Inbreeding depression of sexually selected traits and attractiveness in the zebra finch

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An underexploited approach to study condition dependence of sexually selected ornaments is to use inbreeding to manipulate individual genetic quality. Because of differences in genetic architecture, sexually selected traits are expected to suffer stronger inbreeding depression than morphological traits. We used full-sib mating to investigate the effects of inbreeding on viability, attractiveness, morphology and potentially secondary sexual traits in male and female zebra finches, *Taeniopygia guttata*. Unexpectedly, the genetic load from lethal alleles (leading to early mortality) was low. In males, we found strong negative effects of inbreeding on song rate, beak colour, body size and choice chamber attractiveness, whereas body condition, fat deposition, song structure and plumage ornaments were unaffected. In females, beak colour, body size, fat deposition and choice chamber behaviour showed significant inbreeding depression. These results indicate that some but not all adult phenotypes are affected by inbreeding, most probably because of the homozygous expression of slightly deleterious mutations. Our findings complement alternative approaches to study the condition dependence of traits that may be relevant in mate choice or competition.

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To determine the indicator function of ornaments is of central importance to sexual selection research. To be useful in mate choice, ornaments should honestly signal aspects of an individual's condition or quality (Zahavi 1975; Grafen 1990; Getty 2006). If the marginal costs of ornament elaboration decrease with increasing male quality, high-quality signallers will be more effective at converting ornaments into fitness. This will lead to condition-dependent expression of secondary sexual traits (e.g. Johnstone 1995; Rowe & Houle 1996). Condition in this context can have genetic and environmental components, and ornaments can thus evolve to reflect either genetic quality ('indirect benefits') or environmental quality ('direct benefits'), or both (Iwasa & Pomiankowski 1999). There are multiple ways to assess whether ornaments reflect condition or quality.

First, one can investigate the effect of environmental quality on ornament expression. For example, many studies have manipulated the early rearing environment and tested how this influenced the trait of interest (e.g. Nowicki et al. 2002; Hunt et al. 2004). Second, one can study correlations between traits and fitness (Bolund et al., 2010). Third, one can study the effects of nonadditive genetic

quality by assessing relationships between levels of inbreeding or estimates of individual genetic diversity and the expression of ornamental traits (Cotton et al. 2004). Additive genetic quality, in contrast, is more difficult to study because the heritability of fitness is predicted to be low (Falconer & Mackay 1996). Manipulating genetic quality remains an underexploited means to study the genetic basis of condition dependence (Cotton et al. 2004). In some species it is possible to use genetic manipulation techniques. For example, in *Drosophila*, Chippindale et al. (2001) cloned haploid genomes, expressed them in outbred males and females and measured adult reproductive success and offspring viability of these individuals. This provides an ingenious means of estimating genetic quality. In a similar approach, lines of *Drosophila* can be made homozygous for specific chromosomes, thus simulating different levels of inbreeding (Miller & Hedrick 1993).

Breeding between close relatives often results in a decline in mean phenotype and fitness, known as inbreeding depression (Charlesworth & Charlesworth 1987; Lynch & Walsh 1998). This phenomenon is mainly due to an increase in homozygosity, which can decrease fitness either by exposing the mutational load of deleterious recessive alleles (Lynch & Walsh 1998; Roff 2002) or by reducing the frequency of superior heterozygotes (Charlesworth & Charlesworth 1987). The former has received most support (Charlesworth & Willis 2009). Deleterious alleles can be lethal (leading to early mortality) or have slightly deleterious effects. The

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collective effect of slightly deleterious alleles at many loci should lead to a reduction in efficiency at converting energy into fitness, that is, it will lower the condition of the individual. If population-wide tests show that traits suffer inbreeding depression, the ornament can be considered condition dependent, because different individuals carry different recessive deleterious mutations and will become homozygous at different loci. Thus, the ornament will reflect, or 'capture', the genetic variance in condition, which is due to the cumulative effect at many loci (sensu the 'genic capture hypothesis', Rowe & Houle 1996). Alternatively, if sexual traits are not condition dependent, inbreeding may still affect these traits because of the effects of reduced heterozygosity at loci that directly code for sexual traits (e.g. Drayton et al. 2007).

Since inbreeding depression results from the interaction between parental genotypes, and not from the additive effects of individual genotypes, the effects on different types of traits will differ depending on the genetic architecture of the traits (where genetic architecture means the number of loci influencing a trait and the nature of interactions between alleles within and between loci, Merilä & Sheldon 1999). For example, life history traits and sexually selected traits (which are presumed to be closely related to fitness) should suffer higher levels of inbreeding depression than morphological traits (Hamilton & Zuk 1982; Zuk et al. 1990; Cotton et al. 2004). This is because traits closely related to fitness are assumed to be under strong directional selection, and are therefore predicted to have depleted additive genetic variation and therefore a high ratio of dominance to additive variation (Roff 1997; Lynch & Walsh 1998; De Rose & Roff 1999). Furthermore, life history traits are the result of the combined effects of many underlying traits. Thus, the combined multiplicative effect of the inbreeding depression on each of the underlying traits can produce very large inbreeding depression in fitness (Roff 1997). This broader genetic basis of fitness-related traits also means that they constitute larger mutational targets, since they are influenced by more loci than nonfitness traits (Rowe & Houle 1996: Merilä & Sheldon 1999). In contrast to fitness-related traits, morphological traits (or other nonfitness-related traits) are assumed to be under stabilizing selection and are therefore predicted to have relatively less dominance variance (Lynch & Walsh 1998; Merilä & Sheldon 1999). As predicted from theory, the level of dominance variance is much higher in life history than in morphological traits (Crnokrak & Roff 1995; Van Buskirk & Willi 2006) and life history traits commonly show higher inbreeding depression than morphological traits (De Rose & Roff 1999; Wright et al. 2008).

Surprisingly few studies have investigated the effects of inbreeding on secondary sexual traits such as courtship behaviour and ornamentation (e.g. Aspi 2000; Joron & Brakefield 2003; van Oosterhout et al. 2003; Drayton et al. 2007). Instead, the majority of studies on the effects of inbreeding have focused on primary fitness traits such as development time, survival and fecundity (Lynch & Walsh 1998; Keller et al. 2002). In birds, several studies have found effects on fertility and hatchability of eggs (reviewed by Spottiswoode & Møller 2004) and survival or recruitment of young (Amos et al. 2001; Kruuk et al. 2002). A complementary line of support comes from studies that look at the relationship between heterozygosity and fitness, although the link between heterozygosity and inbreeding may be weak (reviewed in Pemberton 2004; Kempenaers 2007; Chapman et al. 2009).

We studied the effects of one generation of full-sibling mating (inbreeding coefficient, $F = 0.25$) on viability, morphology, secondary sexual traits, attractiveness and mate choice in one of the model species in studies of sexual selection, the zebra finch. Surprisingly, no previous study has looked at effects of inbreeding on sexual characters in the zebra finch and only one study has tested effects of inbreeding on clutch failure (Fetherston & Burley

1990). Because inbreeding may lead to increased early mortality, a nonrandom subset of inbred birds may survive to adulthood. To investigate this possibility, we first looked at the genetic load leading to mortality of embryos and chicks, before turning our attention to inbreeding depression in adult traits.

Courtship song rate and beak colour have received much attention in the extensive zebra finch literature, and are considered the strongest candidates for sexually selected traits in this species (Houtman 1992; Zann 1996; but see Forstmeier & Birkhead 2004). Females are assumed to prefer males with redder beaks and higher song rates. Song structure and plumage ornaments have also been suggested to be sexually selected, although the evidence is mixed (e.g. Zann 1996). Therefore, our main aim was to test the magnitude of inbreeding depression on a variety of male and female traits to separate quality indicators from nonquality indicators. We predicted (1) that inbreeding would have stronger effects on sexually selected traits and traits closely related to fitness, and (2) that inbreeding depression would be stronger in males than females, reflecting more intense sexual selection in males.

METHODS

Subjects and Housing

All subjects originated from a large captive breeding population with wild-type plumage held at Seewiesen since October 2004. Rooms were maintained at a constant temperature of $24 \pm 1\,^{\circ}\text{C}$. Birds received 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. The average level of inbreeding in this laboratory population is relatively low ($F = 0.03$, based on an 18-generation pedigree, Forstmeier et al. 2004).

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

General Study Design

To produce inbred and outbred offspring, we chose 72 birds originating from 12 full-sib families (six siblings per family), and arranged them in six free-flight aviaries, so that each aviary housed six males and six females. In each aviary, three males and three females belonged to one family and the other three males and females to a second family. Thus, each bird had the opportunity to pair with one of three siblings or one of three unrelated birds. All birds had been cross-fostered at the egg stage and were reared with unrelated birds. Birds were arranged such that all individuals in one aviary were unfamiliar to each other. Hence, we expected no or only modest inbreeding avoidance (Schielzeth et al. 2008). A total of 74 birds were used (two birds died and were replaced). Birds were allowed to breed for 12 weeks, resulting in a total of 430 eggs. Of these, 74 could not be sampled for DNA because eggs disappeared ($N = 33$) or dried out ($N = 5$), or because embryos either died too early to obtain enough DNA for correct parentage assignment ($N = 9$) or eggs did not show any signs of development ($N = 27$). We categorized these latter eggs as infertile and assigned them to their social mother (this leads to a low level of incorrect assignment because 9.6% of eggs with assigned parentage resulted from intraspecific brood parasitism). The paternity of the remaining 356 eggs (embryos or offspring) was determined using 18 highly variable microsatellite loci (Forstmeier et al. 2007b, unpublished data). Blood for parentage analyses was sampled from all parents and offspring (5–10 µl from the brachial vein) and tissue samples were taken from all dead chicks and embryos. Offspring were reared by