



Female resistance to male seduction in zebra finches

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A recent revival of the theory of sexual conflict leads to new interest in the question of how much females control copulation. Using the zebra finch, *Taeniopygia guttata*, as a model, I investigated the mechanisms with which males tried to persuade females to copulate with them. Analysing the outcome of a large number of experimental male–female encounters, I concluded that apart from some effect of male choosiness (preference for certain females), females were fully in control of copulation. Males did not differ in their abilities to overcome female resistance, either in terms of sensory exploitation (variation in ornamentation and display) or coercion (use of aggression). Variation in beak colour had no influence on male mating success, but male song rate may be a sexually selected trait: males that sang more frequently obtained more copulations, but this was because other males missed opportunities by not even attempting to display to females. Whenever a male started displaying, female responsiveness, and hence male copulatory success, was not influenced by how persistently he sang. These findings support a theory of chase-away sexual selection, rather than a good-genes theory, which has typically been invoked to explain variation in male song rate. Between-individual differences in male song rate may reflect alternative reproductive strategies (mating effort versus parental effort) rather than variation in male quality. Finally, I found marked differences in sexual responsiveness between females, which is not anticipated by current models of sexual selection and hence urges further investigation.

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The fact that males can fertilize many more eggs than females can produce leads to a conflict over mating between the sexes (Parker 1979). In most situations, males will try to persuade females to copulate, but females will usually try to resist these persuasive attempts. This conflict of interests may give rise to antagonistic coevolution (Holland & Rice 1998; Chapman et al. 2003), and it is therefore important to understand to what extent females are in control of mating (here meaning a copulatory act in which sperm is transferred). Males can influence the probability of obtaining copulations in a variety of ways. Males may use violence or threaten females with violence to render the potential costs of female resistance larger than the costs of giving in (Clutton-Brock & Parker 1995). Males may also wear down female resistance if male display hinders the female from performing other activities, making female resistance costly in terms of time loss

(Clutton-Brock & Parker 1995). Males may use signals (display or ornaments) that exploit the sensory system of females to increase their sexual responsiveness (Endler & Basolo 1998). In situations where there are more mating opportunities than males can use, males may also be choosy and reject some females/opportunities in favour of others (Dewsbury 1982; Sæther et al. 2001).

While it seems unlikely that females will benefit from male violence or male choosiness, it is debatable whether females profit from selecting the most ornamented male or the most persistently displaying male (Holland & Rice 1998; Chapman et al. 2003; Kokko et al. 2003). Whether females profit from choosing attractive males depends on what ornamentation tells about its bearer. If ornamentation and persistent display are costly to males and expressed in a condition-dependent manner, these signals could honestly reflect male genetic quality (Zahavi 1975; Jennions et al. 2001). Under such circumstances, females might profit from selecting ornamented and persistent males in terms of increased offspring fitness (good-genes hypothesis). Under the good-genes hypothesis, female mating preferences for ornamented or persistent males

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would be favoured, and one would predict that the degree of male ornamentation or persistence largely determines whether copulation takes place.

Alternatively, females may incur costs rather than benefits from mating with the most persuasive males (Chapman et al. 2003). This might, for instance, be the case if promiscuous males carry more sexually transmitted diseases (Birkhead & Møller 1992) or provide fewer direct benefits (Ketterson & Nolan 1999), or if there are indirect costs through sexually antagonistic genes (Chapman et al. 2003). If the costs of mating with these males are larger than the benefits, female resistance to sensory exploitation will be favoured (chase-away selection; Holland & Rice 1998). Resistance can evolve in two distinct ways (Rice & Holland 1999). First, preferences may become more stringent, with females requiring even greater trait exaggeration to become aroused. This may lead to a runaway process of increasing trait exaggeration with increasing stringency of preferences, which differs from Fisherian or good-genes processes only in that females suffer survival or fecundity costs from selecting the most persuasive male (Rice & Holland 1999; Kokko et al. 2003). Second, females may evolve to ignore the male trait by losing the preference (Rice & Holland 1999). This second possibility has been overlooked by some authors (Rosenthal & Servedio 1999; Kokko et al. 2003), who wrongly stated that, under the chase-away model, females would always continue to prefer the most exaggerated ornament. When proposing the model of chase-away selection, Holland & Rice (1998) illustrated the evolution of female resistance by referring to empirical studies showing the loss of a preference.

Studies on sexual selection in birds most commonly support the following view: male violence is thought to be important in only a few bird species (McKinney et al. 1983; Westneat et al. 1990), and in most bird species, females are thought to be largely in control of mating (Birkhead & Møller 1992; Johnson & Burley 1998). However, females are typically believed to have preferences for persistent (Ryan & Keddy-Hector 1992; Searcy & Yasukawa 1996) and ornamented males (Ligon 1999). Male choosiness appears to be less widespread, but may play a role in some species (e.g. Monaghan et al. 1996; Sæther et al. 2001).

My aim in the present study was to put this common view to a test, using the zebra finch, *Taeniopygia guttata castanotis*, as a model. Although the zebra finch mating system is probably the most intensively studied of any bird (Zann 1996), it is unknown to what extent variation in male and female attractiveness affects female responsiveness and male motivation to copulate and how these determine whether a mating takes place. Of the many possible pathways of causation, the most relevant are illustrated in Fig. 1.

In one possible scenario of total female control, sexual responsiveness varies between females (effect of female identity), rather than being influenced by male attractiveness (measured as a consistent effect of male identity) or male song rate (reflecting the male's motivation to copulate), and female responsiveness determines whether a copulation takes place (Fig. 1a). The opposite situation is

entire male control (Fig. 1b). In probably the most widely accepted scenario, applicable to most bird species, females are in control of copulation, yet their readiness to mate depends on male attractiveness (male identity: Fig. 1c). Support for this view comes from evidence that female zebra finches prefer males with dark red beaks (an ornament) over males with more orange beaks (Burley & Coopersmith 1987; Houtman 1992; de Kogel & Prijs 1996; Blount et al. 2003; but see Balzer & Williams 1998). However, in other studies, researchers have claimed that this apparent preference for red beaks is caused only by a correlation with male song rate, which is thought to be the real target of female mating preferences (Houtman 1992; Collins et al. 1994) i.e. responsiveness depends on song rate (Fig. 1d). Song rate differs consistently between males (Birkhead & Fletcher 1995; effect of male identity in Fig. 1d), but also depends on female attractiveness (Monaghan et al. 1996; Jones et al. 2001), which is an indication of male choosiness (effect of female identity in Fig. 1d). Hence, for the zebra finch, empirical studies mostly support scenarios 1c or 1d.

To gain insights into the mechanisms of male persuasion and female resistance, I conducted pairwise mating trials and measured female responsiveness, male song rate, male aggression and the occurrence of copulations. Using this observational approach, I tested the following hypotheses. (1) The occurrence of copulation depends on female responsiveness rather than on male sex drive or male violence (female control; scenarios 1a, 1c, 1d versus 1b). (2) Female responsiveness depends on male identity and male beak colour in particular (preference for ornamented males; 1c versus others). (3) Female responsiveness depends on male song rate (preference for persistent males; 1d versus others). (4) Male song rate partly depends on female identity (male choosiness; 1d versus others).

METHODS

Subjects and Housing

Subjects were from a large, relatively outbred population of wild-type plumage zebra finches maintained at the University of Sheffield, U.K. Birds were kept in cages (60 × 40 cm and 45 cm high) that could be joined together into rows of two (120 cm long) or four (240 cm). Birds were maintained at $21 \pm 2^\circ\text{C}$ and were fed ad libitum on a mixture of seeds: pearl white millet, panicum millet, Japanese millet, canary seed, red millet and yellow millet. Seeds and drinking water were freshly provided every day, lettuce and Abidec vitamin supplement were given once per week. Cuttle fish, fine oyster shells and mineralized tonic girt were provided ad libitum. Rooms were illuminated by full-spectrum fluorescent light (Osram Biolux L 36W/72-965; 14:10 h light:dark photoperiod). This source of light has a spectral distribution that closely matches that of natural sunlight and includes ultraviolet radiation (Osram GmbH, Munich, Germany, personal communication). The birds were bred from pairs housed in individual cages between August 2001 and May

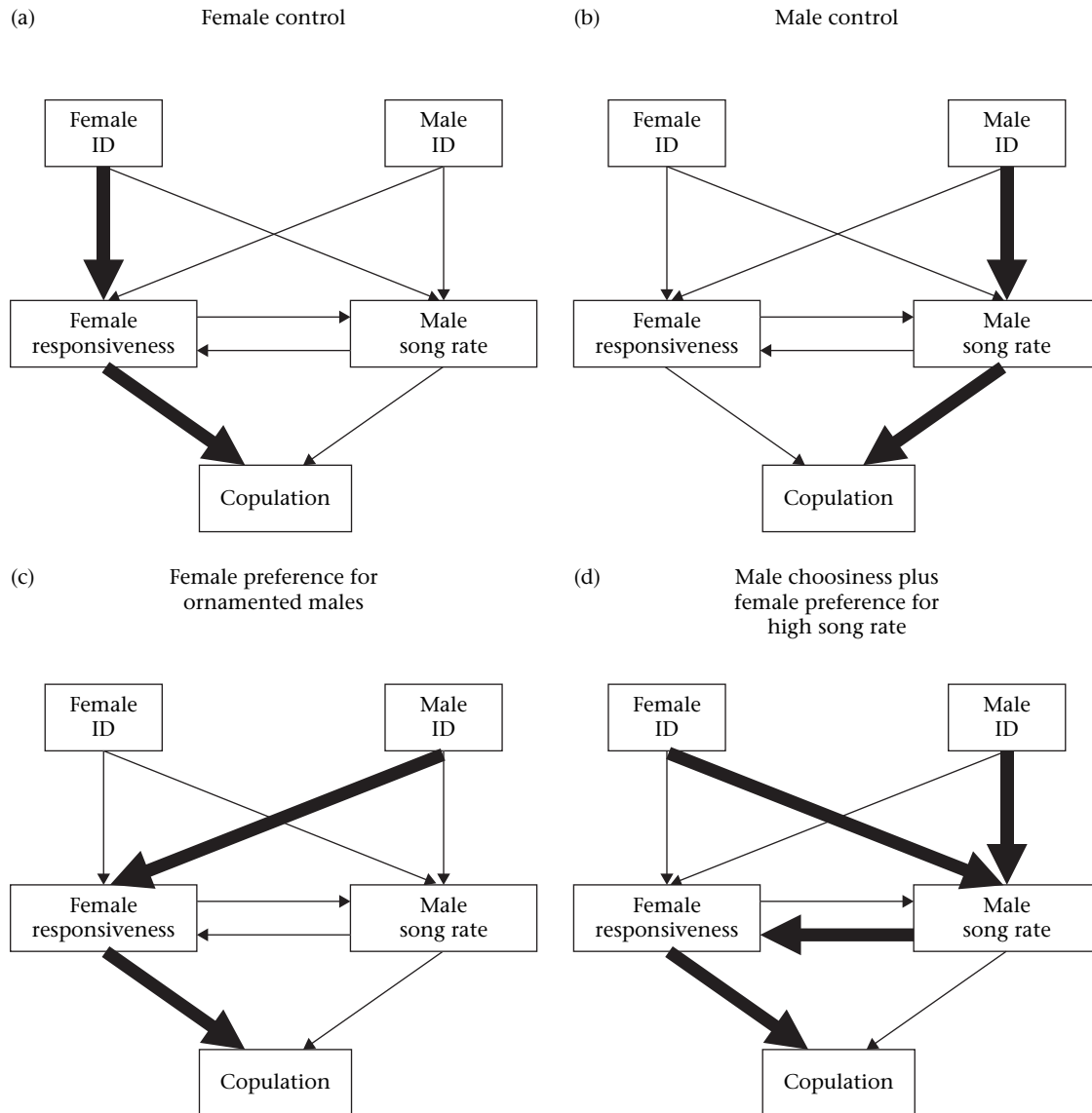


Figure 1. Four simplified scenarios of how the outcome of male–female encounters is determined. Whether copulation takes place depends on the sex drive of the male (measured as song rate) and the readiness of the female to copulate (responsiveness). Song rate depends on male identity (ID, reflecting consistent between-male variation) and is influenced by female identity (reflecting female attractiveness) and female responsiveness. Female responsiveness depends on female identity, male identity and male song rate. Arrows indicate the direction of causal effects, not correlation. Bold arrows indicate where factors are thought to have a dominant effect.

2002. Most of them (72%) were hatched and reared by foster parents, and the remainder were reared by their true parents. From the age of about 35 days onwards, the subjects were kept in unisexual groups to keep them sexually inexperienced.

Mating Trials

When birds were sexually mature (at least 4 months old; Zann 1996), I divided 104 males and 104 females into 13 groups, each comprising eight males and eight females (sexes kept separately). This was done in such a way that no male was related to any female of its group by more than $r = 1/16$, where r is the coefficient of relatedness

when examining the pedigree back to the great-grandparents. Groups of eight birds were maintained in double cages (120 cm). All males received the opportunity to exercise sexual display over 5 days, by creating groups of eight females not otherwise used in this study and releasing each group into a quadruple row of cages together with a group of eight males. To avoid the formation of pairs, I introduced males to a new set of novel females every day. After the period of practice, males were caged in separate pairs, and females remained in groups of eight in double cages.

Tests involving pairwise encounters started 2 weeks or more after the practice period ($\bar{X} \pm \text{SD}$ age, males: 333 ± 78 days, females: 314 ± 96 days when trials started). Within each group ($N = 13$), each of the eight

females was tested with each of the eight males, resulting in 832 trials ($13 \times 8 \times 8$). The eight trials with each individual were carried out on 8 successive days, always between 1200 and 1730 hours (lights were on from 0530 to 1930 hours). Encounters took place in the double cages in which females were maintained by introducing a divider into the double cage, separating the experimental compartment from the rest of the cage. Focal females were placed in the experimental compartment and 1–2 min later the focal male was introduced for 5 min. The experimental compartment contained two perches, a water fountain, a seed dish and a small microphone attached to the front. All trials were videotaped. From these videos, data on male song rate, female sexual responsiveness and the occurrence of copulations were obtained.

Male Behaviour

Using a stopwatch, I measured the number of seconds of directed song within the first 300 s after closing the cage door behind the introduced male. I use the term 'song rate' rather than 'proportion of time spent singing', because in the birdsong literature 'song rate' typically refers to how much a bird sings (regardless of how this amount is measured; e.g. Collins et al. 1994). After square-root transformation, mean song rates of the 104 males were normally distributed (Kolmogorov–Smirnov test: $Z = 0.60$, $N = 104$, $P = 0.87$). A substantial number of trials were without song ($N = 181$), so square-root-transformed song rates of single trials were still not normally distributed ($Z = 4.36$, $N = 832$, $P < 0.0001$), but they were if the zero trials were omitted ($Z = 0.94$, $N = 651$, $P = 0.34$). Undirected song hardly ever occurred and was not recorded. Male aggression (attack with the beak forcing the female to flee) was scored as either present or absent.

Female Behaviour

I use the term female responsiveness in the sense of a female's observed inclination to copulate with a male that is introduced to her. Responsiveness here refers to the female's response to the presence of a male, not necessarily to the display of a male, because a female may indicate her inclination to copulate even when the male is not displaying. Female responsiveness was scored using behavioural cues that were classed as either positive or negative (Morris 1954; Zann 1996). Negative cues were as follows: (1) aggressive supplanting or chasing of the male, (2) threat display with lowered head and sometimes an open beak, (3) offensive beak fencing and (4) fleeing from the male, which is any movement faster than the normal speed of hopping between perches. Positive cues were as follows: (1) tail quivering, (2) wiping the beak on the perch, (3) approaching the singing male and (4) ritualized hopping between the two perches. For each trial, female responsiveness received the score of +1 if only positive cues occurred and –1 if only negative cues were observed. A score of 0 was given when both positive and negative cues occurred. In 121 of 832 trials (15%) no cues of responsiveness were observed. Normally these

were treated as missing values, but in some analyses they were treated as zeros (see Results).

Copulation

I recorded whether successful copulation occurred and whether it had been solicited (preceded by tail quivering). Copulations were regarded as successful if the male's cloacal region made contact with the female's cloacal region. I also recorded incidences of refused copulation attempts, that is, when the male hopped on to the female's back, but the female escaped by jumping off the perch.

Beak Colour

On the day before an 8-day series of trials began, I measured the beak colour of all participating males. As described in Birkhead et al. (1998), beak colour was scored subjectively on a scale ranging from 0 (light orange) to 6 (dark red) using Munsell colour chips. Scoring was done under artificial light with spectral characteristics specially designed for colour matching. To determine the repeatability of this estimate, I scored a random subset of 38 males a second time 14 days later, blind to the previous data. Estimates were highly repeatable within both sexes (males: repeatability = 0.91, $F_{37,38} = 21.8$, $P < 0.0001$).

Statistics

Statistics were calculated using exact tests (StatXact 4.0, Cytel Software Corporation, Cambridge, Massachusetts, U.S.A.) as well as various versions of generalized linear models (GLM), including ANOVAs and ANCOVAs performed by R 1.6.2 (Free Software Foundation, Inc., Boston, Massachusetts, U.S.A.), following Crawley (2002). I assumed normally distributed errors for analyses of song rates and female responsiveness, and I used a binomial error structure in models analysing the occurrence of copulations. To test whether the violation of the assumption of normality in the case of responsiveness scores (Table 1) led to biased estimates of variance components or P values, I repeated the analyses using a Poisson error distribution, where, after I added +1 to the scores explained above, responsiveness took value of 0, 1 or 2 (data were not overdispersed). This led to very similar results (not shown) apart from one significance test (see Results). All tests reported are two tailed.

RESULTS

On average, males sang for a mean \pm SD of 27 ± 25 s (range 0–144 s, $N = 832$) of directed song during trials lasting 300 s. In 181 of 832 trials (22%; Table 1) males did not sing at all. Seven of 104 males did not sing during any of the eight trials, although all of them were capable of singing. Female responsiveness was positive in 151 trials (18%) and successful copulations occurred in 89 of these (59%; Table 1). In the remaining 62 trials, no successful copulations occurred despite positive female

Table 1. Frequencies of various male and female behaviours during pairwise mating trials ($N = 832$)

Behaviour	Trials with that behaviour (N_1)	$N_1/832$ (%)	Of these: trials with successful copulation (N_2)	N_2/N_1 (%)
Directed song	651	78	97	15
No directed song	181	22	0	0
Responsiveness score				
+1	151	18	89	59
0	192	23	8	4
-1	368	44	0	0
No data	121	15	0	0
Solicitation	77	9	64	83
Unsolicited copulation attempts	173	21	51	29
Male aggression	37	4	3	8

responsiveness for several reasons: In nine cases (15%) males attempted to copulate (eight solicited, one unsolicited) but failed because they lost their balance (not caused by female resistance). In 13 cases (21%) males did not sing. Most commonly (40 cases; 65%), males sang but did not attempt to copulate, sometimes even despite female solicitation (four cases). Eight successful copulations occurred when female responsiveness was ambiguous (intermediate score), but none occurred when responsiveness was negative (Table 1). Fifteen males (14%) behaved aggressively at least once, and three of these males did so on a regular basis (four to eight trials). Successful copulations occurred in three of 37 trials involving male aggression (Table 1), but in all cases aggression occurred only after successful copulation, and never vice versa.

Figure 2 shows how the 97 trials leading to successful copulation were distributed between the 104 males and the 104 females. Females deviated significantly from a random binomial distribution (nested within groups;

goodness-of-fit test based on 10^6 Monte Carlo simulations: $\chi^2_8 = 145.8$, $P = 0.0026$), with more females than expected having either no copulations or copulations with more than four males, and fewer than expected having copulations with one or two males. There was no such heterogeneity between males ($\chi^2_8 = 1.8$, $P = 0.884$), indicating that variation in male mating success was no greater than expected by chance alone.

Factors Determining Male Copulatory Success

Females varied greatly in their copulation frequency (Fig. 2), so male copulatory success was strongly dependent on the experimental group to which they belonged, that is, with which females they were tested (GLM with binomial errors, successes versus failures by each of $N = 104$ males: total deviance = 156.6, effect of group: $\chi^2_{12} = 40.3$, $P < 0.0001$). Hence, all subsequent models testing for male copulatory success include group as a factor.

Copulatory success was related to whether males behaved aggressively towards females (GLM: $\chi^2_1 = 3.9$, $P = 0.047$), with aggressive males ($N = 15$) copulating, on average, with 1.19 females and nonaggressive males ($N = 89$) copulating with 0.89 females. To test whether this effect was because aggressive males were generally more active, I excluded all trials where males did not sing at all (because these trials never led to copulation; Table 1). Based on this data set (651 trials by 97 males that sang at least once), aggressive males had no higher success in obtaining copulations (17.0%) than did nonaggressive males (14.2%; GLM: $\chi^2_1 = 1.6$, $P = 0.227$), suggesting that aggression itself did not enhance copulatory success.

Male song rate, averaged over the eight trials of a male, had a highly significant positive effect on male copulatory success (GLM: 832 trials, $\chi^2_1 = 22.9$, $N = 104$ males, $P < 0.0001$; Fig. 3a). However, this effect was absent when excluding trials without song (GLM: 651 trials, $\chi^2_1 = 2.1$, $N = 97$ males, $P = 0.151$; Fig. 3b). This means that in cases where males sang to females, their success was independent of how much they sang to them.

Male beak colour had no significant influence on male copulatory success (GLM based on 832 trials: $\chi^2_1 = 2.3$, $N = 104$ males, $P = 0.133$), yet the trend was in the

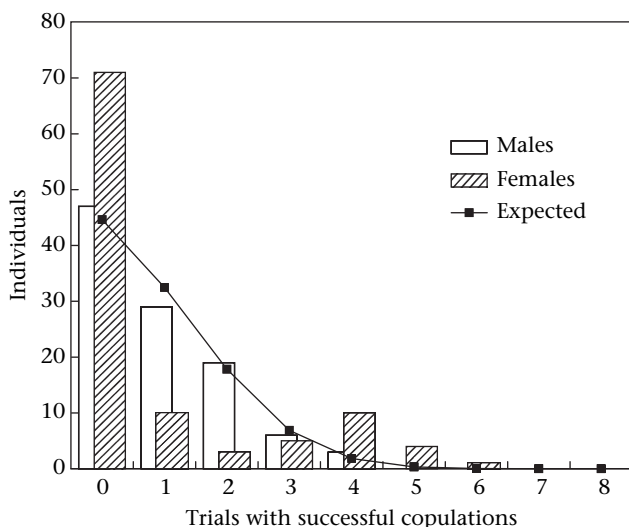


Figure 2. Number of males and females having copulations in 0, 1, 2 etc., of eight pairwise mating trials, each involving a different partner. Expected values represent a random binomial distribution of the successful trials between the individuals, nested within experimental groups.

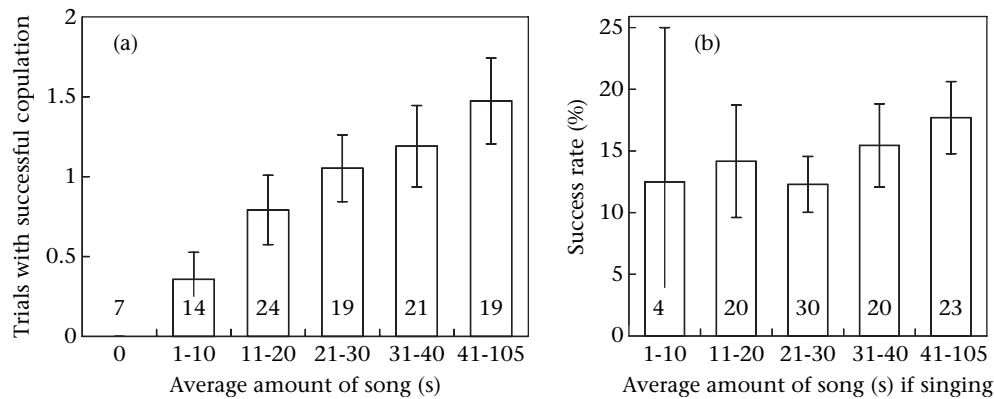


Figure 3. Copulatory success of individual males in relation to their average song rate. (a) Number of trials (of eight) \pm SE in which males copulated successfully in relation to song rate. Males ($N = 104$) are grouped according to their average amount of song delivered (averaged over eight trials for each male). Numbers in bars show the number of males in each category. (b) Same as in (a), but omitting trials without song. Males ($N = 97$) are now grouped according to their average amount of song in trials in which they were singing (excluding the seven males that never sang). Success rate is the percentage of trials in which males copulated successfully.

expected direction, with red-beaked males having more copulations than orange-beaked males. This trend, however, may follow from a weak but significant positive correlation between beak redness and song rate (Pearson correlation: $r_{102} = 0.20$, $P = 0.044$).

Responsiveness

Responsiveness was highly repeatable within females (ANOVA based on 711 trials with responsiveness scores: $F_{103,607} = 12.7$, $P < 0.0001$, repeatability = 0.63). This figure is still high if trials with missing values (no positive or negative signs of responsiveness) are assigned a score of zero (i.e. the intermediate score; $F_{103,728} = 10.3$, $P < 0.0001$, repeatability = 0.54). A multifactorial analysis suggested that responsiveness was also influenced by male identity (GLM, female identity: $F_{91,509} = 12.8$, $P < 0.0001$; male identity: $F_{90,509} = 1.4$, $P = 0.016$), although this effect was much smaller than that of female identity (female identity explained 64.6% and male identity 6.9% of the total variation). In this model, there was no effect of order of testing (test day: $F_{7,509} = 1.0$, $P = 0.443$) and no effect of male song rate ($F_{1,509} = 0.01$, $P = 0.914$) on female responsiveness. This finding was robust: a more parsimonious model, excluding male identity and test day, still showed that responsiveness was independent of song rate ($F_{1,606} = 1.34$, $P = 0.248$). Variation in male beak colour also did not influence female responsiveness (ANCOVA, beak colour nested within females: $F_{1,606} = 0.35$, $P = 0.55$). Repeating these analyses under the assumption of Poisson-distributed rather than normally distributed errors yielded similar effect sizes (female identity explained 67.1% and male identity 6.7% of total deviance), but the male effect was nonsignificant ($\chi^2_{90} = 51.9$, $P = 1.00$).

Song Rate

Male song rate was highly repeatable within males (ANOVA: 832 trials, $F_{103,728} = 12.7$, $P < 0.0001$, repeatability = 0.59), and this repeatability remained

high even when excluding trials without song (based on 95 males with more than one measurement, $F_{94,554} = 7.4$, $P < 0.0001$, repeatability = 0.48). The full factorial analysis of the whole data set requires that missing values for female responsiveness are set to zero (see [Methods](#), [Table 1](#)), which means that the absence of positive and negative female signs is interpreted as an intermediate value of responsiveness. This analysis shows that song rate was significantly influenced by female identity (GLM, male identity: $F_{91,629} = 15.0$, $P < 0.0001$; female identity: $F_{91,629} = 2.0$, $P < 0.0001$). This finding was robust to the exclusion of trials without song (female identity: $F_{91,463} = 1.8$, $P < 0.0001$), the test condition where the assumption of normally distributed errors was met (see [Methods](#)). There was significant heterogeneity between test days ($F_{7,629} = 16.2$, $P < 0.0001$), and a plot of daily averages indicated that song rate declined over the 8-day period. The model also includes a significant effect of female responsiveness on male song rate ($F_{1,629} = 10.0$, $P < 0.0001$). However, this latter effect is spurious, because it results from assigning zeros to missing values of female responsiveness. When the same model was restricted to the 711 trials with real estimates of female responsiveness, there was no effect of responsiveness on song rate ($F_{1,509} = 0.01$, $P = 0.914$; see above).

Copulations

The occurrence of copulations depended primarily on female responsiveness (GLM with binomial errors: 832 trials, total deviance = 599.2, effect of responsiveness: $\chi^2_1 = 319.9$, $P < 0.0001$), but also to some extent on male song rate ($\chi^2_1 = 27.1$, $P < 0.0001$). Test day had no effect on the occurrence of copulations ($\chi^2_7 = 8.2$, $P = 0.312$).

These findings are summarized in [Fig. 4](#), where the percentage of variance explained by each factor is given and, in the case of the binomial model for copulation, the percentage reduction in deviance for each factor is shown. All analyses were carried out in two versions, one based on the 711 trials where estimates of female responsiveness

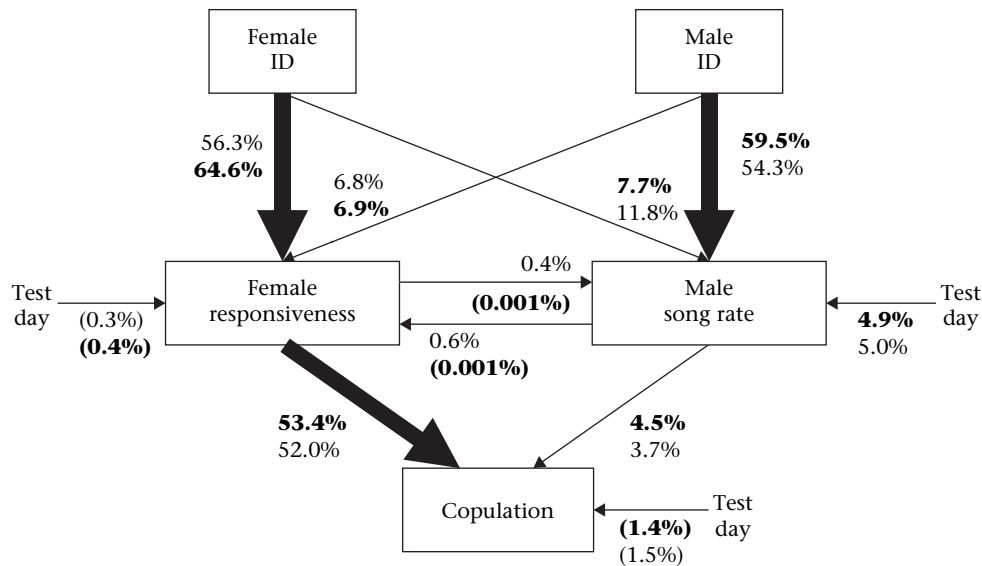


Figure 4. Explanatory power of factors influencing the outcome of male-female encounters. Numbers show the percentage of variance explained in ANCOVAs (for 'responsiveness' and 'song rate'), or, respectively, reduction in deviance in bivariate GLMs (for 'copulation'). The analyses were carried out twice, first based on all 832 trials (first percentage values), and second based on the 711 trials where estimates of female responsiveness were available (second values). In the first analysis, missing values for responsiveness were set to zero. For some relations, the first type of analysis is more meaningful, for others the second is (highlighted in bold). Values in parentheses were not significantly different from zero. Male song rate and female responsiveness were hardly influenced by the identity (ID) of the individual of the opposite sex; variation in responsiveness largely decided whether copulations occurred.

were available, and the other on all 832 trials, assigning zeros to missing values of responsiveness. The two analyses produced similar results except for the spurious correlation between song rate and responsiveness.

DISCUSSION

Female Control

The results strongly support the view that female zebra finches are largely in control of copulation. Aggression towards females did not seem to enhance male copulatory success. Successful copulations never occurred without females showing some positive inclination towards mating. However, male choosiness also played a role: males consistently sang more to some females than to others, and males did not take advantage of every opportunity to copulate (i.e. where females signalled readiness to copulate). Nevertheless, whether successful copulations occurred or not depended more on the readiness of the female than that of the male, thus rejecting the scenario in Fig. 1b. Hence, females seem to play the key role in deciding about copulations, just as the final choice in pair formation seems to lie largely with the female (Immelmann 1962; Clayton 1990).

Resistance versus Preference

Female readiness to copulate was remarkably consistent within individual females and did not depend on either male beak colour or male song rate (rejecting scenarios 1c and 1d), even though most studies in other laboratories have shown female preferences for males with dark red

beaks (Burley & Coopersmith 1987; Houtman 1992; de Kogel & Prijs 1996; Blount et al. 2003; but see Balzer & Williams 1998) and high song rates (Houtman 1992). If other, unmeasured, male characteristics influenced female responsiveness, one would have predicted a consistent effect of male identity on female responsiveness. Such an effect was weak at best, and its statistical significance seems doubtful, given the nonnormal error structure of responsiveness scores. The absence of a consistent male effect means that females showed either very little agreement in their preferences or no meaningful preferences at all. These findings are more consistent with chase-away sexual selection (Holland & Rice 1998; Rice & Holland 1999) than with good genes, where all females should be most responsive towards good-genes males.

Issues of Experimental Design

One limitation of the experimental design used in the present study is the short duration of trials (5 min). Hence, measures of song rate and responsiveness may also reflect variation in mating speed. It is difficult to predict how behaviours would change if trials lasted for several hours or days. For instance, male copulatory success was independent of male aggression, but it is not clear whether male aggression might have long-term effects on female resistance. Nevertheless, the trial duration used closely represents the natural situation. Zebra finches breed in dense colonies, and in the wild, females are frequently courted for short periods by males seeking extrapair copulations (Birkhead et al. 1988a; T. R. Birkhead, personal communication). In such situations, females have to decide within seconds or minutes whether to copulate

with the courting male. In the present experiments, female readiness to copulate could often be recognized within the first 10–20 s of a trial, and in trials with successful copulation, the median latency to copulate was 40 s. Earlier trials lasting up to 1 h indicated that copulations are unlikely to occur if they have not happened within the first 5 min (Birkhead et al. 1988b; T. R. Birkhead, personal communication).

Most studies of female mating preferences have used choice-chamber experiments, where a female can move between separately caged males that are presented to her simultaneously (e.g. Burley & Coopersmith 1987; Houtman 1992; Collins et al. 1994; de Kogel & Prijs 1996; Blount et al. 2003). In many of these experiments, however, it is unclear whether the female, by sitting next to one male rather than the others, is actually expressing her sexual preference (Burley et al. 1982, 1990; Sullivan 1994). In the present study, this problem was largely avoided because variation in female readiness to copulate should primarily reflect her mating preferences. In both experimental set-ups, choice-chamber tests and pairwise encounters, females have to compare males by visiting them sequentially, but in choice-chamber trials females can inspect males repeatedly, and the intervals between seeing two males are shorter than in the set-up that I used (one male presented per day). The importance of these experimental issues for the measurement of mating preferences depends on how information about males is stored by the female and whether females have preexisting thresholds of response towards given levels of male attractiveness (Wagner 1998; Raffa et al. 2002). At least, the present study showed that males did not differ considerably in their capability of exploiting any potentially existing biases of the sensory system of females. The experiments suggest that males have limited means of increasing the sexual responsiveness of females beyond the baseline levels that are elicited by any male zebra finch.

To test whether the apparent lack of common mating preferences was caused by the experimental design, I conducted classical choice-chamber trials, each lasting 3 h, with the same birds directly after the experiments reported here (Forstmeier & Birkhead 2004). Using this more conventional set-up, I found little between-female agreement in choice and no support for the idea that female agreement might increase with increasing sexual experience (unpublished data).

Female Preference for Red Beaks

Male beak colour was unrelated to female responsiveness and to male copulatory success. The present study is not the first to find no female preference for red-beaked males (Balzer & Williams 1998). However, most other studies have reported such a preference (Burley & Coopersmith 1987; Houtman 1992; de Kogel & Prijs 1996; Blount et al. 2003), except those in which beak colour was manipulated using paint (Collins et al. 1994; Sullivan 1994; Weisman et al. 1994; Vos 1995; but see Burley & Coopersmith 1987). It is possible that beak colour per se is

not used to assess male quality, but rather that beak colour is correlated with another male trait that females find attractive (e.g. Blount et al. 2003) in at least some captive zebra finch populations. The inconsistency of findings obtained from different study populations (see also Collins & ten Cate 1996) is remarkable, and more generally, it seems common that female preferences are found in one but not in another population of the same species (Parker & Ligon 2003; Forstmeier & Leisler 2004).

Female Preference for High Song Rate

Researchers in several studies have claimed that female zebra finches prefer males that sing at a high rate (Clayton & Pröve 1989; Houtman 1992; Collins et al. 1994; de Kogel & Prijs 1996; Balzer & Williams 1998). However, the evidence is not convincing. Males are stimulated to sing by the presence of a female (Collins 1994), so song rate should not be measured during choice-chamber trials, but rather in forced pairwise encounters lasting for a fixed period; otherwise, one is bound to obtain a spurious association between male song rate (stimulated by female presence) and female preference (measured in terms of female presence; e.g. experiment 1 in Collins et al. 1994; de Kogel & Prijs 1996; Balzer & Williams 1998). Other evidence is weak because it suffers from pseudoreplication (experiment 2 in Collins et al. 1994), or because the effects of song rate in playback experiments could not be separated from the effects of strophe length or repertoire size (Clayton & Pröve 1989). Thus only Houtman (1992) clearly demonstrated that females in a choice chamber preferentially approached males that, independently of the choice experiment, sang at a high rate during pairwise encounters. However, this preference could indicate simply that females looking for a partner preferentially visit certain males for a closer inspection, which, by singing frequently, also signal their interest in mating (see also Collins 1994). Hence, the present study was the first to test whether female sexual responsiveness itself was related to the rate of male singing, and this relation was not found.

Male Choosiness

The observed male choosiness in terms of singing intensity and utilization of opportunities to copulate (when female responsiveness is positive) is unlikely to be caused by sperm limitation (Dewsbury 1982; Sæther et al. 2001), because the rate of copulation in the present experiment was very low (Birkhead et al. 1995). Furthermore, males were no more likely to pass up an opportunity to copulate if they had copulated on the previous day (31% of 13 trials) than if they had not (42% of 138 trials, referring to the 151 trials with positive responsiveness listed in Table 1). I therefore suggest that males were selective because they were unpaired and were probably looking for a social mate and not just a single copulation. Future experiments will have to evaluate whether paired males are less selective when seeking extrapair copulations.

Individual Differences in Male Song Rate: Alternative Strategies?

Males differed consistently in how much they sang to females. The widespread idea of song rate as an honest indicator of male genetic quality (Catchpole & Slater 1995; Searcy & Yasukawa 1996; Gil & Gahr 2002) assumes that (1) song is costly to produce, and hence that (2) males in good condition sing more than males in poor condition. Assuming that there is heritable variation in male condition, females are thought to profit from selecting males with a high song rate, and hence (3) all females are expected to prefer to copulate with these males. However, little evidence supports any of these assumptions. (1) Recent studies suggest that the metabolic cost of singing in zebra finches is not high, and that costs should arise primarily because the time spent singing cannot be used for other tasks (Oberweger & Goller 2001). (2) Song rate of zebra finches, when measured appropriately, was not affected by nutritional stress experienced during early development (Kilner 1998; Birkhead et al. 1999), suggesting little dependence on condition. (3) A female preference to copulate with males that sing at a high rate has never been shown directly (not even by Houtman 1992), including the present study.

There is another possible explanation for the maintenance of variation in male song rate. Experimental evidence suggests that the song rate of male zebra finches is proximately triggered by androgen levels (Pröve 1978; Harding et al. 1983), and that androgens mediate the trade-off between parental effort and mating effort in birds in general (reviewed by Ketterson & Nolan 1999). Such trade-offs can potentially result in alternative mating strategies, which are maintained by frequency-dependent selection. Males that do not sing to females other than their partner will never obtain extrapair copulations (Fig. 3a), but they may profit from investing the time they save in mate guarding (ensuring paternity) and parental care (enhancing offspring viability). In contrast, males with a high song rate may benefit from obtaining extrapair copulations, but the time that they spend courting other females cannot be invested in brood care. The benefits that males can obtain from seeking extrapair copulations are frequency dependent, being greatest when few other males follow the same strategy and least when all males pursue this strategy.

This alternative explanation is no less speculative than the much-invoked good-genes scenario; it is merely intended as an outline of what is already known and what kind of information is needed in the future. It would be important to show that frequently singing males obtain more extrapair paternity and that rarely singing males provide more paternal care.

Individual Differences in Female Responsiveness

Female responsiveness was highly consistent within individuals over the 8-day testing period. Females were tested again when breeding pairs were formed about 6 months later, and responsiveness scores were highly

correlated with the earlier ones (unpublished data), indicating a high stability of between-individual differences over time. Jennions & Petrie (1997) reviewed possible reasons for individual differences in female mating behaviour, but too little is known about such variation in the zebra finch to determine the causes of this.

Evidence for Chase-away Selection?

Responsiveness varies considerably between females and it hardly seems to be affected by the males' effort to seduce females. In the zebra finch, male ornamentation and sexual display seem to have evolved through intersexual selection rather than intrasexual selection, because these signals are not used in male-male competition (Burley & Coopersmith 1987; Zann 1996; Etman et al. 2001). Although we lack evidence for female resistance to male seduction building up over evolutionary periods (Holland & Rice 1998), the observed degree of female resistance is best explained by chase-away sexual selection. In zebra finches, resistance is achieved not by making female preferences more stringent (Rosenthal & Servedio 1999; Kokko et al. 2003), but by ignoring the variation in male effort to seduce (Holland & Rice 1998; Rice & Holland 1999). This apparently widespread mechanism of resistance (Holland & Rice 1998) has not received adequate attention in theoretical models of sexual conflict (e.g. Kokko et al. 2003).

Conclusion

It is surprising how little is known about the sources of between-individual variation in mating behaviour of both male and female zebra finches. These individual differences seem to be a much better predictor of the outcome of mating trials than the assumption that all females prefer to mate with the highest-quality male. A promising approach would be to study the heritability and condition dependence of individual differences in sexual behaviour and to examine how these behavioural differences relate to fitness under seminatural aviary conditions.

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