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Differential allocation in relation to mate song quality in the Bengalese finch

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Abstract

Postmating sexual selection plays an important part in the evolution of secondary sexual characters. Based on differential allocation theory that predicts biased reproductive investment of females depending on the attractiveness of mates, a number of previous studies have shown that egg production is related in various ways to ornamental sexual traits of males, but evidence for behavioral sexual traits is less abundant. In this study we examine such maternal effects in relation to birdsong. Because the Bengalese finch is a monomorphic songbird, courtship song serves a key role in mate choice. To take into account individual female differences in egg production performance, we sequentially paired naïve, captive, female Bengalese finches to two different males, and investigated if their reproductive investment (clutch size, egg mass and hatchling sex ratio) was related to the song traits of their mates. We found that clutch size and egg mass were highly repeatable within individual females while sex ratio was not. Despite the inflexibility of egg mass within each female, egg mass increased when females were mated to males with longer songs. In addition, we found a non-significant weak tendency toward male-biased sex ratio in relation to longer song duration of mates. Our findings suggest that females mated to better mates adjusted their reproductive investment by producing heavier eggs and possibly offspring of the more costly sex.

Keyword: Bengalese finch, cryptic female choice, differential allocation, egg, sex ratio, songbird

1. Introduction

Courtship songs serve as an indicator of male quality in many songbirds. The acoustic features of songs have been shown to reflect various properties of potential mates, such as early developmental conditions (Nowicki et al., 1998; Buchanan et al., 2003; Spencer et al., 2003; Soma et al., 2006; Zann & Cash, 2007), body condition (Lampe & Espmark, 1994; Hoi-Leitner et al., 1995; Kipper et al., 2006), genetic quality (Hasselquist et al., 1996), and parental care (Welling et al., 1997; Buchanan & Catchpole, 2000). Because these qualities and conditions of individuals are likely to affect their offspring either directly (e.g., through resource provisioning) or indirectly (i.e., genetically), the song quality of the father can be used as a factor to predict offspring fitness. Female songbirds therefore show preferences toward males with particular song traits (Catchpole & Slater, 2008), and are expected to adjust their reproductive efforts and investments differentially depending on the attractiveness of mates, in order to maximise lifetime reproductive success (Burley, 1988; Sheldon, 2000).

In accordance with predictions of the differential allocation hypothesis (Sheldon, 2000), many previous studies in birds have reported that females paired with attractive or preferred males increased their reproductive investment by laying larger clutches or heavier eggs (e.g., peahen, *Pavo cristatus*: Petrie & Williams, 1993; Chinese quail, *Coturnix chinensis*: Uller et al., 2005; mallard, *Anas platyrhynchos*: Cunningham & Russell, 2000; zebra finch, *Taeniopygia guttata*: Balzer & Williams, 1998; Gilbert et al., 2006; Holveck & Riebel, 2010; canary, *Serinus canaria*: Leitner et al., 2006). In addition, it has been revealed that the attractiveness of mates affects the concentration of egg yolk components thought to contribute to the early physical development of offspring, such as testosterone (Gil et al., 1999; Gil et al., 2004; but see Mazuc et al., 2003; Michl et al., 2005) and immune factors (Saino et al., 2002b). These findings confirm that females invest more in reproduction when the prospective fitness return is high.

In contrast with the differential allocation hypothesis, the compensation hypothesis predicts enhanced reproductive investment of females that were mated to non-preferred males (Gowaty et al., 2007; Gowaty 2008). Because offspring viability was lower when females were forced to breed with non-preferred males, females compensated for this by increasing clutch size or egg mass (e.g. Bluhm & Gowaty

2004, Bolund et al. 2009). Even though the majority of studies so far supported the differential allocation hypothesis rather than the compensation hypothesis especially in birds, the direction of differential allocation can be either positive or negative depending on species ecology or experimental setups (Harris & Uller 2009; Ratikainen & Kokko, 2010).

Sex allocation also has an important role in maternal reproductive strategy. Since Trivers and Willard (1973) proposed that parents in good condition should invest more in the sex with greater prospective fitness returns (i.e., usually sons) compared to the other sex, this hypothesis has been discussed or employed to explain various adaptive patterns of sex allocation (Leimar 1996; Cockburn et al., 2002). In avian species, paternal quality reflected in physical conditions or secondary sexual characters can contribute to a biased offspring sex ratio (reviewed by Komdeur & Pen, 2002; West & Sheldon, 2002; Cassey et al., 2006). According to the attractiveness hypothesis (Cockburn et al., 2002), it would be adaptive for females mated to attractive males to produce broods biased toward sons, because sons sired by such males are expected to achieve higher reproductive success than daughters (Burley, 1981; Burley, 1986). Previous studies have supported this hypothesis by showing that brood sex ratio was biased toward males in relation to the ornamentation or body size of the father (Ellegren et al., 1996; Kölliker et al., 1999; Sheldon et al., 1999; Saino et al., 2002a; Abroe et al., 2007). Other studies, however, have reported mixed (Radford & Blakey, 2000; Griffith et al., 2003; Korsten et al., 2006) or negative results (Grindstaff et al., 2001; Leech et al., 2001; Rosivall et al., 2004; Ferree, 2007). Most of these previous studies have concentrated on the physical traits of males, and behavioral sexual traits such as courtship songs have received less attention. Only two studies so far have investigated the effects of song quality on sex allocation, with results showing that song repertoire size had no significant effect on sex allocation in the great reed warbler (Westerdahl et al., 1997), but the proportion of sons in the brood was positively related to song strophe length in blue tits (Dreiss et al., 2006).

Differential sex allocation has been intensively investigated in model species of songbirds in captivity (notably the zebra finch), where it is easy to experimentally control male physical traits and female conditions. However, evidence for whether or not mothers invest more in sons than daughters under favorable breeding circumstances (e.g., better nutrition or mates) remains controversial especially in the

zebra finch (Burley, 1981; Burley, 1986; Kilner, 1998; Rutstein et al., 2004; McGraw et al., 2005; Rutstein et al., 2005b). In addition, laying sequence, which is associated with sibling rivalry resulting from asynchronous hatching, can have confounding effects on differential sex allocation (Rutkowska & Cichoń, 2002; Rutstein et al., 2005a). Cockburn et al. (2002) suggested that sex-specific vulnerability should be taken into account when considering the discrepancies between different studies. Because female zebra finches are more vulnerable than males during development (De Kogel & Prijs, 1996; Kilner, 1998), female offspring are more costly. Hence it can be adaptive for mothers to produce the less costly sex (males) under some unfavorable reproductive situations, and vice versa.

Our study species, the Bengalese finch, *Lonchura striata* var. *domestica*, belongs to the same family (Estrildidae) as the zebra finch, but shows opposite sex-specific vulnerability. We found that male chicks were more likely to suffer from sibling competition in larger broods than females (Soma et al., 2006), because of the relatively higher growth rate of males (Soma et al., 2007). Thus, in Bengalese finches, having sons is more expensive for parents, but might lead to a higher fitness return in exchange for considerable reproductive investment. We therefore considered this species to be suitable for testing the hypothesis that females in optimum reproductive situations invest more in male offspring.

In the present study, we examined sex allocation and differential egg investment in relation to song traits of mates. The study species is sexually monomorphic, except for body size, which is slightly smaller in males (Soma et al., 2007), and therefore songs would likely serve a key role in mate choice. The Bengalese finch song consists of discrete notes and can be characterized by the types and order of notes included in a song (Honda & Okanoya, 1999). Behavioral experiments suggested that female Bengalese finches prefer males with syntactically complex songs (Okanoya, 2004a; Okanoya, 2004b; Morisaka et al., 2008). In addition, female zebra finches have been shown to prefer males that sing longer songs (Clayton & Pröve, 1989; Neubauer, 1999), suggesting that similar preferences for song bout length could also exist in our subject species. It has been demonstrated that song duration in the Bengalese finch reflects body condition (Soma et al., 2006).

We sequentially paired females to two different males, using a crossover design, and asked if variation in egg mass, clutch size and hatchling sex ratio between

the two broods could be explained by the difference in the song traits of the two males. To experimentally control for individual variation of learning-based song traits, the two males mated to each female were foster siblings that were reared by, and hence learned songs from, the same adults. Based on the differential allocation hypothesis (Sheldon, 2000) and the attractiveness hypothesis (Cockburn et al., 2002), we predicted that females mated to males with longer and more complex songs should increase investment in their eggs and bias the sex ratio toward males. However, the direction of differential allocation could be opposite when the compensation hypothesis held true (Gowaty et al. 2008). Because the Bengalese finch is an asynchronously hatching species, and we previously found that egg mass increased with laying order while sex ratio was unaffected (Soma et al., 2007), the laying order was also studied.

2. Material and Methods

2.1. Study birds and pairings

Bengalese finches (male: N = 16, female: N = 16) bred and maintained in our laboratory were paired and allowed to breed twice at approximately 3-month intervals. All the subject birds were between 1–1.5 years old and had no breeding experience prior to the initial pairing for this study.

The 16 study males consisted of eight pairs of foster siblings that were unrelated but had been brought up together and learned songs from the same set of two tutor males (see Soma et al., 2009 for detailed fostering manipulation). Each female was randomly mated to one of the foster siblings in the first breeding session, and to the other in the next. Hence, we assumed that differences in the learned acoustic features of the songs of the two males mated to each female reflected the song-learning ability of the individuals, rather than the social environment they had experienced in the song learning period.

2.2.Breeding conditions

All study birds were kept in single-sex cages (45 × 45 × 45 cm) before and after breeding. Each pair was introduced into an individual breeding cage (45 × 45 × 45 cm) equipped with one nest box, which was isolated visually but not audibly from other cages. Throughout the study, the birds were maintained in a controlled environment

suitable for breeding (temperature 24±3°C, humidity 30–50%, 14L:10D photoperiod) and provided with finch seed mixture, foxtail millet coated with egg yolk, water, shell grit, and green vegetables *ad libitum*. Each nest was checked every morning (i.e., 10:00–11:00 am), and newly laid eggs were weighed to the nearest 0.1 mg on an electronic balance, and marked with nontoxic waterproof colored pens for identification. Newly hatched chicks were marked in the same way and were sexed molecularly using DNA extracted from toenail clippings (for details see Soma et al. 2007). In most cases, only one chick hatched in a single day in a nest, but in rare cases when two or more chicks hatched on the same day, hatchlings could be assigned to eggs based on the dampness of their nestling down. Unhatched eggs were not sexed. We collected data from 174 eggs from 32 broods (clutch size: mean ± SD = 5.44 ± 0.72, range = 4-7), of which 119 eggs hatched and were therefore sexed.

2.3 Song analysis

Song recordings were made before the birds were paired. Each male was placed singly in a soundproof room and its vocal output was recorded using a directional microphone (Sony, ECM-MS975) and a digital audio recorder (Marantz, PMD390) with a sampling rate of 44.1 kHz and 16-bit resolution. At least ten bouts of undirected songs were obtained for each individual, and were analyzed using the sound analysis software Raven 1.2 (Charif et al., 2004). Based on the computed sonograms, song bout duration and note-type repertoire were calculated for each song bout and averaged for each individual. To estimate the syntactical complexity of note orders, we also calculated the linearity index (Scharff & Nottebohm, 1991). The linearity index is measured as the number of different note types divided by the number of different note-to-note transition types, and has smaller values when songs are syntactically more complex and involve more transition patterns.

2.4. Statistical analysis

Because the experimental design used within-individual comparisons, we checked the repeatability of female reproductive investment (i.e., average egg mass, clutch size, sex ratio) between the two different partners by calculating intraclass correlation coefficients (Lessells & Boag, 1987), and compared female reproduction between the first and second broods using paired *t*-tests (average egg mass) and Wilcoxon signed

rank tests (clutch size and sex ratio) to determine the effect of gaining reproductive experience.

To test whether female reproductive investment was affected by the song features of different mates, we used generalized linear mixed models (GLMM) with backward stepwise model selection. For the analyses of clutch size, we entered the identity of foster sibling pair used as mates, female identity and brood order as random effects, to deal with the lack of independence of the data from the same females or the same mate combinations. For the analyses of egg mass and hatchling sex, we included egg order in addition to aforementioned three variables as random effects because egg mass in this species has been shown to be affected by laying order (Soma et al., 2007). A Gaussian error distribution was adopted for the analysis of egg mass, a Poisson distribution with log link for clutch size, and binomial distribution with logit link for sex ratio. In all the models, three song variables (i.e., song duration, note repertoire, and linearity) were entered as fixed effects. The significance of the factor was assessed by the change in deviance associated with dropping the effect from the model, and non-significant factors were excluded in a backwards stepwise fashion with interactions excluded first. All statistical analyses were performed using R 2.12.0 (R Development Core Team 2008), with α set at 0.05.

3. Results

All females (N = 16) laid one clutch when paired to each of two different males (N = 16), and a total of 174 eggs from 32 clutches were therefore analyzed in the present study. Among these, 119 eggs from 29 clutches hatched and were sexed (male: N = 62, female: N =57). Among 55 unhatched eggs, 26 eggs showed some traces of dead embryos while the rest was in bad condition, which made it impossible to determine if they were infertile or not. In order to consider the possibility that this relatively high proportion of unhatched eggs with unknown sex had confounding effects on the results, we preliminarily checked whether hatching success was associated with male song traits. Using GLMM with binomial distribution, we found that hatching success was not linked to any of song traits (note repertoire: $\chi^2 = 0.43$, $p = 0.51$; linearity: $\chi^2 < 0.01$, $p = 0.92$; song duration; $\chi^2 = 0.90$, $p = 0.34$), and also that random effects were statistically non-significant (female identity; $\chi^2 = 2.54$, $p = 0.11$, brood order: $\chi^2 = 2.13$, $p = 0.14$, egg order: $\chi^2 = 0.33$, $p = 0.56$).

3.1. First and second broods

Female reproductive investment, measured as egg size, clutch size, and sex ratio, did not differ significantly between the first and second broods ($p > 0.15$, Table 1). Significant positive intraclass correlations between the data from the two broods showed that egg mass and clutch size were highly repeatable within individual females ($r > 0.6$, $p < 0.005$, Table 1). However, the sex ratios of the first and second broods were not significantly associated ($r = 0.22$, $p = 0.43$, Table 1).

3.2. Male song and clutch size

GLMM analyses revealed that clutch size did not vary with any male song traits ($p > 0.4$, Table 2). This was partially because of the inflexibility of clutch size within females (Table 1). In particular, 13 out of 16 females laid the same number of eggs in both broods, while the remaining three females laid more eggs in the second brood.

3.3. Male song and egg mass

Egg mass showed a significant increase with increasing male song duration (Figure 1, Table 2) but not with note repertoire and linearity (Table 2).

3.4. Male song and offspring sex

None of the song variables had statistically significant associations with the sex ratio of hatchlings (Table 2). However, as seen for the analysis of egg mass, the sex ratio of hatchlings was marginally affected by male song duration. When song duration was longer, offspring sex tended to be more male-biased. As our sex ratio data did not involve unhatched eggs, we also conducted similar analyses on imputed data. Assuming that all unhatched eggs were females, the relationship between offspring sex and song duration was more pronounced and statistically significantly ($\chi^2 = 4.07$, $p = 0.04$). In contrast, when we assume that sex of unhatched eggs were all males or random, there were no significant associations between song duration and sex ratio (all male: $\chi^2 = 1.52$, $p = 0.22$; random: $\chi^2 = 3.21$, $p = 0.07$).

4. Discussion

Differential reproductive allocation by females can have an important role in the

evolution of male secondary sexual characters, including both physical and behavioral traits. In the present study, we focused on multiple song features of males and tested their effects on female allocation decisions in Bengalese finches. Although mating with males with longer song duration led to increased egg mass, the relationship between male-biased hatchling sex ratio and song duration was not statisitically significant. Overall, these results support the idea of the differential allocation hypothesis but not the compensation hypothesis, and indicate that females mated to favoured mates with longer song durations allocated more resources to reproduction by increasing egg volume and possibly by producing male offspring, which are more costly to rear. However, it should be noted that this study without any experimental manipulation of song traits did not show direct evidence that females birds were responding to songs.

Facultative sex allocation was initially proposed some time ago (Trivers & Willard, 1973). However, evidence regarding the ability of birds to adaptively adjust primary sex ratio is still controversial, because of inconsistencies among studies and an incomplete understanding of the underlying mechanisms (Cockburn et al., 2002; West & Sheldon, 2002; Pike & Petrie, 2003; Ewen et al., 2004; Cassey et al., 2006). Because we did not determine the sex of unhatched eggs in the current study, our results could not provide firm evidence that the primary sex ratio was affected. Observed moderate biases in hatchling sex ratio could be caused either by biases in egg sex ratio, or by sex-specific embryo mortality (Pike & Petrie, 2003). Considering that male chicks are more vulnerable than females in this species (Soma et al., 2006), it is possible that male embryos are also subject to sex-specific mortality under particular reproductive conditions (cf., Love et al., 2005).

We found similar reproductive biases in egg mass, which suggests the possibility that a mechanism is shared between sex allocation and egg production. A possible explanation for our results is sexual dimorphism of egg size, which has been previously reported in various avian species (e.g., American kestrel, *Falco sparverius*: Anderson et al., 1997; house sparrow, *Passer domesticus*: Cordero et al., 2000; brown songlark: Magrath et al., 2003). However, we did not find any differences in egg mass between offspring sexes (Two-sample t test: $t = 0.227$, $p = 0.820$; male egg mass: mean ± SD = 1.065 ± 0.073 g, $N = 63$; female egg mass ± SD = 1.062 ± 0.091 g, $N = 56$), indicating that heavier eggs did not necessarily contain sons. Although it is also possible that laying order could mediate the link between egg mass and sex, a past

study found that egg mass but not sex ratio was skewed in relation to laying order (Soma et al., 2007), which holds true for the present data. Therefore, we assume that egg mass and sex ratio were not directly associated with each other.

In contrast with the results for song duration, it is surprising that we found no evidence of reproductive allocation in relation to song complexity, despite female preference for these traits (Okanoya, 2004b; Okanoya, 2004a). This might be related to the degree to which each trait reflects the current physical condition of the males: Repertoire size and syntactical complexity are mostly determined by the song learning capacity of individuals, and change little throughout an individual's lifetime after song crystallization in age-limited song learners. It has been shown in Bengalese finches that these song features reflect early developmental conditions when song learning takes place, but are not related to body conditions in adulthood (Soma et al., 2006). By contrast, song duration is more plastic within individuals, and is known to reflect the body size of individuals (Soma et al., 2006). These observations imply that female Bengalese finches adjust their reproductive investment based on a particular song trait that reflects the current physical state of the male. A small number of previous studies have examined the relationships between differential reproductive allocation and song, including sex allocation. However, their results are only partially consistent with our present findings in that female reproductive investment was affected by some aspects of song performance, that is, non-learning-based traits (song strophe length in blue tits: Dreiss et al., 2006; song rate and duration in zebra finches: Balzer & Williams, 1998; singing interaction between male canaries: Garcia-Fernandez et al., 2010), but not by repertoire size, a learning-based trait (great reed warblers: Westerdahl et al., 1997; song sparrows: Potvin & MacDougall-Shackleton, 2010). Exceptionally, however, in canaries, it has been reported that egg mass and the concentration of yolk testosterone increased when females were exposed to recordings of so-called "sexy syllables", which are acquired through vocal learning (Gil et al., 2004; Leitner et al., 2006; but see Marshall et al., 2005).

Finally, another reason why we could not observe any effect of song complexity on female reproductive investments might relate to pairing combinations. Because we aimed to test female preference for song learning capacity rather than for particular song phenotypes, we sequentially paired each female to two different males that had been tutored by the same adult males. This experimental setting allowed us to

reduce the unexpected influence of individual differences in song phonological phenotypes (i.e., note types) coming from song models, by using two males that differed in the number and ordering of note types but shared most note type repertoires. However, as a consequence, there was less variation in song complexity between two males, which might have made it difficult to detect differential allocation of females in response to it.

In conclusion, our results does not show direct evidence but support the idea that differential allocation of female reproductive investment is one of the essential factors explaining intersexual selection pressure on oscine songs. The results, along with other studies focusing on birdsong and differential allocation, indicate that performance-related song traits are more influential on post-copulatory mate choice than elaboration-related traits. When we look at pre-copulatory mate choice, song elaboration is associated with social mating success, but not with genetic mating success (reviewed by Soma & Garamszegi, 2010). It would thus appear that each multifaceted song trait would differentially affect female preferences in different reproductive stages.

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References

Abroe, B., Garvin, J.C., Pedersen, M.C., Whittingham, L.A. & Dunn, P.O. (2007). Brood sex ratios are related to male size but not to attractiveness in common yellowthroats (*Geothlypis trichas*). — Auk 124: 176–184.

Anderson, D.J., Reeve, J. & Bird, D.M. (1997). Sexually dimorphic eggs, nestling growth and sibling competition in American Kestrels *Falco sparverius*. — Funct. Ecol. 11: 331–335.

Balzer, A.L. & Williams, T.D. (1998). Do female zebra finches vary primary reproductive effort in relation to mate attractiveness? — Behaviour 135: 297–309.

Bluhm, C.K. & Gowaty. P.A. (2004). Reproductive compensation for offspring

viability deficits by female mallards, *Anas platyrhynchos*. — Anim. Behav. 68: 985-992.

Bolund E., Schielzeth H. & Forstmeier W. (2009) Compensatory investment in zebra finches: females lay larger eggs when paired to sexually unattractive males. — Proc. Roy. Soc. Lond. B: Biol. 276: 707-715.

Buchanan, K.L. & Catchpole, C.K. (2000). Song as an indicator of male parental effort in the sedge warbler. — Proc. Roy. Soc. Lond. B: Biol. 267: 321–326.

Buchanan, K.L., Spencer, K.A., Goldsmith, A.R. & Catchpole, C.K. (2003). Song as an honest signal of past developmental stress in the European starling (*Sturnus vulgaris*). — Proc. Roy. Soc. Lond. B: Biol. 270: 1149–1156.

Burley, N. (1981). Sex-ratio manipulation and selection for attractiveness. — Science 211: 721–722.

Burley, N. (1986). Sex-ratio manipulation in color-banded populations of zebra finches. — Evolution 40: 1191–1206.

Burley, N. (1988). The differential-allocation hypothesis: an experimental test. — Am. Nat. 132: 611–628.

Cassey, P., Ewen, J.G. & Møller, A.P. (2006). Revised evidence for facultative sex ratio adjustment in birds: a correction. —Proc. Roy. Soc. Lond. B: Biol. 273: 3129–30.

Catchpole, C.K. & Slater, P.J.B. (2008). Bird song: biological themes and variations, 2nd edn. — Cambridge University Press, Cambridge.

Charif, R.A., Clark, C.W. & Fristrup, K.M. (2004). Raven 1.2 User's Manual. — Cornell Laboratory of Ornithology, Ithaca, NY.

Clayton, N. & Pröve, E. (1989). Song discrimination in female zebra finches and Bengalese finches. — Anim. Behav. 38: 352–354.

Cockburn, A., Legge, S. & Double, M.C. (2002). Sex ratios in birds and mammals: can the hypotheses be disentangled? — In: Sex Ratios: Concepts and Research Methods. (Hardy, I.C.W. ed). Cambridge University Press, Cambridge, p. 266–286.

Cordero, P.J., Griffith, S.C., Aparicio, J.M. & Parkin, D.T. (2000). Sexual dimorphism in house sparrow eggs. — Behav. Ecol. Sociobiol. 48: 353–357.

Cunningham, E.J. & Russell, A.F. (2000). Egg investment is influenced by male attractiveness in the mallard. — Nature 404: 74–7.

De Kogel, C.H. & Prijs, H.J. (1996). Effects of brood size manipulations on sexual attractiveness of offspring in the zebra finch. — Anim. Behav. 51: 699–708.

Dreiss, A., Richard, M., Moyen, F., White, J., Møller, A.P. & Danchin, E. (2006). Sex ratio and male sexual characters in a population of blue tits, *Parus caeruleus*. — Behav. Ecol. 17: 13–19.

Ellegren, H., Gustafsson, L. & Sheldon, B.C. (1996). Sex ratio adjustment in relation to paternal attractiveness in a wild bird population. — Proc. Natl. Acad. Sci. USA 93: 11723–11728.

Ewen, J.G., Cassey, P. & Møller, A.P. (2004). Facultative primary a lack of evidence sex ratio variation: a lack of evidence in birds? — Proc. Roy. Soc. Lond. B: Biol. 271: 1277–1282.

Ferree, E.D. (2007). White tail plumage and brood sex ratio in dark-eyed juncos (*Junco hyemalis thurber*i). — Behav. Ecol. Sociobiol. 62: 109–117.

Garcia-Fernandez, V., Amy, M., Lacroix, A., Malacarne, G. & Leboucher, G. (2010). Eavesdropping on male singing interactions leads to differential allocation in eggs. — Ethology 116: 662–670.

Gil, D., Graves, J., Hazon, N. & Wells, A. (1999). Male attractiveness and differential testosterone investment in zebra finch eggs. — Science 286: 126–128.

Gil, D., Leboucher, G., Lacroix, A., Cue, R. & Kreutzer, M. (2004). Female canaries produce eggs with greater amounts of testosterone when exposed to preferred male song. — Horm. Behav. 45: 64–70.

Gilbert, L., Williamson, K.A., Hazon, N. & Graves, J.A. (2006). Maternal effects due to male attractiveness affect offspring development in the zebra finch. — Proc. Roy. Soc. Lond. B: Biol. 273: 1765–1771.

Gowaty, P.A., Anderson, W.A., Bluhm, C.K., Drickamer, L.C., Kim, Y.K. & Moore, A.J. (2007). The hypothesis of reproductive compensation and its assumptions about mate preferences and offspring viability. — Proc. Natl. Acad. Sci. USA 104: 15023–15027.

Gowaty. P.A. (2008). Reproductive compensation. — J. Evol Biol. 21: 1189-1200.

Griffith, S.C., Ornborg, J., Russell, A.F., Andersson, S. & Sheldon, B.C. (2003). Correlations between ultraviolet coloration, overwinter survival and offspring sex ratio in the blue tit. — J. Evol. Biol. 16: 1045–1054.

Grindstaff, J.L., Buerkle, C.A., Casto, J.M., Nolan, V. & Ketterson, E.D. (2001).

Offspring sex ratio is unrelated to male attractiveness in dark-eyed juncos (*Junco hyemalis*). — Behav. Ecol. Sociobiol. 50: 312-316.

Harris, W.E. & Uller, T. (2009) Reproductive investment when mate quality varies: differential allocation versus reproductive compensation. — Phil. Trans. Roy. Soc. B 364: 1039–1048.

Hasselquist, D., Bensch, S. & von Schantz, T. (1996). Correlation between male song repertoire, extra-pair paternity and offspring survival in the great reed warbler. — Nature 381: 229–232.

Hoi-Leitner, M., Nechtelberger, H. & Hoi, H. (1995). Song rate as a signal for nest site quality in blackcaps (*Sylvia atricapilla*). — Behav. Ecol. Sociobiol. 37: 399–405.

Holveck, M. J. & Riebel, K. (2010) Low-quality females prefer low-quality males when choosing a mate. —Proc. Roy. Soc. Lond. B: Biol. 277: 153-160.

Honda, E. & Okanoya, K. (1999). Acoustical and syntactical comparisons between songs of the white-backed munia (*Lonchura striata*) and its domesticated strain, the Bengalese finch (*Lonchura striata* var. *domestica*). — Zool. Sci. 16: 319–326.

Kilner, R. (1998). Primary and secondary sex ratio manipulation by zebra finches. — Anim. Behav. 56: 155–164.

Kipper, S., Mundry, R., Sommer, C., Hultsch, H. & Todt, D. (2006). Song repertoire size is correlated with body measures and arrival date in common nightingales, *Luscinia megarhynchos*. — Anim. Behav. 71: 211–217.

Kölliker, M., Heeb, P., Werner, I., Mateman, A.C., Lessells, C.M. & Richner, H. (1999). Offspring sex ratio is related to male body size in the great tit (*Parus major*). — Behav. Ecol. 10: 68–72.

Komdeur, J. & Pen, I. (2002). Adaptive sex allocation in birds: the complexities of linking theory and practice. — Phil. Trans. Roy. Soc. B 357: 373–380.

Korsten, P., Lessells, C.M., Mateman, A.C., van der Velde, M. & Komdeur, J. (2006). Primary sex ratio adjustment to experimentally reduced male UV attractiveness in blue tits. — Behav. Ecol. 17: 539–546.

Lampe, H.M. & Espmark, Y.O. (1994). Song structure reflects male quality in pied flycatchers, *Ficedula hypoleuca*. — Anim. Behav. 47: 869–876.

Leech, D.I., Hartley, I.R., Stewart, I.R.K., Griffith, S.C. & Burke, T. (2001). No effect

of parental quality or extrapair paternity on brood sex ratio in the blue tit (*Parus caeruleus*). — Behav. Ecol. 12: 674–680.

Leimar, O. (1996) Life-history analysis of the Trivers and Willard sex-ratio problem. — Behav. Ecol. 7: 316-325.

Leitner, S., Marshall, R.C., Leisler, B. & Catchpole, C.K. (2006). Male song quality, egg size and offspring sex in captive canaries (*Serinus canaria*). — Ethology 112: 554–563.

Lessells, C.M. & Boag, P.T. (1987). Unrepeatable repeatabilities: a common mistake. — Auk 104: 116–121.

Love, O.P., Chin, E.H., Wynne-Edwards, K.E. & Williams, T.D. (2005). Stress hormones: a link between maternal condition and sex-biased reproductive investment. — Am. Nat. 166: 751–766.

Magrath, M.J.L., Brouwer, L. & Komdeur, J. (2003). Egg size and laying order in relation to offspring sex in the extreme sexually size dimorphic brown songlark, *Cinclorhamphus cruralis*. — Behav. Ecol. Sociobiol. 54: 240–248.

Marshall, R.C., Leisler, B., Catchpole, C.K. & Schwabl, H. (2005). Male song quality affects circulating but not yolk steroid concentrations in female canaries (*Serinus canaria*). — J. Exp. Biol. 208: 4593–4598.

Mazuc, J., Chastel, O. & Sorci, G. (2003). No evidence for differential maternal allocation to offspring in the house sparrow (*Passer domesticus*). — Behav. Ecol. 14: 340–346.

McGraw, K.J., Adkins-Regan, E. & Parker, R.S. (2005). Maternally derived carotenoid pigments affect offspring survival, sex ratio, and sexual attractiveness in a colorful songbird. — Naturwissenschaften 92: 375–380.

Michl, G., Török, J., Peczely, P., Garamszegi, L.Z. & Schwabl, H. (2005). Female collared flycatchers adjust yolk testosterone to male age, but not to attractiveness. — Behav Ecol. 16: 383–388.

Morisaka, T., Katahira, K. & Okanoya, K. (2008). Variability in preference for conspecific songs with syntactical complexity in female Bengalese Finches: towards an understanding of song evolution. — Ornithol. Sci. 7: 75–84.

Neubauer, R.L. (1999). Super-normal length song preferences of female zebra finches (*Taeniopygia guttata*) and a theory of the evolution of bird song. — Evol. Ecol. 13: 365–380.

Nowicki, S., Peters, S. & Podos, J. (1998). Song learning, early nutrition and sexual selection in songbirds. — Am. Zool. 38: 179–190.

Okanoya, K. (2004a). Song syntax in Bengalese finches: proximate and ultimate analyses. — Adv. Stud. Behav. 34: 297–346.

Okanoya, K. (2004b). The Bengalese finch: A window on the behavioral neurobiology of birdsong syntax. — Ann. NY Acad. Sci. 1016: 724–735.

Petrie, M. & Williams, A. (1993). Peahens lay more eggs for peacocks with larger trains. — Proc. Roy. Soc. Lond. B: Biol. 251: 127–131.

Pike, T.W. & Petrie, M. (2003). Potential mechanisms of avian sex manipulation. — Biol. Rev. 78: 553–574.

Potvin, D.A. & MacDougall-Shackleton, E.A. (2010). Paternal song complexity predicts offspring sex ratios close to fledging, but not hatching, in Song Sparrows. — Wilson J. Ornithol. 122: 146–152.

R Development Core Team (2007). R: a language and environment for statistical computing. —R Foundation for Statistical Computing, Vienna, Austria. Available from: http://www.R-project.org.

Radford, A.N. & Blakey, J.K. (2000). Is variation in brood sex ratios adaptive in the great tit (*Parus major*)? — Behav. Ecol. 11: 294–298.

Ratikainen, I.I. & Kokko, H. (2010). Differential allocation and compensation: who deserves the silver spoon? — Behav. Ecol. 21: 195–200.

Rosivall, B., Török, J., Hasselquist, D. & Bensch, S. (2004). Brood sex ratio adjustment in collared flycatchers (*Ficedula albicollis*): results differ between populations. — Behav. Ecol. Sociobiol. 56: 346–351.

Rutkowska, J. & Cichoń, M. (2002). Maternal investment during egg laying and offspring sex: an experimental study of zebra finches. — Anim. Behav. 64: 817–822.

Rutstein, A.N., Gilbert, L., Slater, P.J.B. & Graves, J.A. (2004). Mate attractiveness and primary resource allocation in the zebra finch. — Anim. Behav. 68:1087–1094.

Rutstein, A.N., Gilbert, L., Slater, P.J.B. & Graves, J.A. (2005a). Sex-specific patterns of yolk androgen allocation depend on maternal diet in the zebra finch. — Behav. Ecol. 16: 62–69.

Rutstein, A.N., Gorman, H.E., Arnold, K.E., Gilbert, L., Orr, K.J., Adam, A., Nager, R.

& Graves, J.A. (2005b). Sex allocation in response to paternal attractiveness in the zebra finch. — Behav. Ecol. 16: 763–769.

Saino, N., Ambrosini, R., Martinelli, R., Calza, S., Møller, A.P. & Pilastro, A. (2002a). Offspring sexual dimorphism and sex-allocation in relation to parental age and paternal ornamentation in the barn swallow. — Mol Ecol. 11: 1533–1544.

Saino, N., Bertacche, V., Ferrari, R.P., Martinelli, R., Møller, A.P. & Stradi, R. (2002b). Carotenoid concentration in barn swallow eggs is influenced by laying order, maternal infection and paternal ornamentation. — Proc. Roy. Soc. Lond. B: Biol. 269: 1729–1733.

Scharff, C. & Nottebohm, F. (1991). A comparative-study of the behavioral deficits following lesions of various parts of the zebra finch song system: implications for vocal learning. — J. Neurosci. 11: 2896–2913.

Sheldon, B.C. (2000). Differential allocation: tests, mechanisms and implications. — Trends Ecol. Evol. 15: 397-402.

Sheldon, B.C., Andersson, S., Griffith, S.C., Örnborg, J. & Sendecka, J. (1999). Ultraviolet colour variation influences blue tit sex ratios. — Nature. 402: 874–877.

Soma, M., Takahasi, M., Ikebuchi, M., Yamada, H., Suzuki, M., Hasegawa, T. & Okanoya, K. (2006). Early rearing conditions affect the development of body size and song in Bengalese finches. — Ethology. 112: 1017–1078.

Soma, M., Saito, D.S., Hasegawa, T. & Okanoya, K. (2007). Sex-specific maternal effect on egg mass, laying order, and sibling competition in the Bengalese finch (*Lonchura striata* var. *domestica*). — Behav. Ecol. Sociobiol. 61: 1695–1705.

Soma, M., Hiraiwa-Hasegawa, M., Okanoya, K. (2009). Early ontogenetic effects on song quality in the Bengalese finch (*Lonchura striata* var. *domestica*): laying order, sibling competition, and song syntax. — Behav. Ecol. Sociobiol. 63: 363–370

Soma, M. & Garamszegi, L.Z. (2011). Rethinking birdsong evolution: meta-analysis of the relationship between song complexity and reproductive success. — Behav. Ecol. 22: 363–371.

Spencer, K.A., Buchanan, K.L., Goldsmith, A.R. & Catchpole, C.K. (2003). Song as

an honest signal of developmental stress in the zebra finch (*Taeniopygia guttata*). — Horm. Behav. 44: 132–139.

Trivers, R.L. & Willard, D.E. (1973). Natural selection of parental ability to vary the sex ratio of offspring. — Science. 179: 90–92.

Uller, T., Eklöf, J. & Andersson, S. (2005). Female egg investment in relation to male sexual traits and the potential for transgenerational effects in sexual selection. — Behav. Ecol. Sociobiol. 57: 584–590.

Welling, P.P., Rytkönen, S.O., Koivula, K.T. & Orell, M.I. (1997). Song rate correlates with paternal care and survival in willow tits: Advertisement of male quality? — Behaviour 134: 891–904.

West, S.A. & Sheldon, B.C. (2002). Constraints in the evolution of sex ratio adjustment. — Science. 295: 1685–1688.

Westerdahl, H., Bensch, S., Hansson, B., Hasselquist, D. & VonSchantz, T. (1997). Sex ratio variation among broods of great reed warblers *Acrocephalus arundinaceus*. — Mol. Ecol. 6: 543–548.

Zann, R. & Cash, E. (2007). Developmental stress impairs song complexity but not learning accuracy in non-domesticated zebra finches (*Taeniopygia guttata)*. — Behav. Ecol. Sociobiol. 62: 391–400.

Table 1. Comparison between first and second broods for the response variables average egg mass, clutch size, and sex ratio.

	1st brood		2nd brood		Comparison of broods	Repeatability
	Mean ± SD	Median (range)	Mean ± SD	Median (range)		
Egg mass (g)	1.053 ± 0.084	1.055 (0.930–1.205)	1.064 ± 0.061	1.063 (0.982–1.180)	$p = 0.40$ [a]	$\boldsymbol{r = 0.77}$ $\boldsymbol{p = 0.0002}$
Clutch size	5.3 ± 0.70	5 (4–6)	5.6 ± 0.72	6 (4–7)	$p = 0.17$ [b]	$\boldsymbol{r = 0.64}$ $\boldsymbol{p = 0.0041}$
Sex ratio (proportion of males)	0.57 ± 0.26	0.60 (0.00–1.00)	0.49 ± 0.23	0.50 (0.00–0.80)	$p = 0.28$ [b]	$r = 0.22$ $p = 0.43$

a: Paired *t*-test, b: Wilcoxon signed rank test.

Table 2. Results of the GLMM analyses on the response variables clutch size, egg mass, and brood sex ratio in relation to male song traits.

			Clutch size ($N = 32$)		Egg mass ($N = 174$)		Sex ratio ($N = 119$)	
Fixed effect	Mean ± SD	(range)	χ^2	p	χ^2	p	χ^2	p
Note repertoire	7.9 ± 1.7	(4.0–10.9)	0.56	0.45	2.90	0.09	0.19	0.66
Linearity	0.50 ± 0.06	(0.39–0.57)	< 0.01	0.96	2.12	0.15	0.29	0.59
Song duration	7.57 ± 2.28	(4.58–11.47)	0.07	0.79	10.23	**< 0.005**	3.63	< 0.057
Random effect								
Mate pair identity			< 0.01	> 0.99	0.46	0.50	< 0.01	> 0.99
Female identity			< 0.01	> 0.99	65.33	**< 0.001**	< 0.01	> 0.99
Brood order			< 0.01	> 0.99	0.25	0.62	< 0.01	> 0.99
Egg order			—	—	12.54	**< 0.001**	< 0.01	> 0.99

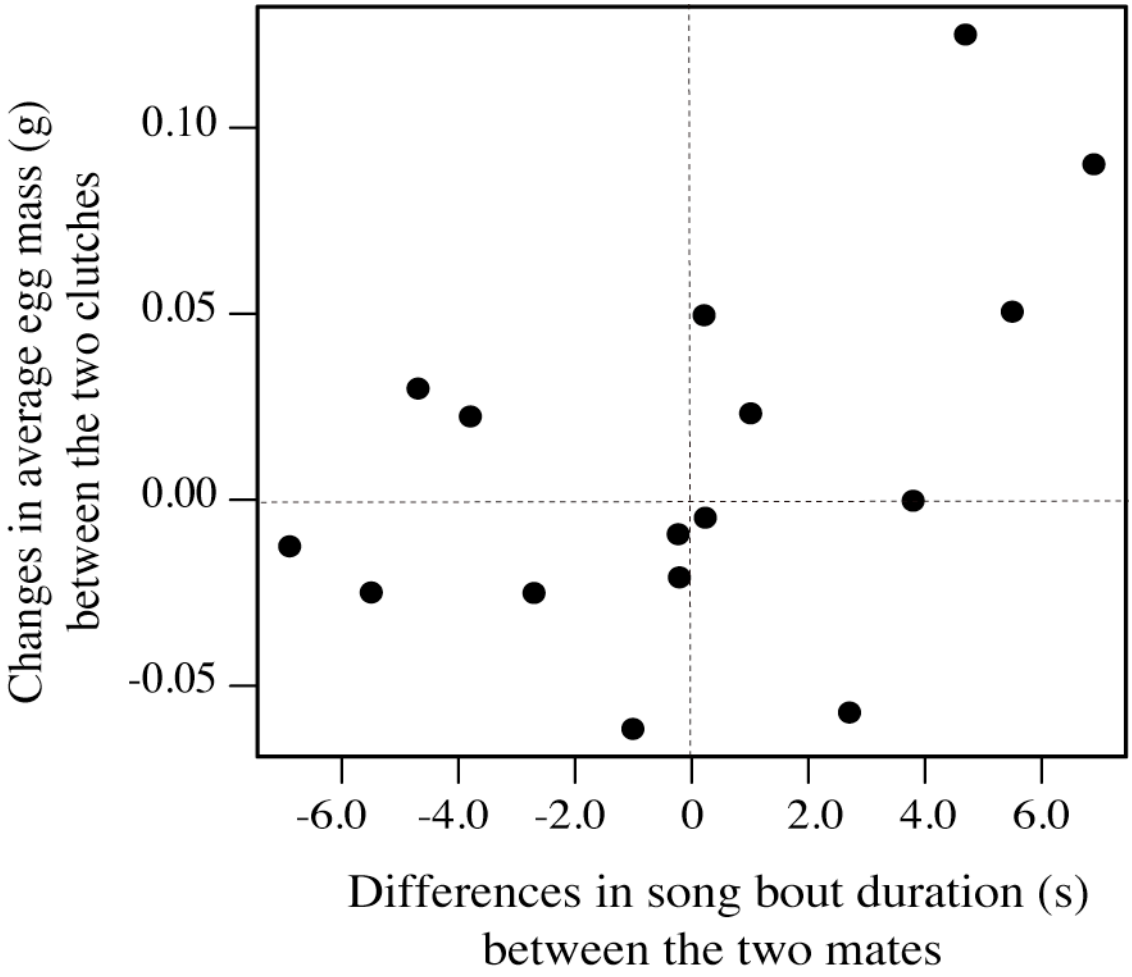

Figure 1. Changes in average egg mass of the two clutches each female (N = 16) laid were positively associated with the differences in song duration of the mates with which each female was paired in the two breeding sessions (Spearman's rank correlation: r_s = 0.523, p = 0.040).

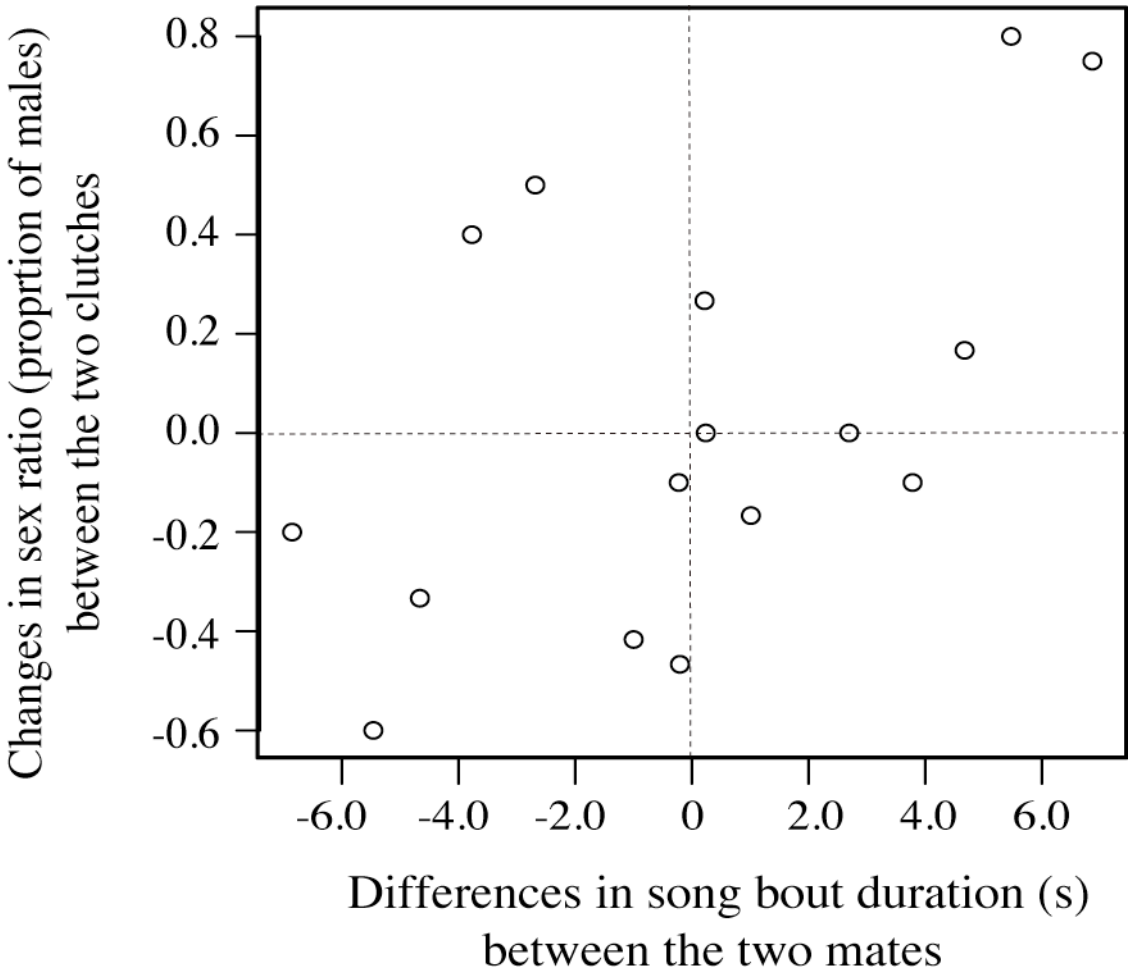

Figure 2. Changes in sex ratio (proportion of males) were positively associated with the differences in song duration of the mates with which each female was paired in the two breeding sessions (Spearman's rank correlation: $r_s = 0.548$, $p = 0.028$).