (WP-success), we define EPP-success as the number of eggs sired outside the pair-bond with other paired females and WP-success as the proportion of the eggs laid by the partner of a male that the male sired. The EPP-rate was 22% of all eggs. The measures of male success were highly repeatable between seasons, i.e. when each pair faced a new social environment. We calculated repeatabilities from linear mixed effect models, which allow controlling for the sex ratio treatment effect. The variance components of individual identity are the intra-class correlation coefficients and represent the individual repeatability. The variance component for the individual was 0.64 (LRT: $\chi^2_1 = 29.1$, $P < 0.0001$) for overall fertilisation success and 0.40 (LRT: $\chi^2_1 = 5.61$, $P = 0.018$) for EPP-success. The repeatability of EPP-success is reduced compared to that previously reported from the same experiment ($R = 0.58 \pm 0.09$, Bolund et al. 2009) due to the stricter definition of EPP used in the present paper.

Quantitative genetics

We used animal models in REML-VCE 6.0.2 (Groeneveld et al. 2008) to separate the phenotypic variance in tarsus, song rate and beak colour into additive genetic, maternal and early environmental effects. We used a 5-generation pedigree with N = 1374 birds to estimate additive genetic components (phenotypic data were available for $N_{\text{tarsus}} = 1054$ males and females, $N_{\text{beak colour}} = 1019$ males and females, $N_{\text{song rate}} = 586$ males), mother identity was entered to estimate maternal effects (additional to the additive genetic contribution of the mother, $N_{\text{tarsus}} = 251$ mothers, $N_{\text{beak colour}} = 212$, $N_{\text{song rate}} = 203$), foster parent identity was entered to estimate rearing environment effects ($N_{\text{tarsus}} = 350$ foster families, $N_{\text{beak colour}} = 303$, $N_{\text{song rate}} = 279$). For song rate and beak colour, peer group identity (i.e. the group the bird was kept in from independence at day 35 until full maturity at around day 100) was entered to estimate peer group effects ($N_{\text{beak colour}} = 82$ peer groups, $N_{\text{song rate}} = 56$). The heritability (the ratio of additive genetic variance to total phenotypic variance, Lynch and Walsh 1998) of tarsus was 0.55 ± 0.046, of beak colour 0.33 ± 0.041 and of song rate 0.26 ± 0.062. While the heritability is a population level parameter, it is possible to estimate, for each individual, the expected effect of the genes that the individual passes on to its offspring: the breeding value (BV) of the trait (Falconer and Mackay 1996, Kruuk 2004). If an individual is mated to a number of individuals taken at random from the population, then its BV is twice the mean deviation from the population mean. The deviation has to be doubled because the parent in question provides only half the genes in the progeny, the other half coming at random from the population. Thus, for each trait, we used the PEST software (Groeneveld et al. 1992), which uses the REML estimates of variance components to estimate best linear unbiased predictors (BLUPs) of the breeding value.

We address the issue pointed out by out by Postma (2006) by removing the phenotype of the individual in question from the data file when calculating its BV. Thus, for the 68 males in this experiment, we calculated two types of BVs and two types of environmental deviations (figure 2). On the one hand the ‘traditional’ BVs, with the phenotypes of all individuals included in the data file (“BVincl”), and on the other hand, ‘adjusted’ BVs, calculated by removing the phenotype of one male at a time from the data file while extracting the BVs ("BVexcl"), consequently, this was done 68 times for each trait, once for each male.

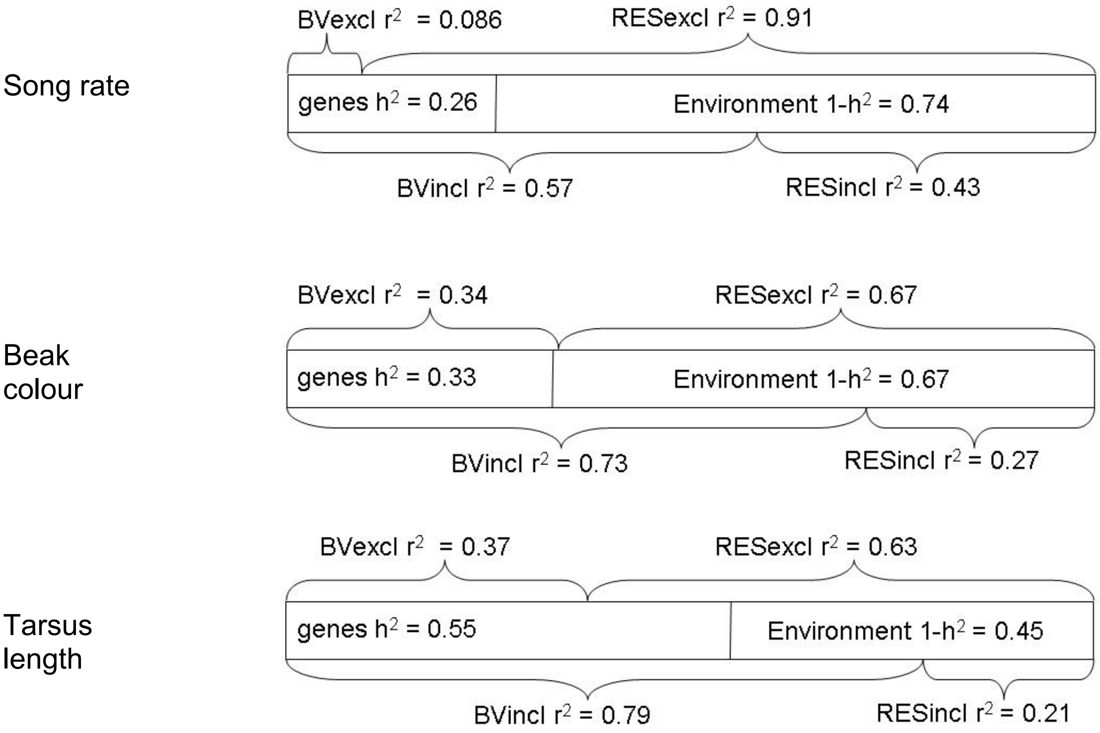

Figure 2.
The animal model approach divides the variation of a trait into a genetic (breeding value, BV) and an environmental (residual) component. However, if the BV of a focal individual is calculated with the knowledge of the individual's phenotype, this BV (BVincl) will contain some part of the individual specific environmental component in addition to the genetic component. This can be remedied by excluding the focal individual's phenotype from the data file while calculating its breeding value (BVexcl). However, the BV then reflects only part of the genetic variation. The figure illustrates how the genetic and environmental components of the three traits (song rate, beak colour and tarsus length) are represented by the two types of BVs and their corresponding residuals (RESincl and RESexcl, respectively).

Selection analyses

We use linear mixed effect models to estimate standardised selection gradients for each of the three traits of interest (Arnold and Wade 1984). The selection gradient for a trait expresses the strength of the linear selection acting on that trait, while controlling for correlated selection acting on other traits that are simultaneously entered into the model (Lande and Arnold 1983). We focus on linear selection gradients, since our interest is in directional selection on sexually selected traits. To obtain standardised selection gradients, we use relative success within the aviary as the response and z-standardised (mean = 0, SD = 1) traits as predictors. Standardisation ensures that slope estimates are expressed as change in units of phenotypic standard deviations in the response variable per changes in units of phenotypic standard deviations in the predictor variables. The slope of the linear term of the trait represents the linear selection gradient, β. We controlled for pairing status in two levels, paired or unpaired, and the number of days present (since some birds died and were replaced) as fixed effects. To obtain estimates for the different sex ratio treatments, we included the interaction between trait and sex ratio treatments. Male identity was entered as a random effect, since the observations from the two breeding rounds were treated as separate data points. Since slopes as well as intercepts of the three traits can differ between aviaries, we fitted, for each trait, the interaction between trait and aviary as a random effect (Schielzeth and Forstmeier 2009). To obtain meaningful selection gradients, no further transformation of the response is recommended after conversion to relative fitness (Lande and Arnold 1983). However, since significance testing assumes a normal distribution of errors from the regression, we use Markov Chain Monte Carlo (MCMC) simulations to obtain more robust p-values and standard errors for the estimates. Models were run once with the phenotypic values of the traits, and once with each of the breeding values and residuals.

Components of total success

We decomposed male total success into EPP and WP-success. The analyses of EPP-success were identical to those of total fertilisation success, with relative EPP-success within the aviary as the response. Thus, the estimates can be interpreted as the strength of the selection on the trait of interest acting via EPP-success. The WP-success was expressed as a proportion, transformed with angular transformation to approach normality.

Attractiveness to females in courtships

To obtain measures of courtship success in the aviaries, we used video surveillance data from the 2006 breeding season. We used 279 h (31 h per aviary) of early-morning observation over the season, with a total of 856 EP-courtship events by 65 males. We use data from the first 30 minutes after the lights were turned on, when the success of courtships (the percentage of courtships resulting in copulation) is higher than during the day as a whole (success-rate of EP-courtships during the first 30 minutes was 8%, compared to 5% over the whole day, in a different data set). Each male participated in on average 13 ± 13 courtships (mean ± SD, range 1-66). Female response to each courtship was scored on a scale from -1 to +1 in 0.5 increments, as described in Forstmeier (2007). To obtain one measure of average female responsiveness to a specific male, we used the random effect estimates from linear mixed effect models accounting for observer identity, day of the experiment, time of the day, sex ratio treatment and female fertility (in two levels: peak fertility, i.e. day -4 to -1 in relation to the day the first egg (day 0) of a clutch was laid, versus non-fertile) as factors and male and female identity as random effects. This results in one value per male of male extra-pair attractiveness to females. To see how male attractiveness was related to the three focal traits, we used the same approach as in the selection analyses described above, with z-standardised EP-attractiveness as the response.

Statistics

We used SPSS (SPSS for Windows, Rel. 15.0.1. Chicago: SPSS Inc.) and R 2.7.0 (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria) for statistical analyses. All statistical tests are two-tailed. For selection gradient analyses, we used the lmer function from the lme4-package in R 2.7.0 (Bates et al. 2008). We ran variance component analyses in R 2.7.0 using the lme function from the nlme package (Pinheiro et al. 2008). Random effect estimates were obtained with the lmer function (beak colour, EPP-attractiveness) and the lme function (song rate). We ran MCMC simulations using the pvals.fnc function from the languageR package (Baayen 2008).

RESULTS

Selection gradients

There was significantly positive selection on the phenotypic values of tarsus, but not on the phenotypic values of song rate and beak colour (Table 1). However, a different picture emerges when breaking down the phenotypic variation into the genetic and environmental components of the traits. We found significant positive selection on the breeding value (BVexcl) of beak colour and song, thus indicating the potential for evolutionary change (Figure 3). The selection on the residuals (RESexcl) of beak colour and song rate showed weak negative trends. The selection for longer tarsus was due largely to selection on the environmental component of tarsus length (Figure 4). Table 1 and Figure 2 illustrate how the estimation of selection gradients is influenced by the design of the breeding values and residuals. The BVexcl and the RESincl, representing the pure genetic and environmental contribution, respectively, show the strongest effects, while the BVincl and the RESexcl, which both reflect a mix of the genetic and the environmental components, show generally weaker trends.

The selection gradients generally differed only slightly among sex ratio treatments (Table 1). However, the selection estimates for tarsus were strongest under a male-biased sex ratio.

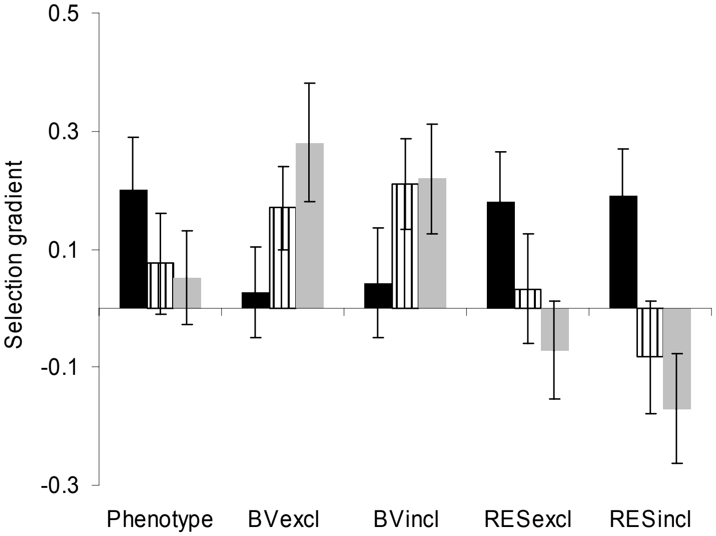

Figure 3.
The relationship between fitness (success at fertilizing eggs under aviary conditions) and each of the three traits: tarsus length (black bar), directed song rate (hatched bar) and beak colour (gray bar). The graph shows the standardised selection gradients (*β* ± SE from a linear mixed effect model that accounts for the sex ratio treatment). In addition to selection on the phenotypic value, the genetic and environmental components of each trait are represented by the breeding value calculated with the individual's phenotype excluded from the pedigree data file (BVexcl), the residual from the phenotype over this BV (RESexcl), the BV including the individual's phenotype (BVincl), and the residual from the phenotype over this BV (RESincl). The BVexcl and the RESincl thus represent only (but not the entire) genetic versus only (but not the entire) environmental variation, while the BVincl and the RESexcl represent a mixture of genetic and environmental variation.

Table 1.
Standardised selection gradients (estimate ± SE from a linear mixed effect model) of the three focal traits under three different sex ratio treatments. Sex ratios were female biased (0.4), even sex ratio (0.5) and male biased (0.6). Shown is also the overall mean effect controlled for treatment. The phenotype is subdivided into the genetic component (BVexcl) and the environmental component (RESincl).

		Sex ratio 0.4	Sex ratio 0.5	Sex ratio 0.6	Mean effect		
		β ± SE	*β* ± SE	*β* ± SE	*β* ± SE	*T*	*p*
Pheno-type	Tarsus	0.16±0.23	0.11±0.16	0.28±0.22	0.20±0.09	2.13	0.036
	Beak colour	0.078±0.2	0.047±0.13	0.067±0.19	0.052±0.08	0.55	0.58
	Song rate	0.095±0.2	0.095±0.08	0.05±0.21	0.076±0.085	0.78	0.44
BVexcl	Tarsus	0.004 ±0.19	-0.044 ±0.14	0.086 ±0.19	0.026±0.077	0.31	0.76
	Beak colour	0.22±0.25	0.28±0.19	0.31±0.25	0.28±0.1	2.25	0.026
	Song rate	-0.01±0.19	0.20±0.12	0.29±0.16	0.17±0.07	2.22	0.029
RESincl	Tarsus	0.22±0.22	0.032±0.18	0.28±0.21	0.19±0.078	2.39	0.019
	Beak colour	-0.20±0.23	-0.13±0.16	-0.16±0.22	-0.17±0.092	-1.66	0.10
	Song rate	-0.024±0.24	0.043±0.17	-0.21±0.23	-0.083±0.095	-0.76	0.45

Components of total success and the role of female choice

Two components of total success are EPP-success and WP-success. We found that the patterns for EPP-success largely reflect those found for overall success, while the trends for WP-success were generally weak (Table 2). To pinpoint the female choice component of EPP-success, we looked at the average female responsiveness to each male in EP-courtships (Table 2). Male attractiveness to females was not influenced by tarsus length, while beak colour showed similar trends as for total success, albeit weaker. The song rate showed an interesting pattern. Males with a higher phenotypic song rate were clearly rejected by females (Figure 5). This was driven by the strong negative relationship between environmental song rate and attractiveness, while the breeding value for song rate showed a positive trend. Results remained qualitatively the same when using partial correlation between male trait and male attractiveness controlling for the actual number of courtships that the male performed (data not shown), indicating that the results reflect female choice rather than being a by-product of individual differences in male extra-pair mating effort.

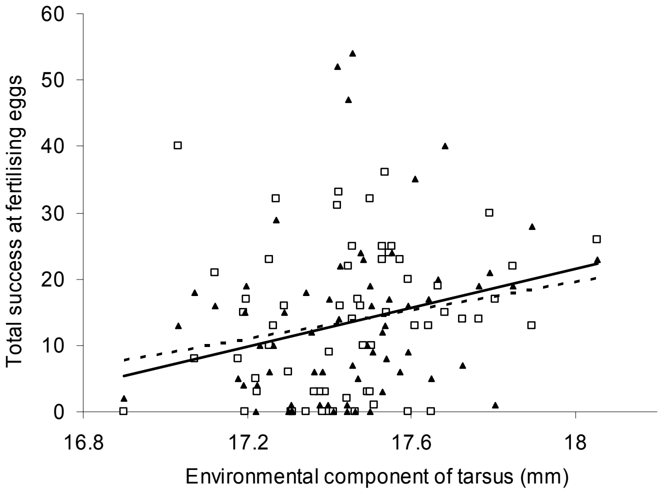

Figure 4.
The environmental component of tarsus length (RESincl) showed the strongest relationship with fitness. Both seasons are included to illustrate the consistency of the effect. Values are coded by season (season 1 open squares and dashed regression line, season 2 filled triangles and solid regression line).

Table 2.
The relationship between the three focal traits and two sub-components of total success: extra-pair (EPP) success and within-pair (WP) success, and the attractiveness in extra-pair courtships (EP-attractiveness). The overall mean effect controlled for sex ratio treatment is shown. For illustration, the phenotype of each trait is subdivided into the genetic component (BVexcl) and the environmental component (RESincl).

		EPP-success			WP-success			EP-attractiveness		
		β ± SE	*t*	*p*	β ± SE	*t*	*p*	β ± SE	*t*	*p*
Pheno-type	Tarsus	0.27±0.17	1.55	0.12	0.071±0.11	0.56	0.57	0.081±0.16	0.62	0.54
	Beak colour	-0.006±0.16	-0.033	0.97	0.15±0.12	1.46	0.25	0.053±0.15	0.38	0.71
	Song rate	0.093±0.17	0.48	0.63	0.026±0.11	0.20	0.84	-0.47±0.14	-3.78	0.0004
BV excl	Tarsus	0.025±0.16	0.15	0.88	-0.070±0.10	-0.59	0.56	-0.052±0.19	-0.21	0.83
	Beak colour	0.28±0.16	1.58	0.12	0.26±0.11	2.10	0.039	0.23±0.15	1.89	0.064
	Song rate	0.42±0.15	2.43	0.017	0.19±0.10	1.58	0.12	0.22±0.16	1.75	0.086
RES incl	Tarsus	0.31±0.16	1.90	0.060	0.17±0.11	1.33	0.19	0.062±0.17	0.36	0.72
	Beak colour	-0.35±0.19	-1.72	0.088	-0.051±0.11	-0.39	0.70	-0.11±0.14	-0.82	0.42
	Song rate	-0.22±0.18	-1.10	0.27	-0.14±0.11	-1.12	0.27	-0.47±0.17	-2.68	0.001

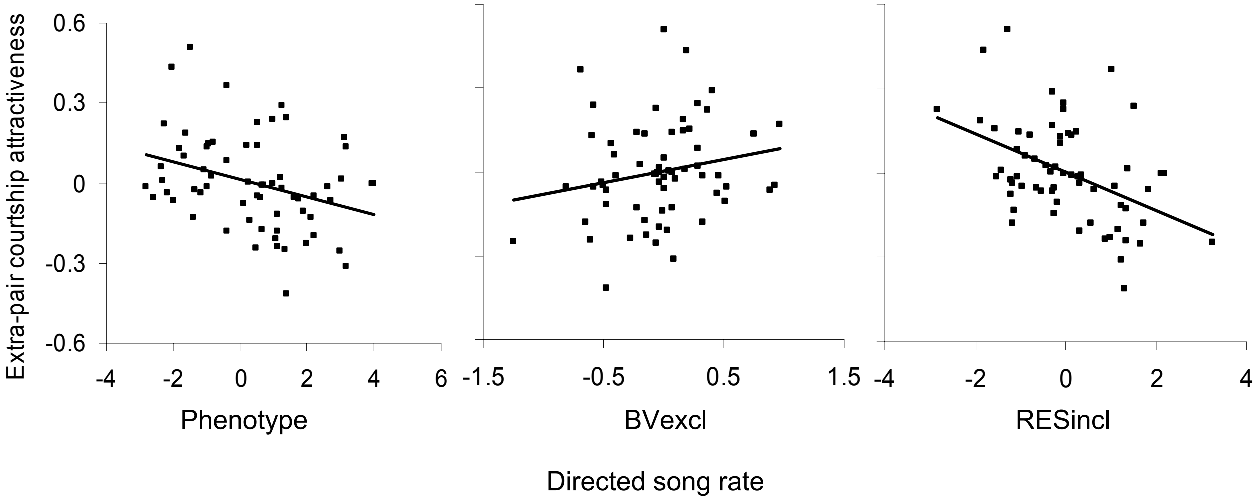

Figure 5.
The relationship between male average attractiveness to females in extra-pair courtships and (a) the phenotypic value of directed song rate, (b) the breeding value (BVexcl) and (c) the residual (RESincl).

DISCUSSION

Decomposing the phenotypic variation into its genetic and environmental components revealed a significant difference in selection on the breeding value (BV) versus the environmental component for all three traits. We found significant positive selection gradients for residual tarsus and for the breeding values of song rate and beak colour. However, the positive selection on the breeding values of song rate and beak colour was counteracted by the negative trends on the residuals, resulting in very weak trends on the phenotypic level. For tarsus, selection was mainly acting on the environmental component, thus the potential for evolutionary change would be very low (Kruuk 2004). Further, by removing the phenotype of one individual in turn from the pedigree data file while calculating its estimated BV, we obtained BVs that reflect the purely genetic contribution to the trait. Thus, we were able to estimate selection pressures acting on the purely genetic versus the purely environmental components of the traits, leading to an improved estimation of the selection pressures (Figure 2).

We expected that selection patterns on the BVincl would more closely reflect the selection acting on the phenotype, while selection on the BVexcl should not be biased towards the phenotype. Conversely, selection acting on the RESexcl should more closely reflect the selection patterns on the phenotype compared to the RESincl. The observed selection patterns partly correspond to this expectation, however, the differences are mostly minor and some are in the opposite direction (Figure 3). This might partly be due to the increased imprecision of the BVexcl (and correspondingly RESincl) because the BVexcl reflects only part of the genetic contribution to the trait. Thus, the gain from excluding the environmental component might in many cases be outweighed by the cost of 'loosing' some of the genetic variation. Thus, the use of traditional BVs seems to often be justified.

Our results of selection on the phenotypic level can be compared to one previous study which used social parentage under aviary conditions and found significant positive selection acting on the phenotypic value of male beak colour, while selection on male tarsus length was significantly negative (Price and Burley 1994).

The patterns for EPP-success were similar to those for total success for all traits. The patterns for WP-success were also similar to the overall success, but very weak, only the BV of beak colour was significant. Hence, it appears that female propensity to have EPP might be influenced more by the attractiveness of the available EP-males than by the attractiveness of the social partner, as was indicated in a previous study on this population (Forstmeier 2007). Since success at obtaining EPP does not necessarily reflect female choice, especially under skewed sex ratios, where male-male interaction might have heightened importance, we looked at male attractiveness to females in EP-courtships. We found no female preference for tarsus length in EP-courtships. Thus, the higher success of larger males might be due to male-male competition (see Bolund et al. 2007). This idea is supported by the stronger effects when sex ratios were biased, with presumably more intense competition. Selection on beak colour showed very little difference among sex ratios, and the effects on the song rate were strongest in the male-biased sex ratio.

For both beak colour and song rate, we found a nearly significant female preference for males with higher BVs. This means that these should increase over evolutionary time. However, females behaviourally discriminated strongly against males with an environmentally high song rate, thus resulting in a significant avoidance on the phenotypic level. For beak colour, females tended to avoid males with environmentally higher values, resulting in neither preference nor avoidance on the phenotypic level. This lack of a pattern on the phenotypic level was expected given that the measure of beak colour we used reflects a life-time average nonbreeding beak colour, while the actual beak colour during the experiments mostly reflected male breeding status (zebra finch beak colour in both sexes substantially declines over the breeding cycle, Burley et al. 1992).

Female choice acts on phenotypes, since the genetic and environmental contributions to a trait cannot be judged directly. Thus, a preference for high breeding values could result if females use other cues that are visible on the phenotypic level and genetically correlated with song and beak colour, for example ‘good gene’ indicators like health or vigour. Current selective pressures will depend on the specific environmental conditions of the experiment. This population of birds has been bred (almost) exclusively in cages for at least 20 generations, with one force-paired pair per cage, thus minimising the opportunity for female choice to be expressed. For the current experiment, birds were allowed to breed in free-flying aviaries, allowing for complex social interactions. This drastic environmental change will likely be reflected in current selection pressures as genes that were selective neutral under cage conditions might become ‘good genes’ under the new environmental conditions. In other words, a female preference for ‘good gene’ indicators will reflect a preference for genes that are ‘good’ under the current environmental conditions, and not necessarily reflecting ‘general good genes’ such as health and vigour.

The avoidance of males with environmentally high song rates and redder beaks could similarly be achieved if environmental conditions that lead to a high song rate and more male-like beak colour also affect other traits or behaviours that were not measured by us but judged by the females. The very strong avoidance of males with an environmentally high song rate was not simply due to an avoidance of males with a high courtship rate, since the effect was still strong after accounting for number of courtships. Note that song rate was measured under standardised conditions long before the birds were released into the aviaries.

Interestingly, the strong female avoidance of high song rate males was not reflected in the EPP-success, where the trend was even weakly positive. Despite the substantial number of courtships recorded, the number of copulations was too low (1.4 ± 2.3 per male, mean ± SD) to give a clear picture of the connections between female responsiveness to the male, copulation success and fertilisation success. Thus, we refrain from speculating about how these connections might be influenced by day time effects, the female reproductive cycle, or processes such as sperm competition.

In conclusion, we found positive selection on the phenotypic value of tarsus, but no selection on the phenotypic value of song rate or beak colour. However, the breeding values revealed a different picture, with no selection on tarsus, but positive selection on song rate and beak colour. This illustrates the importance of separating the genetic and environmental components of traits. Further, we found that female choice was not mediating the patterns for tarsus, while the patterns for song rate and beak colour are likely to some extent mediated by female choice for correlated characters.

ACKNOWLEDGEMENTS

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Chapter 4

Honest signalling turned upside down: high song rate may reflect low male quality

Elisabeth Bolund, Holger Schielzeth, Wolfgang Forstmeier

ABSTRACT

Males of many bird species continue to sing after pair formation. If song rate honestly signals quality through condition dependence, it seems likely that singing at a high rate after pair formation would attract extra-pair females, minimize the own partner's interest in extra-pair copulations, and thereby deter rivals from cuckolding attempts. We test this hypothesis in a captive population of zebra finches breeding in large aviaries. Contrary to the prediction, we find that males with the lowest, not highest, song rates were most successful in siring extra-pair offspring, and also tended to keep more paternity within the pair bond. We therefore experimentally test a complementary hypothesis that has received only scant attention, namely that the amount of song stimulates the own female's investment into eggs in a quantitative way. Indeed, we find that females produced higher-quality eggs when paired to a high song-rate male. Based on these findings, we suggest that males may allocate their resources depending on their quality; highly attractive males should search for extra-pair mating opportunities, while low-quality males should concentrate on stimulating their partner by singing more. We hypothesize that females might either be manipulated by male song due to a sensory bias or that females could benefit from such compensatory investment if low genetic-quality offspring are more dependent on maternal resources. We conclude that mated zebra finch males sing to stimulate their partner and not to signal their quality to extra-pair females.

INTRODUCTION

It is commonly accepted that song has two primary functions: mate attraction (before social pairing) and territory defence (before and after social pairing) (Andersson 1994; Searcy and Yasukawa 1996; Slater 2003; Catchpole and Slater 2008). Support for these functions comes from studies on a wide range of species and it is assumed that song rate honestly signals the quality of a male (reviewed by Searcy and Andersson 1986; Kroodsma and Byers 1991). A signalling system requires reliability of signals to be maintained (Searcy and Nowicki 2005) and honest signalling theory generally assumes that signalling costs ensure this reliability (Zahavi 1975; Grafen 1990a, b). In the case of song rate, condition dependence would ensure honesty. However, there is scant direct support for condition dependence of song rate (reviewed in Gil and Gahr 2002). Nonetheless, a positive correlation between song rate and territory quality (e.g. Radesäter and Jakobsson 1989) or measures of male success (Møller et al. 1998) has led to the view of high song rate as an honest signal of male quality to predominate in the literature (Hardouin et al. 2007; Catchpole and Slater 2008).

Given this reasoning, why do many species, even species with no territory defence, continue to sing also after social pair formation? If high song rate is an honest indicator of male quality (Møller 1991) and female choice is based on male quality, the mate attraction function would not only be relevant for attracting social partners, but also for extra-pair partners. This leads to two clear predictions: High song-rate males should have (1) higher extra-pair paternity success, since they should be more attractive as extra-pair copulation partners for females and (2) higher paternity within the pair bond, since females mated to high song-rate males should be less inclined to have extra-pair copulations. In species with territories, high song-rate males will thus suffer fewer territory intrusions than low song-rate males. The need for paternity assurance is the highest during the fertile period of the partner, thus song rate should be the highest during this period (Greig-Smith 1982; Mace 1987; Møller 1991). Indeed, a large number of species studied to date do exhibit a pronounced peak in song activity during the fertile period of the female (Mace 1987; Møller 1988; Cuthill and Macdonald 1990; Welling et al. 1995; Dunn and Zann 1996a; Currie et al. 1998; Pinxten and Eens 1998; Mota 1999; Forstmeier and Balsby 2002; Ballentine et al. 2003; Bodily and Neudorf 2004).

A complement to the mate-attraction function of song is mate-stimulation (Kroodsma 1976; Kroodsma and Byers 1991). This would also predict a peak in singing activity during the fertile and egg laying periods of the female. The role of song in stimulating female reproductive physiology has been addressed by several studies (reviewed in Catchpole and Slater 2008). The focus has been on features of song such as structure (Kroodsma 1976; Marshall et al. 2005) and familiarity to the female (Hinde and Steel 1976; Leboucher et al. 1998). It seems plausible that song rate could affect female reproductive investment in a quantitative way, so that increased song rate of the partner could stimulate the female to invest more into the current reproductive attempt. One could then predict a linear relationship between male song rate and female investment. This could either be selectively neutral from the female perspective or, if song reflects male quality, increased investment when paired to a high song rate male might be adaptive (the differential allocation hypothesis, Burley 1986, 1988). However, under some circumstances, increased investment would be expected when paired to a male of lower quality (compensatory investment, Bolund et al. 2009; Navara et al. 2006).

Here, we test the functions of song after pair formation in a species where song is not used in territory defence. We employ two separate approaches on a laboratory population of zebra finches. To test if song rate is an honest indicator of quality, we studied paternity patterns under free-flying aviary conditions. We expect that paired males with higher song rates should have higher success at fertilising eggs outside the pair-bond (extra-pair paternity), and keep more paternity within the pair-bond. We use total fertilisation success of eggs as a proxy for fitness, and assume that such attractive males are of higher (genetic) quality. Compared to a more comprehensive fitness measurement (like the number of grandchildren), fertilisation success neglects fitness components such as parental qualities. However, we found no correlation between song rate and measures of parental quality in this study (see Methods), indicating that focusing on fertilisation success should not lead to a consistent bias in our estimation of fitness. To test if song stimulates female reproductive investment in a quantitative way, we allowed females to breed under cage conditions subsequently with two males that differ in song rates. We expect females to lay larger eggs with more nutrients when paired to a high song rate male.

METHODS

Study species

The zebra finch is a small, colonially breeding Australian Estrildid that forms life-long socially monogamous pair bonds (Zann 1996). There is no territory defence in this species, and males sing throughout the year, also in the non-breeding season, regardless of their pairing status (Zann 1996). Levels of extra-pair paternity are high in the zebra finch under laboratory conditions (Bolund et al. 2009; Burley et al. 1996), but much lower in the wild (Birkhead et al. 1990). Song rate peaks during the fertile period of the partner (Dunn and Zann 1996a; this study). The zebra finch is a close-ended learner with a short motif of about 0.8 seconds that is sung repeatedly. The song is highly stereotyped within, but greatly variable among individuals. A distinction is usually made between courtship song ('directed song' i.e. song directed at the female) and song sung in any non-courtship context ('undirected song') (Zann 1996). To facilitate comparisons with other species, we here apply the more intuitive terms 'courtship rate' (for directed song) and 'song rate' (for undirected song) and focus mainly on song rate, not courtship, rate. Song has been suggested to function in the attraction of social mates as well as extra-pair females (Dunn and Zann 1996b, a).

Aviary experiment, general design

All subjects were kept at the Max Planck Institute for Ornithology in Seewiesen, Germany, since October 2004. For details of housing conditions, see Bolund et al. (2007). For details of rearing conditions for these birds, see Forstmeier (2005). Six females and 6 males in each of 9 aviaries were allowed to breed for three months in September–November 2005. As part of a sex-ratio treatment, three aviaries had an additional three females, three aviaries an additional three males, while the remaining three had no additional birds added, giving sex ratios of 0.4, 0.5 and 0.6, respectively. After a non-breeding break without nesting opportunities, breeding pairs were swapped among aviaries and sex-ratio treatments, such that each pair faced five new unfamiliar pairs, and allowed to breed for another three months in April-June 2006. In total, due to the replacement of deaths, 68 males and 71 females were used in this experiment. The paternity of eggs or offspring was determined using 10 microsatellite loci (Forstmeier et al. 2007), and assigned to parents as described elsewhere (Forstmeier et al. manuscript). Calculations are based on 1727 eggs. The extra-pair paternity rate (EPP-rate) was 22% of all eggs. EPP was defined as eggs sired by a paired male with a paired female other than his partner (note that this definition of EPP is stricter than the definition used in Bolund et al. 2009, where EPP was defined as a paired male siring eggs with any females other than his partner). Each pair was allowed to rear one clutch (or 2-3 if unsuccessful), per breeding round. Eggs were fully cross-fostered between the aviary pairs and 72 foster pairs kept in cages, and each aviary clutch was restricted to 2-3 eggs (2.44 ± 0.53, mean ± SD). In total, 275 fertile eggs were fostered into the aviaries and they resulted in 109 fledged offspring.

Measures of male success

For each male, we calculated overall success at fertilising eggs as a proxy of fitness (square root transformed to approach normality). This includes eggs laid by the partner, by extra-pair females and by unpaired females. To look specifically at EP-success and success at keeping paternity within the pair-bond (WP-success), we define EP-success as the number of eggs sired outside the pair-bond with other paired females (cube root transformed to approach normality) and WP-success as the proportion of the eggs laid by the partner of a male that the male sired (angular transformation to approach normality).

The measures of male success were highly repeatable between seasons, i.e. when each pair faced a new social environment. We calculated repeatabilities from linear mixed effect models, which allow controlling for the sex ratio treatment effect. The variance components of individual identity are the intra-class correlation coefficients and represent the individual repeatability. The variance component for the individual was 0.64 (LRT: $\chi^2_1 = 29.1$, $P < 0.0001$) for overall fertilisation success and 0.40 (LRT: $\chi^2_1 = 5.61$, $P = 0.018$) for EP-success. The repeatability of EPP is reduced compared to that previously reported from the same experiment (R = 0.58 ± 0.09, Bolund et al. 2009) due to the stricter definition of EPP used in this paper.

Male parental quality is another potentially important component of fitness. We obtained a measure of male parental quality as described in Bolund et al. (2009). Song rate was not correlated with parental quality in this experiment ($b = -0.039 \pm 0.63$, $t_{32} = -0.061$, $P = 0.95$, linear mixed-effect model with song rate and sex ratio treatment as fixed factors, male identity as a random effect and song rate controlled for status (see below) as a continuous predictor). Thus, we did not include parental quality in our fitness proxy. This should introduce noise, but no consistent bias, to our estimation of male fitness.

Measures of song rate

Each aviary was observed for periods of 5 minutes several times per week (37 out of 83 days in 2005, 53 out of 87 days in 2006). Aviaries were observed in random order and at different times of the day (0-8 hours after lights on). Song in a non-courtship context was scored as present or absent during each 30 second interval of the 5 minutes. This type of song comprised the majority of total song output (87 ± 12%, mean ± SD), with the remainder being courtship song. Since females might either judge male absolute song rate, or song rate accounting for a male's breeding status (e.g. unpaired vs. incubating), we used two measures of song rate: the raw values (square root transformed to approach normality) and song rate corrected for male pairing and breeding status. Since pairs were breeding throughout the experiment, we accounted for male state on a daily basis, with two states for pairing status: 'paired' or 'unpaired'. Paired males rotated between a non-breeding state and three breeding states: 1) 'incubating eggs', 2) 'feeding young offspring' (< 8 days old) and 3) 'feeding old offspring' (> 8 days old but pre-fledge). We used the random-intercept effect estimates (BLUPs) of song rate for each male obtained from a linear mixed-effect model with state, date and time after lights on as fixed effects and male identity as a random effect.

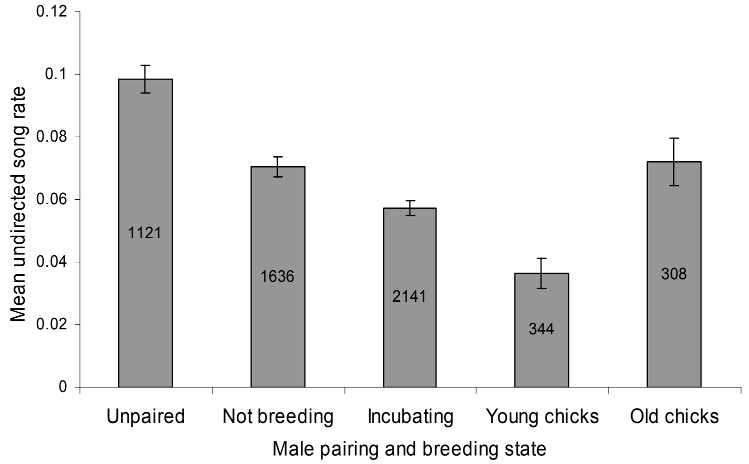

Figure 1.
Male average undirected song rate in aviaries was influenced by their states. Males are divided into five status categories: unpaired, paired but not breeding, incubating eggs, feeding chicks < 8 days old and feeding chicks > 8 days < fledging. Bars represent the raw proportion of 30-second intervals (mean ± SE) where song occurred for each group. Total number of registrations of song activity scored as present or absent for each group is shown inside each bar.

To look at male success in relation to song rate, we used linear mixed-effect models with the relevant success measure as the response, song rate and sex ratio treatment as fixed factors, and male identity as a random effect. Our success measures were overall fertilisation success, EP-success and WP-success. Song rate was either raw values, or random effect estimates controlled for status (see above). Differences in song rate between paired and unpaired males were analysed using a generalised linear model with penalized quasi likelihood (PQL) with song rate on a daily basis as the response, pairing status, date and time after lights on as fixed factors and male identity as a random effect. We included date and time after lights on to control for season and day time effects on song rate. To facilitate comparison of the two groups, we also ran the model with the data limited for paired males to include only days when they were not occupied with breeding.

To obtain measures of courtship rates in the aviaries, we used video surveillance data from the 2006 breeding season, since sample sizes in the live observations described above was limited due to the relatively low frequency of courtship. We used 279 h (31 h per aviary) of early-morning observation over the season, with a total of 2268 courtship events by 65 males. Number of courtships per male was square-root transformed to approach normality.

Cage experiment, general design

The effect of male undirected song rate on female primary investment was investigated in a cage setting. The experiment was first performed with 16 new pairs from our population (henceforth 'Cage 1'). Later the experiment was repeated with a very similar design, this time with 32 pairs, using birds that had participated in the aviary breeding experiment described above (henceforth ‘Cage 2’). This second experiment is described elsewhere (Bolund et al. 2009) and we will describe in detail only the design of the first experiment here.

Sixteen females were paired sequentially to two males in a crosswise design. With each partner, the female was allowed to lay around 3 clutches (3.1 ± 0.85, mean ± SD). Eggs were removed and replaced with dummy eggs on the day of laying. Dummy eggs were removed after a standard incubation period of 15 days after the day on which incubation was initiated. We measured primary investment in terms of egg volume, egg laying rate (eggs laid/number of days), yolk mass, yolk testosterone content and yolk hue with each of the two males. Yolk hue is an index of yolk carotenoid content, for details of this measure and of the testosterone analyses, see Bolund et al. (2009).

Male song rate was scored as present or absent during a 30 second period 4 times per day, with a minimum of 1 h between observations. For each male, the counts of song were then summed over the experimental period and divided by the total number of observation events, giving us a measure of male song rate. Males were assigned to females randomly with respect to song rate. We used the difference in song rate between her two partners as the predictor of the difference in female investment with her two partners. We looked at within-female changes in investment with her two partners using Pearson’s correlation.

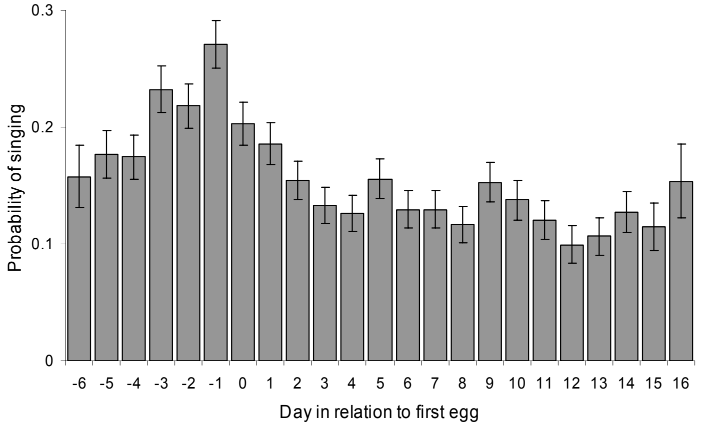

Figure 2.
Undirected song rate peaked during the fertile period of the partner. For illustration, the figure shows mean ± SE of the probability of singing, against the day of the breeding cycle of the female. Day 0 denotes the day the first egg is laid and the fertile period extends through day -6 to day +4. Data from ‘Cage 2’, based on 33 pairs and 9168 registrations of song activity scored as present or absent.

Statistics

We used SPSS (SPSS for Windows, Rel. 15.0.1. Chicago: SPSS Inc.) and R 2.7.0 (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria) for statistical analyses. We ran variance component analyses in R 2.7.0 using the lme function from the nlme package (Pinheiro et al. 2008). The same package was used for the models of success in relation to song rate and parental quality. To obtain random effect estimates of song rate controlled for status, we used the lmer function from the lme4-package (Bates et al. 2008). For PQL estimation, we used the glmmPQL function from the nlme package (Pinheiro et al. 2008). All statistical tests are two-tailed. Repeatabilities and their standard errors were calculated following Lessells and Boag (1987) and Becker (1984).

RESULTS

Repeatability of song rate

Song rate was higher and also more variable among males, in cages than in aviaries, (mean ± SD of 30 sec intervals containing song for males that participated in both the aviary experiment and 'cage 2': cage: 0.15 ± 0.12, CV = 0.79, aviary: 0.064 ± 0.031, CV = 0.48, paired t-test: $t_{29} = 4.14$, P = <0.001). Within males, however, the rate was stable (individual repeatability between breeding rounds in aviaries when neighbours were exchanged: R = 0.62 ± 0.08, $F_{60,67} = 4.27$, $P < 0.001$). Also in cages, the song rate was highly repeatable between breeding rounds with different partners (R = 0.73 ± 0.07, $F_{44,47} = 6.27$, $P < 0.001$). However, when the social context changes dramatically, the song rate is flexible: the repeatability between aviary and cage song rate was R = 0.13 ± 0.18 ($F_{29,30} = 1.31$, $P = 0.12$, after adjusting for the differences in mean song rate between cage and aviary).

Song rate, mating status and partner fertility

In the aviaries, mated males had lower song rates than unmated males (Fig. 1, glmmPQL, t = -4.91, P < 0.001, N = 5563 observations, 68 males). This result was significant also when comparing only non-breeding paired males with unpaired males (glmmPQL, t = -3.22, P = 0.0013, N = 2673 observations, 68 males).

In the cages, song rate peaked during the fertile period of the partner (Fig 2). Song rate was significantly higher during the fertile period (defined in relation to the day of the first egg as day -6 to day +4) than during the non-fertile period (pre-breeding, incubation, and post-breeding phases) (glmmPQL: t = 8.05, P < 0.0001, N =9809 scorings by 33 males, data from 'cage 2').

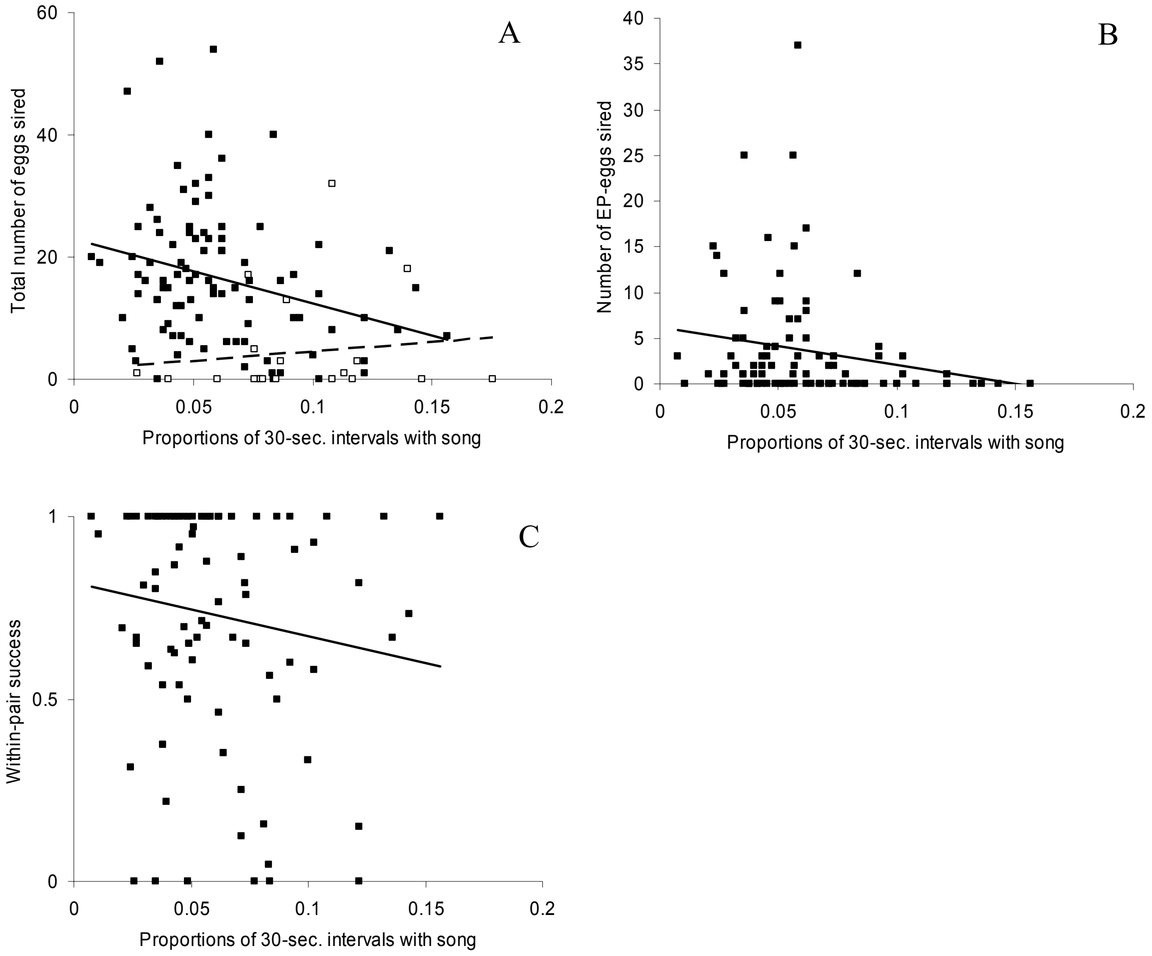

Figure 3.
Male egg fertilisation success in relation to undirected song rate. In paired males, undirected song rate was significantly negatively correlated with fitness (total number of eggs sired, 3A, P = 0.0082) and with extra-pair (EP) success (3B, P = 0.035). Success at keeping paternity within the pair-bond (WP-success) showed a weak trend in the same direction (3C, P = 0.32). For ease of illustration, data from both breeding seasons are shown, so that each data point corresponds to one male in one season. Paired males are indicated by black squares and solid linear regression lines, unpaired males by open squares and dashed linear regression lines.

Song rate and fertilisation success

Among mated males, the overall success at fertilising eggs in the aviary experiment was, unexpectedly, negatively related to song rate (Fig 3a, raw song rate: $b = -2.34 \pm 0.84$, $t_{37} = -2.79$, P = 0.0082, song rate controlled for status: $b = -1.65 \pm 0.59$, $t_{37} = -2.81$, P = 0.0079). We looked at two components of fertilisation success; success outside the pair bond (EP-success) and success at keeping paternity within the pair-bond (WP-success). Again contrary to our expectation, high song-rate males had lower EP-success (Fig 3b, raw song rate: $b = -1.28 \pm 0.58$, $t_{34} = -2.20$, P = 0.035, song rate controlled for status: $b = -0.72 \pm 0.39$, $t_{34} = -1.86$, P = 0.072). WP-success showed a weak and non-significant effect in the same direction (raw song rate: b = -0.28 ± 0.28, t_{34} = -1.02, P = 0.32, song rate controlled for status: b = -0.29 ± 0.19, t_{34} = -1.52, P = 0.14). These patterns were largely consistent between the two separate breeding seasons (Table 1).

Table 1.

Undirected song rate as a predictor of three measures of fertilisation success during two different breeding seasons. Male fertilisation success is measured as total number of eggs fertilised (with any female: fitness), number of extra-pair eggs fertilised (with other paired females: EP-success) and proportion of his partner's eggs that the male sired (WP-success). The predictors are raw values of undirected song rate (SU-rate), or random effect estimates of SU-rate after accounting for breeding and pairing status on a daily basis, for details, see Methods.

			SU-rate			SU-rate corrected for state		
		df	*b* ± *SE*	*t*	*P*	*b* ± *SE*	*t*	*P*
2005	Fitness	42	-1.60±0.98	-1.63	0.11	-0.94±0.67	-1.41	0.16
	WP	40	-0.18±0.36	-0.50	0.62	-0.11±0.24	-0.45	0.65
	EP	40	-1.08±0.73	-1.48	0.15	-0.69±0.49	-1.43	0.16
2006	Fitness	45	-3.32±1.39	-2.39	0.021	-2.16±0.90	-2.39	0.021
	WP	44	-0.61±0.42	-1.46	0.15	-0.44±0.27	-1.64	0.11
	EP	43	-1.74±0.90	-1.93	0.060	-1.10±0.58	-1.92	0.062

Courtship rate

In aviaries, courtship rate tended to be negatively correlated with song rate (r = -0.20, N = 65, P = 0.11). Courtship rate was positively related to fitness (b = 2.53 ± 0.87 t_{64} = 2.91, P = 0.005). However, when looking at paired and unpaired males separately, this relationship was no longer significant (paired males: b =1.47 ± 1.02, t_{48} = 1.44, P = 0.16, unpaired males: b = -1.1 ± 1.53, t_8 = -0.72, P = 0.51).

Female reproductive stimulation

In the cage experiment, change in partner song rate was correlated with change in female investment. As expected, females laid eggs with significantly more orange (as opposed to yellow) yolks and eggs that were larger when paired to the male with the higher song rate (Fig. 4, Both experiments pooled: Yolk hue: r = 0.38, N = 45, P = 0.01, Egg volume: r = 0.35, N = 45, P = 0.021). The two experiments considered separately showed similar trends (Yolk hue: cage 1: r = 0.51, N = 15, P = 0.051, cage 2: r = 0.34, N = 30, P = 0.064, Egg volume: cage 1: r = 0.49, N = 15, P = 0.062, cage 2: r = 0.28, N = 30, P = 0.13). Results remained qualitatively the same when using an alternative analysis with female absolute values of investment as the response and testing for male song rate as a predictor in linear mixed-effect models (data not shown). Number of eggs laid per day, yolk mass and yolk testosterone content were not correlated with change in partner song rate (all r < 0.19, all P > 0.31).

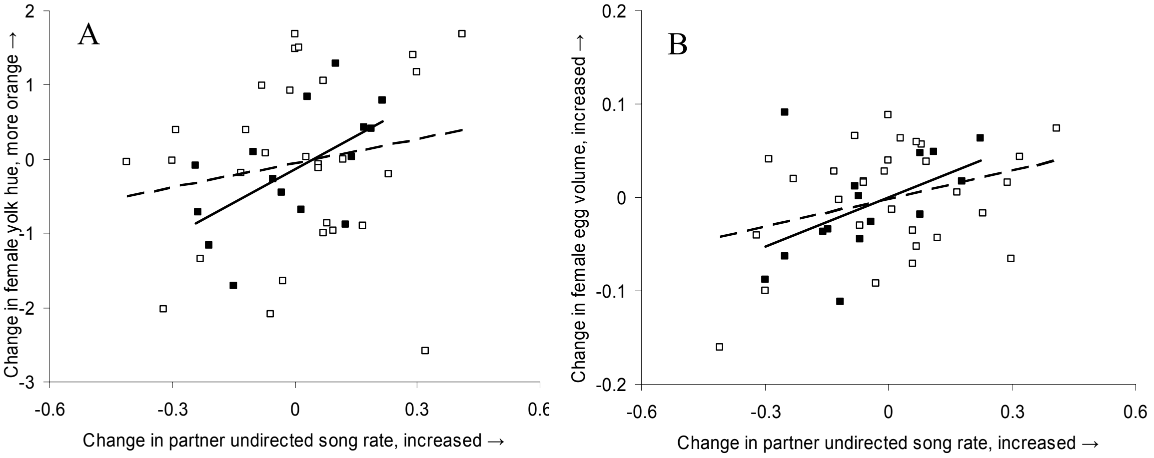

Figure 4:
Female change in investment with partner 2 minus partner 1 related to change in song rate of partner 2 minus partner 1. Females laid eggs with significantly more orange yolks (4A, P = 0.01) and larger egg volume (4B, P = 0.021) with the partner with the higher song rate. For ease of interpretation, higher values of yolk hue correspond to more orange (as opposed to yellow) yolks. Cage experiment 1 is represented by filled squares and solid regression lines, cage experiment 2 by open squares and dashed regression lines.

DISCUSSION

As expected, we found support for the mate attraction function of song. However, to our knowledge, this is the first study to show a significant negative correlation between song rate and a measure of fitness in a bird species. Among paired males, males that sang the most had the lowest success at siring eggs. This was also reflected in the lower extra-pair success of high song-rate males, while there was only a weak trend for high song-rate males to loose more paternity within the pair-bond. This counterintuitive finding suggests that song does not always positively reflect male quality, at least not in our captive zebra finch population.

The honest signalling function of song rate assumes that honesty is ensured by costs (see Introduction). We will therefore review the evidence for condition dependence imposed by energetic constraints on song rate, and discuss whether constraints other than energy costs could limit song rate. The lower success of high song rate males in our study implies that song rate would predict low, rather than high, quality. Why, then, do males continue to sing after social pairing? We offer two possible explanations for the unexpected pattern, and while some suggestions are necessarily somewhat speculative, they offer testable predictions. First, we suggest that song rate could reflect investment into the pair bond. Second, our study found support for the often overlooked reproductive stimulation function of song, since females invested more into reproduction when paired to a male with a higher song rate.

Mate attraction

As expected, unpaired males sang at higher rates than paired males. This confirms the widespread finding that males that stay unpaired the longest have the highest song rates (reviewed in Kroodsma and Byers 1991). Thus, it seems plausible that song functions in mate attraction, as has been suggested for zebra finches (Dunn and Zann 1996b; Tomaszycki and Adkins-Regan 2005). In our experiment, there was limited scope for unpaired males to pair, especially in the male-biased sex ratio treatment, where all available females were paired to other males. Hence, unpaired males had no chance to attract a partner, in spite of a high song rate.

Limitations to high song rates

The energetic costs of singing at a high rate are often assumed to enforce the honesty of song rate as a quality indicator (Catchpole and Slater 2008). Several studies under laboratory conditions, on a number of passerines, including zebra finches, have found remarkably low energy costs of singing (Oberweger and Goller 2001; Franz and Goller 2003; Ward et al. 2003; Ward et al. 2004). Nevertheless, in the wild, song rate is lowered by scarceness of food and low temperatures in several species (reviewed by Gil and Gahr 2002). In zebra finches, there is some evidence for such an effect (Dunn and Zann 1996b; Johnson and Rashotte 2002). This implies that song rate could be an honest signal of quality under some circumstances, but apparently when food is abundant, this is not the case. However, there is also evidence that time constraints between singing and behaviours such as foraging or seeking EP-copulations are a limiting factor to song rates (reviewed in Searcy and Yasukawa 1996). Such trade-offs might actually be more important than direct energy costs. Hence, even under laboratory conditions, song rate might be constrained.

Pair bond maintenance

Zebra finch song is readily individually recognisable (even to the human observer, personal observation), and shows several characteristics typical for traits that are used in individual recognition (Dale et al. 2001). Females have been shown to recognise their partner based on song alone (Miller 1979), and auditory contact (songs and calls) alone is enough to sustain the pair bond also during prolonged periods of visual separation (Silcox and Evans 1982). In the wild, 70% of the total amount of song occurred within 1 meter of the nest and 88% while the female was inside the nest (Dunn and Zann 1996a). Thus, sustained singing during all periods of the year could function in pair bond maintenance. Low quality males might need to invest more into maintaining the pair-bond, necessitating higher song rates. This could produce the unexpected negative correlation between song rate and fertilisation success.

Reproductive stimulation of the partner

We found that females invested more into reproduction when paired to a partner with a higher song rate. This suggests that song physiologically stimulates the female to invest into her eggs. If energy costs do not limit song rate, one could expect that high quality males would sing at least as much as low quality males, in order to increase female investment. However, if a time trade-off exists between singing close to your partner and flying away to seek EP-copulations, high quality males would gain more from focusing their reproductive investment less on their social partner and more on seeking EP-copulations.

Considering the negative relationship between male song rate and our measure of fitness, the female investment pattern is concordant with compensatory investment, where the female invests more when paired to a male of lower quality (Navara et al. 2006). This could reflect sensory exploitation by low quality males, if females are stimulated by song due to a sensory bias. Alternatively, it could be adaptive for the female to invest in a compensatory manner, if offspring sired by a low quality male would especially benefit from increased resource deposition into the eggs. Song rate could mediate this investment trade-off. This idea fits with a previous finding from our population, where females invested more into reproduction when paired to a low genetic quality male than when paired to a high genetic quality male (Bolund et al. 2009). The low repeatability of song rate between aviary and cage conditions means that male genetic quality measured in the aviary was not correlated with his subsequent song rate in cages (data not shown). Thus, the effects of genetic quality (Bolund et al. 2009) and song rate (this study) on female investment that we found under cage conditions, are independent.

Conclusions

Our results do not support the hypothesis that song rate signals male quality through condition dependence in the zebra finch, at least not under *ad libitum* food conditions. Rather it seems that differences in song rates represent alternative strategies for males to maximize reproductive gain, where the time trade-off between singing and seeking EP-copulations is mediated by male quality. This implies that song-rate is not the main determinant of male attractiveness in zebra finches.

Using actual paternity data from a free-flying aviary setting, we were able to relate song rate to success at siring eggs. The unexpected finding that higher song rate was correlated with lower success provided critical insights toward the interpretation of the follow-up study, where females invested more for high-rate singers. Under the traditional view of high song rate as an honest indicator of male quality, we could have interpreted this investment pattern as positive differential allocation. However, the negative correlation between song rate and success in the aviary experiment, considered together with a previous finding from this population (Bolund et al. 2009), suggest that the investment pattern reflects compensatory investment. We are not suggesting that males are directly signalling their low quality by singing at a high rate, but rather that the average low-quality male benefits more from stimulating their partner than the average high-quality male (due to time trade-offs with e.g. seeking EP-copulations), leading to higher song rates in low quality males. Thus, low quality males might be doing the ‘best of a bad job’, investing their time into the reproductive stimulation of the partner, rather than into seeking EP-copulations.

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Chapter 5

Compensatory investment in zebra finches: Females lay larger eggs when paired to sexually unattractive males

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ABSTRACT

The classical version of the differential allocation hypothesis states that, when females reproduce over their lifetime with partners that differ in their genetic quality, they should invest more into reproduction with high-quality males. However, in species with lifetime monogamy, such as the zebra finch, partner quality will typically remain the same. In this case the compensatory investment (CI) hypothesis predicts higher investment for low-quality males, because low genetic-quality offspring are more dependent on maternal resources. Here we show that female zebra finches invested more resources, both in terms of egg volume and yolk carotenoid content, when paired to a low genetic-quality male, as judged from his previous ability to obtain extra-pair paternity in aviary colonies. We also found that females deposited slightly larger amounts of testosterone into eggs when paired to a low parental-quality male, as judging from his previous success in rearing offspring. This is the first experimental support for the CI hypothesis in a species with lifetime monogamy. We stress that in more promiscuous species, the benefits of classical differential allocation may partly be neutralised by the supposed benefits of CI.

INTRODUCTION

Life-history theory predicts that females should adjust their investment in a particular breeding attempt according to the expected pay-offs of that attempt (Williams 1966). Male attractiveness can influence offspring fitness and, hence, should affect female investment into reproduction. Two opposing patterns have been found in empirical studies: either that females invest more when mated with attractive males, or, less commonly, that they invest more for unattractive males. Both these patterns can be referred to as differential allocation in the broad sense, but we suggest that it is conceptually fruitful to clearly separate the two types.

Positive differential allocation

Positive differential allocation (PDA) is built on the logic that females trade off their current versus future reproduction (Trivers 1972) and that this trade-off is influenced by the attractiveness of the partner. If partner quality is expected to vary over an individual's lifetime, the female should invest more when paired to a more attractive male, since good gene effects will increase offspring value and eventually result in more grand offspring and, hence, higher fitness. This is the case in mating systems with high reproductive skew, where females should preferentially invest in the highest quality offspring. PDA has found support in a range of taxa (reviewed by Sheldon 2000). Somewhat unfortunately, the general term differential allocation has been widely accepted in this narrow sense of denoting only PDA.

Compensatory investment

If low genetic-quality offspring are more sensitive to environmental stress (e.g. shortage of nutrients) than high genetic quality offspring, then it may pay females to increase their investment when paired to a male of low genetic quality. This investment pattern is called compensatory investment (CI) and has been observed in a few studies (Saino et al. 2002; Michl et al. 2005; Navara et al. 2006; Gowaty et al. 2007). This is especially likely in a mating system with low reproductive skew, where even low-quality offspring will reproduce successfully. In addition to assessing the genetic quality (indirect benefits) of a partner, females could also judge the situation based on male parental quality (direct benefits). Thus, increased primary investment (for example egg nutrients) could compensate for both low genetic quality and low parental quality.

The benefits of PDA and CI have to be weighted against each other. Hence, the ecology and life-history of a species will determine what process is most likely to occur. Two decisive factors are the likelihood of mating with a higher quality partner in the future and the degree of reproductive skew in the population. In addition to partner quality, a range of environmental factors could influence female investment into reproduction. We therefore use an experimental approach where we can control for a number of such factors, either experimentally (e.g. food availability, time of season, etc.) or statistically (lay order effects).

Previous work on zebra finches

Burley pioneered the field of differential allocation, but in contrast to us, she concentrated on secondary investment (i.e. feeding rate, Burley 1986, 1988) rather than primary investment into eggs. Since her now classical work, the zebra finch has become a model species for studies on differential allocation (Burley 1986, 1988; Swaddle 1996; Balzer and Williams 1998; Gil et al. 1999; Rutstein et al. 2004; Gorman et al. 2005; Rutstein et al. 2005a; Rutstein et al. 2005b; Gilbert et al. 2006; Williamson et al. 2006). However, empirical studies on female primary investment in relation to male attractiveness have found mixed results (Balzer and Williams 1998; Rutstein et al. 2004; Gilbert et al. 2006; Williamson et al. 2006). Three of these studies used artificial ornaments (i.e. colour rings) to manipulate male attractiveness and found no significant main effects, but rather two- or three-way interactions with different investment patterns for attractive versus unattractive males, either with respect to egg laying sequence (Gilbert et al. 2006; Williamson et al. 2006) or with respect to female mass (Rutstein et al. 2004). The biological interpretation of these interactions is not straightforward. Moreover, it is unclear what aspects of male attractiveness are manipulated with coloured rings, since female zebra finches will likely choose males both for indirect ('good genes') and direct (for example parental abilities) benefits. The same is true for choice chamber attractiveness, that was used by Balzer and Williams (1998) and von von Engelhardt (2004). To disentangle the effects of indirect and direct benefits, an experimental approach is needed that uses separate measures of male sexual attractiveness (reflecting good genes) and of male parental quality.

The present study

In the present study, we look at female zebra finch primary investment into reproduction in relation to actual measures of male sexual attractiveness as ascertained by females themselves. To do this, we used birds that had participated in a breeding experiment under free-flying aviary conditions. This allowed us to obtain reliable measures of male sexual attractiveness and parental quality. A male's sexual attractiveness was measured as his success at siring extra-pair (EP) offspring, and parental quality as the success at rearing offspring. Based on these measures, we chose pairs of males with extreme values of sexual attractiveness (matched for parental quality) and pairs of males with extreme values of parental quality (matched for sexual attractiveness). We then paired females sequentially to two males, one from either extreme of one of these axes. Since investment measures differed among previous studies, and since different aspects of the investment could be influenced by either genetic or parental quality, we decided to simultaneously measure a range of egg traits. Thus, as measures of female primary investment, we use egg volume, yolk mass, an index of yolk carotenoid content, yolk testosterone content and the number of eggs laid.

Study species

The zebra finch is an opportunistic breeder with biparental care that forms lifelong pair bonds in nature (Zann 1996). Under laboratory conditions, divorce rates of established pairs is extremely low (Forstmeier 2007), whereas re-pairing readily occurs after partner loss. Typically, several broods are reared during one rainy season (Zann 1996). Mortality between breeding seasons is rather high (Zann 1996), and since pairs remain together also in the non-breeding season, both partners are likely to be affected simultaneously by harsh environmental conditions, such as droughts (Immelmann 1970). Thus, the probability to survive beyond the first partner and reproduce with another partner is rather low. Moreover, it seems likely that there is assortative mating for quality in zebra finches (Burley 1986; Burley and Foster 2006). Thus partner quality is expected to vary only little over an individual's life time. Considered together with the low reproductive skew that characterises the zebra finch mating system, we assume that there has been limited scope for PDA to evolve, while we would expect to find CI.

METHODS

General design

We chose the 32 females with the highest average fecundity from two breeding rounds in a previous aviary breeding experiment. They were split randomly into two groups of 16 females and each female was paired sequentially to two males. Males were chosen from the same aviary experiment based on two criteria: success at siring EP eggs and parental quality. One group of females was assigned males based on EP attractiveness, so that the two partners of a female had maximal difference in EP attractiveness, while the parental quality was as similar as possible between the two partner males, and vice versa for the other 16 females. Within treatments, males were assigned randomly to females, but ensuring that a female had not been in the same aviary as any of her two partners.

Subjects and housing

All subjects were kept at the Max Planck Institute for Ornithology in Seewiesen, Germany, since October 2004. For details of rearing conditions for these birds, see Forstmeier (2005). During the experiments, pairs were housed in cages measuring 60 × 40 cm and 45 cm high in two rooms. For details of housing conditions, see Bolund et al. (2007).

Aviary experiment

We measured sexual attractiveness and parental qualities under free-flying aviary conditions. Briefly, six females and six males in each of nine aviaries were allowed to breed for three months in September–November 2005. As part of a sex ratio treatment, three aviaries had an additional three females, three aviaries an additional three males, while the remaining three had no additional birds added, giving sex ratios of 0.4, 05 and 0.6, respectively. After a non-breeding period without nesting opportunities, breeding pairs were exchanged among aviaries and sex ratio treatments, such that each pair faced five new unfamiliar pairs, and allowed to breed for another three months in April-June 2006. In total, due to the replacement of birds that died, 139 birds were used in this experiment. The paternity of 1622 eggs or offspring was determined using 10 microsatellite loci (Forstmeier et al. 2007). Of these, 99.9 per cent of samples were assigned unambiguously to genetic parents. The EP paternity rate (EPP rate) was 34 per cent of eggs or offspring. We defined EPP as eggs sired by a paired male with any female other than his partner. Each pair was allowed to rear one clutch (or two to three, if initially unsuccessful) per breeding round. Eggs were fully cross-fostered between the aviary pairs and 72 foster pairs kept in cages, and each aviary clutch was restricted to 2-3 eggs (2.44 ± 0.53 mean ± s.d.). In total, 275 fertile eggs were fostered into the aviaries and they resulted in 109 fledged offspring.

Measure of genetic quality

In our population, the most likely reason for females to seek EPP is for indirect benefits, i.e. good genes. This is supported by the high repeatability of male EPP success with different sets of potential EP partners in the aviary experiment (ANOVA: $F_{59,60} = 3.72$, $P < 0.001$, $R = 0.58 \pm 0.09$), suggesting that females are choosing males for EP copulations based on their perception of the male's genetic quality. However, the EPP success of a male depends both on female choice and male sex drive (i.e. the tendency to seek EP copulations). To obtain a measure reflecting female perception of male genetic quality, we used residuals of a male's average EPP success, accounting for sex ratio treatment and correcting for courtship rate measured in standardized trials where a male and a female were allowed to interact for 5 minutes in a cage setting (Male sex drive measured in this way is repeatable over long periods of time, $R \pm \text{s.e.} = 0.60 \pm 0.07$, Forstmeier

2007). We picked the 8 males with the highest and the 8 with the lowest residual EPP scores that also had estimates of parental quality (see below). Out of the 16 males chosen for the current experiment based on their EPP success, the 8 unattractive males sired 1.1 ± 1.8 (range 0-4) EP offspring each, and the 8 attractive sired 29.4 ± 16.5 (range 17-57) EP offspring each (the males in the high attractiveness group sired on average 31% ± 13 of all available eggs in their aviary). Observations carried out in the 2006 breeding season, showed that only one out of 65 males never attempted an EP courtship during 22 hours of early morning observation (195h in total for the nine aviaries). The 8 unattractive males had two unforced copulations (forced copulation attempts are practically never successful, see also Forstmeier 2004; Forstmeier 2007) out of 66 courtships (3% success rate, range from 1 to 25 courtships per male), while the attractive group had 24 unforced copulations out of 133 courtships (18% success rate, range from 4 to 39 courtships per male). Thus, display frequencies were still higher in the attractive group compared to the unattractive group in our experiment, but, importantly, the success rate was much higher for the attractive males. Hence, while the correction for song rate is only partly successful in disentangling female choice from male sex drive, the paternity data is still more reliable than the limited number of observed copulations. Male success at fertilising EP eggs (EP success) was strongly dependent on the average EP female responsiveness (scored as described in Forstmeier 2007) to him in these observations (multiple regression: standardised $\beta = 0.35$, $p = 0.01$), while EP success was not dependent on EP courtship rate in the same observations ($\beta = 0.14$, $p = 0.31$). The high- and low- EP-attractiveness group were matched as closely as possible for parental quality.

Measure of parental quality

Male parental quality is difficult to measure directly, since actual feeding rates are difficult to estimate. However, in our population, despite ad libitum food conditions, readiness to feed the chicks varies greatly among individuals, such that offspring mass at the age of 8 days varied between 1.6 and 12.2g (Schielzeth et al. 2008). Furthermore, some birds let all or most of their offspring starve to death before starting to feed the brood. In a previous study, we measured offspring growth under cage conditions of one to two subsequent clutches for 38 females (mean ± s.d.: 1.36 ± 0.48 clutches) and 29 males (mean ± s.d.: 1.26 ± 0.44 clutches) with each of two different partners, i.e. each bird was a part of two different pairs. To estimate parental quality, we used a combination of the average offspring mass at day 8 (m) and fledging success ($s = n/e$) where n is the number of fledglings and e is the number of fertile eggs put into the nest. We combined these two measures by taking the average of ($m*s$) and (m). This was done to give more weight to the normally distributed m relative to the overdispersed measure s. The resulting term was z-standardized and then multiplied with ($\sqrt{e}$) to weight it by sample size. This measure was repeatable for a male with two different female partners (ANOVA: $F_{30.31} = 2.27$, $p = 0.013$, $R = 0.39 \pm 0.15$, E. Bolund, H. Schielzeth & W. Forstmeier, unpublished data) whereas for females, it was not (ANOVA: $F_{38.39} = 0.99$, $p = 0.52$, $R = 0.007 \pm 0.18$). This suggests that the parental quality of males is predictable, or at least that rearing success depends on male, but not female, identity in a predictable way. Hence, for the present study, we use the same estimate of parental quality, which was measured based on male breeding success during the aviary experiment. We chose the males from the eight best and the eight worst performing pairs out of the 58 pairs with available data. This created two groups that differed substantially in parental performance (e.g. the proportion of fertile eggs resulting in fledged offspring was 0.85 ± 0.13 vs. 0.07 ± 0.1, mean ± s.d. for the two groups, respectively). These extreme high or low levels of parental care require both the male and the female to be either 'good' or 'bad' parents. Our estimate of parental quality is less reliable for intermediate parental quality males (including the males chosen based on EP attractiveness), since an intermediate success can be achieved for example via compensation by the female following low investment of the male. The high and low parental quality group were chosen such that they were matched for EP attractiveness, see figure 1.

Male parental quality was not correlated with his residual extra-pair success ($r = 0.15$, $n = 56$, $p = 0.28$, based on all males from the aviary experiment who had an estimate of both variables).

Experimental procedure

Birds were paired in cages in January 2007, and allowed 42 days to lay two clutches (one female laid no eggs, one female laid one clutch only, while two females initiated a third clutch). After separation, they were given a non-breeding period of 52 days in same-sex groups in aviaries, with no auditory or visual contact with the previous partner. Birds were then paired to the second partner in April 2007 and allowed 40 days to lay two clutches (one female laid no eggs and four females laid only one clutch). On the day of laying, eggs were collected and replaced with plastic eggs, which were removed after a standard incubation period of 15 days after the day on which incubation was initiated. The length and width of the eggs was measured using callipers to the nearest 0.05 mm and egg volume was calculated according to the formula of an ellipsoid ($1/6*\pi*\text{length}*\text{width}^2$). During the experiment, 522 eggs were laid. Three birds died between the two breeding rounds of the experiment, and were replaced with birds fulfilling the above mentioned criteria. These deaths resulted in a total of 67 birds (34 males and 33 females) used for the experiment.

At the initiation of each breeding round, we measured the mass, fat score (clavicular and abdominal fat, scored on a scale from 0 to 5) and beak colour of all birds. We used a principal component type 1 analysis of 6 spectrophotometric traits for beak colour analyses (described in Bolund et al. 2007). As measures of female condition, we used residuals from a regression of mass over tarsus. We used five measures of female investment, egg volume (egg volume is positively related to offspring survival to adulthood in our population, random-intercept model: $t = 6.0$, $p < 0.0001$, $n = 1661$ eggs by 154 females, W. Forstmeier 2008, unpublished data), number of eggs laid per day of the experiment, yolk hue (see below), yolk androgen content and yolk mass. Correlations between female morphology and female investment can be seen in table 1.

Table 1:
Correlations between different aspects of female investment and female morphology. (Correlations are based on female mean values from the two breeding rounds. Yolk hue is used as a proxy for total carotenoid content in eggs. Female beak colour was measured before the onset of breeding with spectrophotometry. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.)

	yolk hue	egg volume	yolk mass	eggs per day	yolk testosterone	female beak colour	female mass	female condition
egg volume	0.14							
yolk mass	-0.12	0.45*						
eggs per day	0.40*	0.12	0.28					
yolk testosterone	-0.30	0.48**	0.01	0.43*				
female beak colour	0.35	0.03	0.20	0.14	0.26			
female mass	0.04	0.31	0.20	-0.20	0.065	-0.13		
female condition	0.05	0.11	0.16	-0.19	0.020	-0.10	0.86***	
female fat score	-0.07	0.05	0.09	-0.21	-0.29	-0.09	0.63***	0.54**

Yolk colour analyses

Carotenoids are pigments that are responsible for the hue of egg yolk and are essential to embryonic and early post-hatching development in birds (Surai 2002). Two studies using HPLC to measure carotenoid content in zebra finch yolks found mainly lutein, zeaxanthin and anhydrolutein, with small amounts of β-cryptoxanthin and β-carotene (McGraw et al. 2002; Royle et al. 2003). Carotenoid content decreases over the laying sequence (e.g. Royle et al. 2003; Williamson et al. 2006), but the relative percentages of component carotenoids remained unchanged in a comparison of first and last eggs in zebra finch clutches (Royle et al. 2003). The hue of zebra finch egg yolks varies dramatically among eggs and changes substantially from orange to yellow over the laying sequence within a clutch, while the saturation invariably was very close to 100 per cent (this study). This suggests that more orange yolks contain a higher total concentration of carotenoids. Studies comparing carotenoid content obtained from biochemical assays with the colour of carotenoid rich materials, like egg yolk or feathers, have confirmed that colour of egg yolks can be used as a reliable indicator of carotenoid content (Saks et al. 2003; Verboven et al. 2005). We thus assume that yolk hue can be used as a proxy for total carotenoid content in zebra finch egg yolks.

Out of 522 egg yolks, 514 were photographed relative to a colour standard (Q-13, Kodak Colour Separation Guide) under standardised conditions (Nikon D1x, Nikon DX80-flash), either on the day of laying or, in 12 per cent of the cases, eggs were refrigerated at 7°C on the day of laying, and photographed 1-2 days later. The albumen was removed with the use of a pipette and the yolk subsequently transferred to a slide for photographing and weighing (to the nearest 0.1 mg). We used a self-written R-function to extract RGB values (red, green, blue) of the yolk and yellow standard. This function sampled a pre-defined area, covering 30-50% of the yolk. Data sampled in this automated way accurately represented the hue of the whole yolk surface in a subset of yolks: $r = 0.99$, $p < 0.001$, $n = 48$. We used the function 'grDevices' in R to convert RGB into hue, saturation and brightness values. Each yolk was photographed twice and we used the averaged hue for further analyses. The repeatability of yolk hue between the two photos of one yolk was extremely high (ANOVA: $F_{516.517} = 235$, $p < 0.001$, $R = 0.99 \pm 0.0007$). Brightness measured on the colour standard varied slightly among photographs (mean ± s.d.: 0.96 ± 0.01). Hence, hue of the yolk was corrected by taking residuals from a regression over brightness of the standard. Hue is a circular variable (0-360 º), but the range covered by variation in egg yolks (33–49 º) is sufficiently small to use linear statistics.

To obtain one measure of yolk hue per female for each of her male partners, we used the random effect estimates for each pair from a linear mixed effect model of residual yolk hue with laying order within clutches as a fixed effect covariate and pair identity and clutch identity as random intercepts. This model accounts for variation over the laying sequence and between successive clutches with the same male. Models were run in R 2.5.1 (R Development Core Team, 2007) using the 'lme' function from the nlme package (Pinheiro et al. 2008).

Yolk testosterone content

Testosterone (T) levels were analyzed in egg 1 and 4 of each clutch where this was possible. In total, 189 eggs were analysed for T. Frozen yolks were thawed and homogenized with an equal amount of double-distilled water. Two hundred microlitres of the yolk-water mixture was used; 1500 dpm 3H-testosterone was added to measure extraction efficiency, incubated overnight, and steroids extracted by freeze decanting using a mixture of 4 ml petroleum ether/diethyl ether (30% vol./70% vol.), repeated twice. The petroleum ether/diethyl ether phase was dried at 39°C under a stream of nitrogen. Then, 90 per cent ethanol was added and samples frozen overnight to precipitate proteins. The next day, the ethanol phase was removed and washed twice with hexane to remove lipids. Then, the ethanol was dried at 50°C under a stream of nitrogen and samples resuspended in phosphate buffered saline with gelatine buffer. Extraction efficiency was 58 ± 8 per cent (mean ± s.d.). T concentrations were measured by radioimmunoassay following Goymann et al. (2006). Because the T antibody has a cross-reactivity of 40 per cent with 5α-dihydrotestosterone our measurement refer to androgens. Samples were analyzed in two assays. The detection limits were 0.6 and 0.9 pg per tube. Intra-assay variations were 5 and 3.1 per cent. The inter-assay variation was 6.1 per cent. T-values (pg per mg yolk) were ln-transformed to approach normality. To obtain one measure of T per female with each of her two partners, we used the same method as for yolk hue, i.e. we used the random effect estimates from a linear mixed effect model with laying order within clutches as a fixed effect covariate and pair identity and clutch identity as random intercepts. T deposition was highly repeatable within females between her two different partners (ANOVA: $F_{27.27} = 6.5$, $p < 0.001$, $R = 0.73 \pm 0.09$). In a subset of 10 females, T levels were highly repeatable between the current experiment (in cages) and the previous experiment in aviaries in 2005-2006 ($r = 0.75$, $p = 0.013$, $n = 10$).

Interaction effects on allocation

Two previous studies looking at female investment in relation to male manipulated attractiveness found different investment patterns over the egg laying sequence for attractive versus unattractive males (Gilbert et al. 2006; Williamson et al. 2006). We therefore tested for interactions between treatment and lay order, using linear mixed effect models. Apparently, previous studies have accounted for the non-independence of data points (eggs laid by the same female) by including female identity as a random effect (allowing females to differ in their means, i.e. intercepts). However, females also differ randomly in their slopes of investment over the laying order. If random variation in slopes is not controlled for, there is a dramatically increased risk of type I errors for the interaction between laying order and treatment (Schielzeth and Forstmeier 2009).

Hence, our linear mixed effect models include random intercepts as well as random slopes for female identity and clutch identity. This accounts for between-individual and between-clutch variation in overall response values as well as laying order slopes. Predictors were male EP attractiveness and lay order including both main effects and the interaction.

Statistics

We used SPSS (SPSS Inc. 2004) and R 2.5.1 (R Development Core Team, 2007) for statistical analyses. All statistical tests are two-tailed and α was set to 0.05. Data was transformed if necessary to approach normality. Repeatabilities and their standard errors were calculated following Lessells and Boag 1987 and Becker 1984. G*Power 3.0.8 (Franz Faul, Universität Kiel, Germany, 2006) was used for power analysis.

RESULTS

The repeatability of female investment between the two different partners was generally very high (table 2).

Table 2.
Repeatability of female investment with two different partners. Yolk hue is used as a proxy for carotenoid content.

	d.f	*F*	*p*-value	repeatability ± s.e.
yolk hue	29, 30	9.90	<0.001	0.82 ± 0.06
egg volume	29, 30	16.4	<0.001	0.89 ± 0.039
yolk mass	29, 30	9.13	<0.001	0.80 ± 0.064
eggs per day	30, 31	6.83	<0.001	0.74 ± 0.080
yolk testosterone	27, 27	7.28	<0.001	0.76 ± 0.083

Effects of extra-pair attractiveness

In the group of females that were assigned males based on EP attractiveness, the change in female investment was correlated with the change in the male trait. When paired to a male with lower EP attractiveness, females laid eggs that were significantly larger (egg volume: $r = -0.69$, $n = 15$, $p = 0.005$, mean ± s.d. with attractive male: 1.20 ± 0.11, mean ± s.d. with unattractive male: 1.23 ± 0.12, this result remained significant after the most conservative Bonferroni correction, i.e. for conducting 10 tests) and produced more orange yolks (yolk hue: $r = 0.52$, $n = 15$, $p = 0.046$ mean ± s.d. with attractive male: 43.3 ± 1.84, mean ± s.d. with unattractive male: 42.9 ± 2.35, lower hue values correspond to more orange yolks). Both relationships remain significant when including the second group of females that were assigned males based on parental quality (figure. 1). Results remained qualitatively the same when using an alternative analysis with female absolute values of investment as the response and controlling for male parental quality in linear mixed effect models (data not shown). Change in T levels in the yolk, yolk mass and number of eggs laid were all not influenced by change in male EP attractiveness (all $r < 0.24$, all $p > 0.38$). There were no significant interaction effects of male attractiveness on female investment over the laying order of a clutch (egg volume: $F_{1,392} = 0.75$, $p = 0.39$, yolk hue: $F_{1,390} = 0.19$, $p = 0.67$).

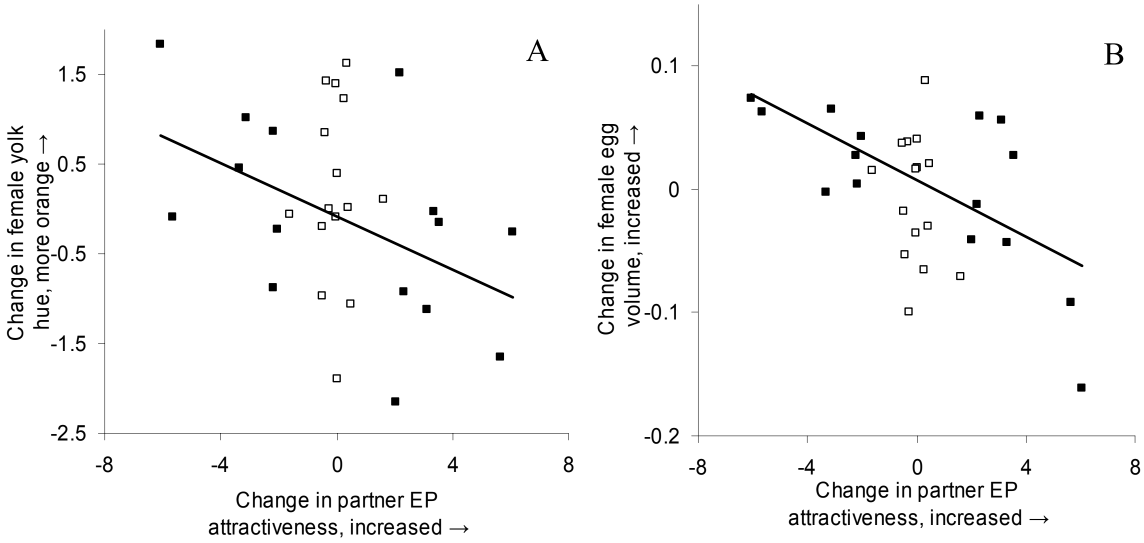

Figure 1.
(A) Change in egg volume when females were paired to two different partners (partner 2 minus partner 1) related to change in extra-pair (EP) attractiveness of the partners (partner 2 minus partner 1). Females laid significantly larger eggs with the partner with lower EP attractiveness (all females pooled: $r = -0.55$, $n = 30$, $p = 0.002$). (B) Females laid eggs with significantly more orange yolks with the less attractive partner. For ease of illustration, higher values of yolk hue correspond to more orange eggs (all females pooled: $r = -0.38$, $n = 30$, $p = 0.038$). The 15 females paired to males with maximal difference in EP attractiveness are represented by filled squares, while the 15 females paired to males with maximal difference in parental quality are represented by open squares.

Effects of parental quality

The change in T levels in the yolk were correlated with the change in male parental quality (figure. 2). However, the changes in T levels in response to this treatment were small compared to the changes observed in the other group of females that were assigned males based on EP attractiveness. Hence, the correlation across all females was clearly non-significant (figure. 2). The change in all other investment traits, i.e. egg volume, yolk hue, yolk mass and number of eggs laid, were not correlated with the change in male parental quality (all $r < 0.31$, all $p > 0.24$). There were no significant interaction effects of male parental quality on female investment over the laying order of a clutch (all $F < 1.75$, all $p > 0.18$).

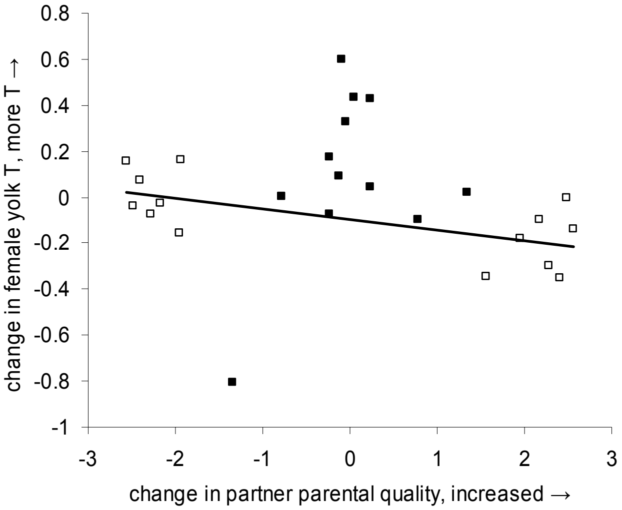

Figure 2:
Change in female yolk testosterone (T) deposition when paired to two different males (partner 2 minus partner 1) related to change in parental quality of the partners (partner 2 minus partner 1). Females assigned males based on parental quality deposited more T when paired to a low parental quality male (r = -0.70, n = 14, p = 0.0052, see regression line). When all females are pooled, this is not significant (r = -0.17, n = 26, p = 0.42). Females paired to males with maximal difference in EP attractiveness are represented by filled squares, while females paired to males with maximal difference in parental quality are represented by open squares.

DISCUSSION

In concordance with the compensatory investment hypothesis, females invested more resources into their eggs (larger eggs and more orange yolks) when paired to a male of lower sexual attractiveness. Females paired to a male of low attractiveness could be making the most of a bad situation. This is similar to the finding of Navara et al. (2006), who concluded that female house finches may alter the condition of lower quality offspring to salvage an otherwise unsuccessful breeding attempt. We found no effect of male attractiveness or parental quality on number of eggs laid or yolk mass. Similarly, in earlier studies of female primary investment in relation to male attractiveness in zebra finches, no or only very weak effects on clutch size have been found (Balzer and Williams 1998; Rutstein et al. 2004; von Engelhardt 2004; Gilbert et al. 2006).

Female T deposition was not related to male attractiveness. Thus, the hypothesis that low genetic quality offspring would not be able to tolerate higher levels of T (see Groothuis et al. 2005) is not supported by our data. Interestingly, females deposited more T in the yolk when paired to a male of low parental quality. While this was highly significant, the effect was only minor in relation to the overall variation, see figure 2. Thus, it is questionable whether this difference in T levels confers any biological relevance. Still it should be mentioned that the large within-female changes in T levels in the EP attractiveness treatment group does not invalidate the significant effect obtained in the parental-quality treatment group, because our estimates of male parental quality may be valid only for extreme cases, i.e. the latter group (see Methods). Nevertheless, the pattern is consistent with the previous finding of Gil et al. (1999), showing that females increased androgen deposition when paired to a red-ringed male compared to when the same females were paired to a green-ring male. Red-ringed males have been found to provide less parental care (Burley 1986, 1988; Burley et al. 1994; Burley et al. 1996). Thus, both studies imply that females might adjust T deposition in response to expected male parental quality rather than in response to male perceived genetic quality. This is in accordance with the idea that females might use T to manipulate partner parental contribution (see Groothuis et al. 2005; Hinde and Kilner 2007), since higher T levels in the yolk causes chicks to beg more intensely (Schwabl 1996; Groothuis et al. 2005). The other primary investment traits measured were not influenced by male parental quality. It might be that egg nutrients are an inefficient compensation for the decreased male chick provisioning. Male parental quality would then likely influence female secondary, rather than primary, investment (see Burley 1988).

Possible ultimate and proximate explanations

If one assumes that CI is an adaptive strategy for the female, this would require that an increase in egg nutrients confers larger fitness benefits for low genetic quality offspring than for high genetic quality offspring. It is possible that this is the case in a mating system with low reproductive skew where most individuals will be able to find a partner. If provided enough resources during development, low genetic quality offspring might be able to reproduce nearly as successfully as high genetic quality offspring, whereas with more restricted resources, they might not reproduce at all. Thus, CI might generally confer a fitness advantage in species with low reproductive skew. In contrast, if reproductive skew is high, disproportionate investment in the highest genetic quality offspring would seem to pay more in terms of the production of grandchildren, since only the highest quality males will be able to reproduce.

A possible proximate explanation to the CI pattern is that male behaviour influences female investment. Males of low EP attractiveness are expected to focus their reproductive investment on their social partner instead of seeking EP copulations. This increase in within-pair investment could stimulate the reproductive physiology of the partner, so that she invests more in egg quality. One pathway of reproductive stimulation would be via stimulatory singing (Kroodsma and Byers 1991). We have some evidence that this is the case in our zebra finch population (Bolund et al. in preparation).

The occurrence of EPP has the potential to shift the expected investment pattern from CI to PDA (see Sheldon 2000). If the availability and genetic quality of EP males varies over the lifetime of a female, she is expected to invest more when having EPP with a high genetic quality male. However, it is questionable whether a female can adaptively adjust her investment in response to EPP, since the clutch will likely contain a mixture of within-pair and EP offspring (Birkhead et al. 2000). Also, in a colonially breeding species, such as the zebra finch, there should always be the opportunity to have EP copulations with a highly attractive male, leading to little variation in the attractiveness of genetic sires between clutches.

Previous studies

Two previous studies have found interaction effects of male attractiveness on female investment patterns over the egg laying sequence of a clutch (Gilbert et al. 2006; Williamson et al. 2006). Caution is required when assessing the statistical significance of such interaction effects (see Methods and Schielzeth and Forstmeier 2009).We found no interaction between male attractiveness and female investment over the lay order. While an increased investment in the last eggs of a clutch could help in alleviating the disadvantages to last hatched chicks, under the framework of CI, all offspring of an unattractive male would be of lower genetic quality and hence would all benefit from increased investment.

Previous studies on differential allocation in zebra finches have found no significant main effects regarding egg size or yolk carotenoid level. One study looked at carotenoid levels in egg yolks, and found a trend for an increased level of carotenoids in eggs laid for green-ringed (i.e. unattractive) males (Williamson et al. 2006). Four studies have looked at effects of natural or manipulated male attractiveness on egg size and found no consistent trends (Balzer and Williams 1998; Rutstein et al. 2004; von Engelhardt 2004; Gilbert et al. 2006).

Our significant results of male attractiveness on female investment beg the question of why such an effect has not been found in previous studies. We can think of three possible explanations that will be dealt with in detail below. (i) differences in experimental design. (ii) the strength of the experimental treatment and (iii) differences in information content of the traits used as indicators of male attractiveness.

Experimental design and effect sizes

Earlier studies on DA in zebra finches have often used a between-female design (Balzer and Williams 1998; Rutstein et al. 2004; Gilbert et al. 2006; Williamson et al. 2006). While this design has the advantage of avoiding possible carry-over effects on female investment, i.e. that females would be ‘primed’ by the attractiveness level of their first partner (Rutstein et al. 2005a), unfortunately, it also confers a considerable loss of statistical power. This is because between-individual differences in investment traits are much larger than within-individual flexibility, as signified by the high individual repeatability of investment (table 2). In our experiment, the observed within-individual change in egg volume (Fig. 1a), for the 16 females assigned males with maximum difference in EP attractiveness, was only 0.27 s.d. of the between-individual variation in our population (mean within-individual change in egg volume: 0.032 ± 0.058 cm^3, between-individual variation in s.d. = 0.11 cm^3). This is not surprising, considering egg size is highly heritable ($h^2 = 0.63 \pm 0.09$ in our population), with a limited flexibility (repeatability = 0.89 ± 0.04 in this study). To detect an effect of $d = 0.27$ using a between-female design, 330 females would be necessary for a power of 80 per cent.

Balzer & Williams (1998) used the more powerful within-female approach, and paired a female sequentially to her most and least preferred male from a six-way choice chamber (i.e. each female was paired to the extremes of six males). In our experiment, each female was instead paired to one of the eight most, and one of the eight least, attractive males out of 58 males. Using a simulated dataset of a male trait drawn from a standard normal distribution, one can compare the expected difference in trait value between a female's two partners if males are chosen in the two different ways described above. With our approach, the mean difference is 3.13 s.d., which can be compared to 2.15 s.d. for the approach by Balzer & Williams. If one assumes that EP attractiveness and choice chamber attractiveness are traits with similar information content to females, the expected effect size in the study by Balzer & Williams would be 0.21. With a power of 80 per cent, 27 females would be required to detect this effect. The actual sample size was close to this, with 22 females, yet no effect was found. This might imply that the assumption above is not correct, i.e. that the information content of choice chamber attractiveness and EP attractiveness might be different to female zebra finches. This is supported by the low correlation between choice chamber attractiveness and EP attractiveness in our population ($r = 0.24$, $p = 0.098$, $n = 50$, E. Bolund & H. Schielzeth 2008, unpublished data).

The use of colour rings to manipulate attractiveness

Using paternity data from a free-flying aviary situation, we were able to determine male actual success at obtaining EPP, thus overcoming the difficulty of obtaining reliable measures of male sexual attractiveness to females. The EP success of a male was highly repeatable under two different social situations, suggesting that it represents female perception of male genetic quality, if we assume that females choose EP males based on additive genetic benefits. In contrast, most previous studies have manipulated male attractiveness with coloured leg rings (Balzer and Williams 1998; Rutstein et al. 2004; Gilbert et al. 2006; Williamson et al. 2006) and it can thus be useful to consider what red and green rings might signal to female zebra finches. While a preference for red over green rings has been found in several populations in choice chamber experiments (Burley et al. 1982; Hunt et al. 1997, but see Ratcliffe and Boag 1987) to our knowledge, Burley and her co-workers are the only ones who have studied the effects of red and green rings on male EP success under aviary conditions. Burley et al. (1994) found that red-ringed males were more successful at obtaining EP copulations in aviaries. The success rate of red males in EP courtships was 2.4 times that of green males (based on 906 EP courtships, with 40 unforced copulations). This can be compared to our data. In the 16 males that were chosen on the basis of their EP success in aviaries, the attractive group had a success rate that was 5.8 times as high as that of the unattractive group (based on 198 EP courtships, with 26 unforced copulations). This weaker treatment effect of rings as compared to success in siring EP offspring would reduce the effect size from 0.27 s.d. to an expected effect size of 0.11 s.d.. Combined with a between-female design, this effect would require approximately 2000 females to be detected with a probability of 80 per cent.

In ringed birds, red-ringed males are more attractive for EP copulations and provide less parental care, while green-ringed males are less sexually attractive, but provide more parental care (Burley 1986, 1988; Burley et al. 1994; Burley et al. 1996). This apparent trade-off between EP attractiveness and parental quality in ringed birds, might not be observable under unmanipulated conditions, since males that are naturally more successful at obtaining EP copulations, will also be of higher genetic quality and hence also able to provide good parental care.

Conclusions

Our study highlights the usefulness of real measures of male quality, as opposed to manipulating unknown aspects of male attractiveness. When we use separate measures reflecting direct and indirect benefits, a new picture of female investment in relation to male attractiveness emerges in the zebra finch. Yet, importantly, our findings are not contradicted by earlier studies in any point (see Discussion above) and some of our findings are even in line with earlier results (Gil et al. 1999; Williamson et al. 2006). Our study further suggests that it is important to consider the mating pattern and life history of a species when investigating patterns of female resource allocation into reproduction. In less monogamous systems than the zebra finch, the benefits of PDA and of CI might have to be balanced against each other. If the benefits cancel each other out, neither PDA nor CI should evolve.

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The study was approved by the animal care and ethics representative of the Max Planck Institute for Ornithology.

General discussion

In this thesis, I set out to conduct a thorough investigation of the condition dependence and fitness consequences of ornaments, size and behaviour in the zebra finch. As is so often the case in science, the results I have presented raise more questions than they can begin to answer, and in this final chapter I will briefly synthesise the findings and suggest future directions. Table 1 offers an overview of the traits I focused on, the data I collected to attempt to pinpoint the function of these traits and the conclusions that can be drawn from this work. Overall, there were pronounced differences between males in reproductive success, but no single trait was strongly correlated with these differences. The strongest predictor of male success was body size, while beak colour, directed song rate and song structure were not important. Unexpectedly, undirected song rate was negatively correlated with male reproductive success. Females invested more into reproduction when they were paired to males of a lower genetic quality, and also when paired to males with a higher undirected song rate, both in line with a compensatory investment pattern. Below, I will review the evidence for condition dependence of key traits and their relationship with fitness.

Table 1.
Overview of focal traits, data collected and conclusions drawn regarding the condition dependence and fitness consequences of traits in the zebra finch. Abbreviations: BV: breeding value i.e. the genetic component of the trait, RES: environmental deviation from the breeding value.

		Traits				
		Courtship song rate	Undirected song rate	Song structure	Beak colour	Body size
Data	Condition dependent	no	no*	no	weakly	yes
	Affects competition	no*	no*	no*	no	yes
	Aviary success	no	negative	no*	no	positive
	Female investment	no*	yes	no*	no*	no*
Concl-usions	(Sexually) selected	on BV	negatively ?	no*	on BV	on RES
	Female indirect benefits	possibly?	?	no*	possibly?	no
	Proposed function	Male strategy	Female reproductive stimulation and pair bond maintenance	Identity signal	Signal of breeding status	Optimized by natural selection

* = Unpublished data from our population, not reported in manuscripts in this thesis

Signalling quality to potential partners and extra-pair females

According to honest signalling theory, females use honest quality indicators to choose high quality males. Honesty is maintained through costs, leading to a condition-dependent expression of ornaments across all members of the population. A common approach to study this is to identify a trait that looks like a sexually selected ornament (e.g. a colourful plumage ornament or an elaborate song) and to then test for condition dependence of this trait, with the expectation to find a positive relationship between condition (and hence quality) and ornament expression across all individuals in the population. This somewhat simplistic view, however, ignores the possibility that such 'ornament-like' traits might have several functions, or not serve as honest quality indicators at all. This possibility changes the expectation about the relationship between condition and ornament expression.

Song

The song of the zebra finch has received much research attention, and is usually seen as an honest indicator of male quality. The honesty of bird song as a quality indicator could be maintained by developmental or performance constraints. Developmental constraints would affect the song learning and hence the complexity of the song, while the time spent singing (i.e. song rate) would be limited by e.g. energy or time constraints. Looking at song rate, Houtman (1992) found that males with a higher directed song rate were more attractive to females in a choice chamber and that males that were more attractive in the choice chamber were also more preferred for EP-copulations. This has become widely cited as evidence for song rate as an honest signal of quality in this and other species.

Importantly, Houtman measured the rate of directed song, which means song that is sung during courtship, while song in any other context is referred to as undirected song. The majority of the song output is undirected song (e.g. 87 % of the total in the aviaries, chapter 4). Most research has focused on the courtship song, while the undirected song has received less attention (despite being the much more frequent song), though it has been suggested to function in mate attraction (Dunn and Zann 1996b). The results from my thesis, and from other studies on our population, challenge this established view.

Undirected song rate

The undirected song is sung regularly throughout the day, on average, about 7 % of 30-second intervals throughout the day contained undirected song in the aviaries (with substantial between-individual variation, chapter 3-4). While this song rate is not very high compared to many other song bird species (e.g. dusky warblers spent about 80% of 1-minute intervals singing during at least parts of the breeding season, Forstmeier and Balsby 2002), still the song rate could potentially be energy limited. However, contrary to the expectations of honest signalling theory (Gil and Gahr 2002), I found that males with a higher undirected song rate were less successful in obtaining paternity in the aviaries (chapter 4). Since the song rate was measured during the breeding seasons in the aviaries, cause and effect remains elusive: are males with a high song rate unsuccessful or are unsuccessful males singing at a high rate? Given that undirected song rate is flexible when the social conditions change (the repeatability between cage and aviary conditions was very low, chapter 4), the latter alternative seems more plausible. In any case, a high song rate clearly does not reflect high male quality across all males in the population. Instead, I found that song functions in the reproductive stimulation of the partner (chapter 4). Because I did not manipulate song rate, I cannot say if song rate is directly linked to female investment, or if characters correlated to the song rate are linked to female investment. To elucidate this further, manipulation of song rate without unwanted simultaneous manipulation of other traits would be ideal. While this would be extremely technically challenging, it would open a range of new possibilities to study the function of song.

Thus, the results from this thesis suggest that the undirected song rate primarily serves other functions than as a signal of high quality. For example, if undirected song stimulates partner investment and strengthens the pair bond, a low quality male might maximise his reproductive success by singing to the partner, while a high quality male might instead focus on seeking extra-pair copulation opportunities. Hence, low quality males might use the song rate as a 'best of a bad job' strategy and song rate could still reflect male quality among the low quality males.

In general, traits that appear to be ornaments and are costly to develop or maintain are thus not always predicted to show a simple relationship between ornament expression and quality across all individuals. This can be illustrated with an example from our own species. It is energetically costly to increase the urgency of our voice, and raising our voice often serves us in winning conflicts. Still, we would not expect that the individual, who on average, speaks with the highest urgency, has the highest status. Quite to the contrary, speaking in a very calm voice might even function as a signal of high status.

Consequently, the simplistic assumption that the condition dependence of honest quality indicators leads to a positive relationship across all individuals between condition and ornament expression needs to be nuanced. If the ornament serves other functions in addition to or instead of as an honest quality indicator, we can expect different relationships (positive, negative or no relationship) between condition and ornament expression on a population level, and also differences among sub-groups of the population.

Directed song rate

I do find some support indicating that directed song is related to male quality, since there was positive selection on the breeding value ('BV', the genetic component) of directed song rate (chapter 3). Moreover, in a study on our population, Martin (2008) found that the directed song rate was affected by inbreeding depression, with birds from full-sibling pairings having significantly lower song rates than outbred birds. These results both support the notion that directed song rate is positively related to male quality ('good genes'). However, I also found strong evidence contradicting the prevailing view. Directed song rate was not dependent on past condition (chapter 1). We also have found no indications that directed song rate is influenced by current condition in our population (unpublished data). This was to be expected, considering that the directed song is only sung during courtship, amounting to only a few minutes of song per day in the aviary experiments (chapters 3-4). Thus, given the low energetic costs of singing (Oberweger and Goller 2001; Franz and Goller 2003) and the ad libitum food, an energy constraint is hard to imagine. In the aviaries, males with a higher phenotypic song rate did not have a higher reproductive success and, somewhat surprisingly, were even avoided by females in extra-pair courtships (chapter 3). The directed song rate is highly individually repeatable and has a moderate heritability (Forstmeier et al. 2004, and unpublished data). Collectively, these findings suggest that song rate reflects male genetic quality only to a small extent (for example inbred birds have lower song rates), and that most of the variation is selectively neutral, reflecting heritable alternative male strategies related to variation in sex drive.

Some intriguing results from our population show that genes for male song rate also influence female behaviour, such that males with a high song rate have female relatives with a high promiscuity level (Forstmeier et al. manuscript). Thus, a promising way forward would be to test for sexually antagonistic selection on breeding values for song rate, since there was positive selection on male BVs in the aviaries.

Song complexity

Many previous studies in zebra finches have assumed that there is selection for increased song complexity. While studies on developmental stress in zebra finches have found some evidence that the complexity of the song could reflect past condition, the evidence is far from compelling (see chapter 1). I found no indications that developmental stress affected the complexity of the song (chapter 1). Also, females did not prefer more complex songs and neither were any aspects of song structure related to male success in the aviaries (unpublished data). The low complexity of the zebra finch song (on average, the full repertoire is presented in less than one second) compared to many other song bird species, indicates that song structure is unlikely to reflect developmental or production constraints. Dale et al. (2001) outlined general properties that are expected to be different between traits that signal quality versus traits that signal individual identity. The song of the zebra finch, by these definitions, clearly falls into the identity signal category, displaying (1) high variability among (but stereotypy within) individuals, (2) no condition dependence, and (3) no relation to fitness. I therefore suggest that song complexity does not signal male quality, but rather functions in individual recognition in this highly social species. This is corroborated by previous findings that females can recognise their partner based on song alone (Miller 1979).

Beak colour

The strikingly red beak of the male zebra finch is commonly viewed as a condition dependent quality indicator that is preferred by females. The results from our population partly support, but also partly contradict, this view. Similar to the directed song rate, I found positive selection on the breeding value of male beak colour (chapter 3). Beak colour was also weakly affected by developmental stress in both sexes (chapter 1). Another study on our population found that beak colour is affected by inbreeding depression (Martin 2008). These results all speak in favour of the possibility of beak colour as a quality indicator. However, I found no evidence that beak colour is preferred by females (chapter 3) and this is in line with previous findings from our population (Forstmeier and Birkhead 2004; Forstmeier 2007). Beak colour also played no role in male-male competition (chapter 2). Thus, considering that beak colour seems a possible candidate for an honest signal of quality, this lack of a function in a sexual selection context is puzzling.

Previous work by Burley and co-workers (Burley et al. 1992) hint at an alternative function of beak colour. They found that beak colour in both sexes substantially changes over the breeding cycle. Thus, in this colonially breeding species, beak colour might signal breeding status rather than for example genetic quality to potential extra-pair females. In the non-breeding season, when much of the social pairing takes place (Zann 1996), beak colour could still potentially reflect male quality, but in that case we would have expected to find a female preference for redder beaks in our population.

Body size

Zebra finch body size is not sexually dimorphic, indicating that it has unlikely been subject to sexual selection in the past. Therefore, it has been largely disregarded in previous studies. Surprisingly, I found that body size is the most promising trait to be used as a condition indicator. Tarsus length was strongly affected by developmental stress (chapter 1), it showed strong inbreeding depression (Martin 2008), it affected male-male competition (chapter 2) and it was related to reproductive success in the aviaries, in males but not females (chapter 3). All this taken together strongly suggests that there is potential for tarsus length to be sexually selected. However, chapter 3 showed that it was the environmental, not the genetic, component of tarsus length that was related to success. This lack of selection on the genetic component explains why sexual size dimorphism will not evolve, despite apparent sexual selection for increased size in males.

Plumage

The plumage ornaments of the zebra finch have received surprisingly little research attention, likely because early studies speculated that they might primarily be important in species recognition (e.g. Morris 1954) and one study found that plumage ornaments were not important for male extra-pair copulation success (Burley and Price 1990). I found that neither the cheek patches, nor the breast band were affected by developmental stress (chapter 1). These plumage ornaments were also not related to reproductive success in the aviaries (unpublished data). Thus, my results add little evidence that plumage ornaments could signal male quality. Burley and Symanski (1998) found that female zebra finches have a latent sensory bias for a novel ornament in the form of a white feather on the head. It seems plausible that the plumage ornaments have evolved via such sensory exploitation of a pre-existing bias in a mate choice context, and that they now function as threshold cues for species recognition. To test this sensory bias hypothesis, a promising approach would be to imprint female offspring on parents of colour strains lacking specific plumage ornaments and then reintroduce the ornament in a mate choice context in adulthood.

Influencing the female after pairing

Many studies have looked at female investment in relation to male attractiveness in zebra finches, all with the expectation to find positive differential allocation, that is, that females invest more for more attractive males. However, results have been mixed, probably partly due to the manipulation of poorly understood aspects of male quality. Therefore, in chapter 5, I decided to use a different approach to this question. The breeding under aviary conditions showed that some males were clearly and consistently more successful at obtaining paternity. Because there was no single most important trait, I used fertilisation success in aviaries as an integrated proxy of male attractiveness and, hence presumably, genetic quality. This allowed me to test the differential allocation hypothesis by assigning males of known genetic and parental quality to females, instead of manipulating unknown aspects of attractiveness. Surprisingly, I found that females invested more when paired to a genetically less attractive male. This investment pattern is known as compensatory investment. To understand these results, one must consider the ecology of the species studied. The zebra finch has life-time monogamy and high mortality between breeding seasons. The low probability of future reproduction with a higher quality partner would favour individuals that make the best of the current situation, and compensate for a low quality partner by investing more. However, the results do not reveal if this is an active or a passive process, that is, if the female investment pattern is an adaptive response to male attractiveness or a passive response to male manipulation.

That males differ in their attractiveness to females indicates that they also differ in their genetic quality, even though the specific traits used by females to judge this remain elusive. Chapter 4 might offer part of an answer to this conundrum by suggesting an alternative function of undirected song. Females invested more into reproduction for a partner with a high undirected song rate, which is consistent with the compensatory investment pattern found in chapter 5, if low quality males spend their time stimulating partner reproductive investment through song, while the high quality males spend their time seeking extra-pair copulation opportunities.

Conclusion

With this thesis, I have opened a window into the process of sexual selection in the zebra finch. I found that the routes to reproductive success for a male zebra finch are diverse and that several of the classical views of this model organism need to be reinterpreted. In particular, I have cast new light on the function of song and on the differential allocation hypothesis. My results illustrate the importance of taking into account the ecology, life history and mating system of the study species in question, since the patterns of sexual selection are often complicated. Therefore, to reach a full understanding of the processes of sexual selection in a species, an integrative approach is needed. The advent of methods that are moving the focus from the phenotypic to the genetic level show promise for the future.

In conclusion, I hope to have made a small contribution to the accumulation of knowledge that drives our field forward and slowly leads us from simplistic views to a more and more nuanced comprehension of the process of sexual selection.

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E. B. contributed with study design, practical work, data analysis and writing; H. S. with practical work, discussion and writing; W. F. with practical work, concept and study design.

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Declaration

Ehrenwörtliche Versicherung

Ich versichere hiermit ehrenwörtlich, dass die vorgelegte Dissertation von mir selbständig und ohne unerlaubte Hilfe angefertigt ist.

München, den ....
Elisabeth Bolund

Erklärung
Hiermit erkläre ich, dass die Dissertation nicht ganz oder in wesentlichen Teilen einer anderen Prüfungskommission vorgelegt worden ist und dass ich mich anderweitig einer Doktorprüfung ohne Erfolg nicht unterzogen habe.

München, den ....
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