Manipulating the perceived opportunity to cheat: an experimental test of the active roles of male and female zebra finches in mate guarding behavior

Leah C. Wilson · John P. Swaddle

Abstract Birds are commonly sexually promiscuous, which can lead to conflict between the sexes and the evolution of paternity assurance strategies, such as mate guarding. Adaptive explanations for mate guarding have tended to focus on fitness consequences for males, but mate guarding and participation in being guarded is also likely adaptive for females in certain contexts. To better understand the adaptive explanations for mate guarding as well as the observed variation in paternity patterns, it is necessary to explore the relative costs and benefits of guarding (and being guarded) from both the male and female perspective. To investigate these costs and benefits, we conducted an experiment with the Australian zebra finch (*Teniopygia guttata*) in which we independently varied the perceived opportunity for each member of a captive breeding pair to engage in extra-pair copulation (EPC) solicitation behavior; as an individual's EPC opportunity increased, the partner's EPC opportunity remained constant. Our results indicate that, for males, mate guarding intensity increases when their female's EPC opportunity increases but decreases when their own (i.e., male) EPC opportunity increases. We did not find evidence of flexible female guarding behavior, but we found that females do not evade their partners more as female EPC opportunity increases.

Keywords Extra-pair copulation · Extra-pair paternity · Mate guarding · Sexual selection · *Teniopygia guttata* · Zebra finch

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Introduction

Extra-pair fertilization (EPF) is an almost ubiquitous avian reproductive tactic. Approximately 90 % of avian species engage in EPF, and interspecific rates of extra-pair offspring range from 1 to 72 % (Griffith et al. 2002). In addition to influencing the strength of sexual selection, EPF has a profound impact on the evolution of many aspects of life history (Griffith et al. 2002). Sexual conflict over EPF has led to the evolution of paternity assurance strategies, including frequent copulation and mate guarding (Birkhead and Møller 1992). When defined from the male perspective as the close following of a fertile female, mate guarding seems to be absent only in those contexts where effective guarding is precluded by an ecological or social factor (e.g., intense nest site competition) (Møller and Birkhead 1991). Exploring the behavioral mechanisms underlying EPF is critical both to understanding the observed variation in rates of promiscuity and to clarifying the adaptive basis of EPF itself (Petrie and Kempenaers 1998).

To the degree that mate guarding is adaptive, it is shaped by the costs and benefits of engaging in the behavior. The two primary costs of mate guarding by the male are the energetic costs (Komdeur 2001) and the opportunity costs of not pursuing EPFs (Hasselquist and Bensch 1991; Dickinson 1997). The chief benefit of mate guarding is the suppression of female EPFs (Komdeur et al. 2007). There is observational and experimental evidence of substantial

plasticity in the expression of mate guarding behavior. Within a species, males guard females *more closely* when male extra-pair copulation (EPC) opportunity decreases (e.g., Johnsen and Lifjeld 1995) or when female EPC opportunity increases (e.g., Dickinson and Leonard 1996). Conversely, males guard females *less closely* when male EPC opportunity increases (e.g., Chuang-Dobbs et al. 2001) or when female EPC opportunity decreases (e.g., Komdeur 2001). To understand the adaptive basis of this plasticity, as well as the relationship between mate guarding intensity and observed paternity patterns, it is necessary to explore how mate guarding behavior is shaped by variation in the costs and benefits of engaging in the behavior.

Although the costs and benefits of mate guarding have mostly been explored from the male perspective, it seems reasonable to suggest that the female guarding of a male is under similar selection in certain environments. Both males and females can limit the promiscuity of their partners by guarding them (e.g., Sandell 1998; Komdeur et al. 2007), and mate guarding is likely adaptive for both sexes when there are costs to mate infidelity (Petrie and Kempenaers 1998). Male infidelity may be costly to females by increasing the risk of sexually transmitted disease (Sheldon 1993) and decreasing paternal investment (Yasukawa and Searcy 1992).

The active role of the female and evolution of female behavioral strategies have not been formally modeled as part of the male–female "mate guarding" interaction. Although the female is usually thought of as a passive object in mate guarding, it is possible that the behavior is strongly influenced by female strategies concerning access to extra-pair mates and her influence of her partner male. Active female participation in being guarded by the male may be adaptive when there are fitness costs to interacting with extra-pair males (Lifjeld et al. 1994). By participating in being guarded, females may avoid both forced EPCs (Low 2005) and harassment by extra-pair males (Kempenaers et al. 1995). Common field measures of male guarding of the female tend to discount the active role of the female; measures of pair proximity do not account for female involvement in maintaining that proximity (e.g., Estep et al. 2005), and some measures of male following fail to account for variation in female evasion (e.g., Chuang-Dobbs et al. 2001). When the benefits of guarding a mate are high and the benefits of pursuing EPFs are low, individuals may actively participate in being guarded (Burley et al.1994; Kempenaers et al.1995). Failure to consider participation in being guarded by both sexes may be part of the reason why we have not been able to detect general patterns in mate guarding and EPF across taxa (Dias et al. 2009). In this paper, we present data from an experiment in which we independently varied the perceived opportunity to gain access to extra-pair partners for both members of a breeding pair. We then assessed variation in guarding behavior and participation in guarding behavior from both male and female perspectives.

Study system

The zebra finch is a small estrildid finch endemic to Australia (Zann 1996). As in other species, EPFs are produced and prevented though the behavioral conflicts between (1) pair members and (2) pair and extra-pair individuals. Frequent pair copulations and mate guarding are both important paternity assurance strategies in the zebra finch (Birkhead et al. 1989). In both the wild and captivity, males guard their females most intensely during the female's fertile period (Birkhead et al. 1989). Males mate guard by attacking intruding extra-pair males, producing alarm calls when the female is out of sight, and following the female's movements (Birkhead et al. 1988, 1989). Approximately 40 % of all EPCs do not result in fertilization because the pair male attacks the extra-pair male (Birkhead et al. 1989). There is evidence that females may participate in being guarded by delaying departure from the nest when the male is absent (Birkhead et al. 1988). Further, females may more readily participate in being guarded by their mates when paired to males that have been experimentally manipulated to appear more attractive (Burley et al. 1994).

Despite male paternity guards and possible female participation in being guarded, EPFs do occur. Rates of EPF range from 1.7 % of offspring in the wild (Griffith et al. 2010) to 27 % of offspring in captivity (Burley et al. 1996). Both males and females commonly solicit EPCs both in the wild and in captivity (Birkhead et al. 1988, 1989; Burley et al. 1994). Forced EPCs are common, but as they are unlikely to result in successful EPFs, they may represent a form of harassment rather than a paternity threat (Birkhead et al. 1989). These data indicate several ideas: (1) low levels of EPF are maintained though male (possibly mutual) mate guarding; (2) forced EPCs may constitute a form of harassment to the female; and (3) the pair male can prevent EPCs, including forced EPCs, and EPFs if he is physically present.

To explore the relative importance of costs and benefits in structuring male and female guarding behavior, we exposed breeding pairs of zebra finches to extra-pair stimulus birds in varying sex ratios. We separately manipulated the opportunity for each member of the pair to engage in an EPC: as an individual's EPC opportunity increased, their mate's EPC opportunity remained constant and vice versa. By symmetrically manipulating EPC opportunity, we were able to assess the degree to which paired males and females respond as if they are sensitive to a potential benefit (keeping their partner from engaging in extra-pair mating) and to a potential cost (lost opportunities to pursue EPFs) of guarding and being guarded. Hence, we could explore the role of the male and female separately in structuring mate guarding behavior as the opportunity for EPC increased. As far as we are aware, this is the first study to manipulate EPC opportunities for both sexes within the same experimental design.

Methods

Pair formation

Excluding siblings and half-siblings, we arbitrarily paired 18 adult males and females from our large, outbred colony. All birds were maintained on a constant 14:10 light/dark photoperiod at approximately 19 °C with a nutritionally complete seed mix, water, cuttlebone, and grit available ad libitum. Each pair was housed in a cage measuring approximately 50 cm×30 cm×40 cm and was provided with a plastic hooded nest box and nesting material. All experimental procedures were approved by our Institutional Animal Care and Use Committee.

We considered the pair bond to be established when the pair built a nest and began laying eggs (Zann 1996). If the pair did not begin nesting behavior within 3 weeks, females were re-paired with a new male. To maximize the probability that the experimental female was fertile, we began the experimental protocol immediately after the female had begun laying. Female zebra finches are fertile between day −11 and day +3 where day 0 is the day the first egg is laid (Birkhead et al. 1989).

Experimental trials

Within the experimental chamber (Fig. 1), a pair could be exposed to up to four extra-pair stimulus birds at a time. By varying the number and sex ratio of stimulus birds, we were able to separately manipulate the perceived opportunity for each member of the pair to pursue an EPC. While stimulus birds were separated from the focal pair by thin metal mesh, all birds were able to interact visually and acoustically. The chamber was 1.54 m across, had 61 cm^2 of floor space, and was 42 cm tall. The outer walls of the chamber were opaque plastic, and the ceiling was Plexiglas in order to allow birds in the chamber to be video recorded from above. To facilitate identification, the focal male in each trial was painted with a small (<3 mm^2) drop of white correction fluid on his cap.

Experimental pairs were moved to the experimental chamber and were given 7 days to acclimate to the space before we began the experiment. Based on a preliminary study using fecal droppings as evidence of visitation, 7 days was enough time for pairs to explore all arms of the chamber. After the acclimatization period, we conducted a control trial ("trial 0") in which no stimulus birds were presented. This baseline trial let us assess whether the pair had preferences for locations within the chamber before the experimental presentation of stimulus birds. We then conducted five experimental trials in which we varied the number and sex ratio of stimulus birds (summarized in Table 1); trial 1 presented zero females and one male; trial 2 presented one female and zero males; trial 3 presented one female and one male; trial 4 presented three females and one male; and trial
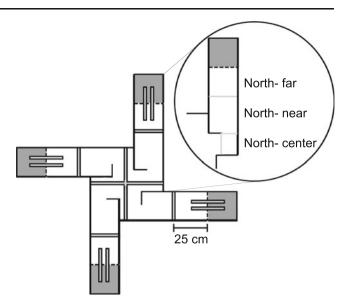

Fig. 1 Experimental arena from above. *Shaded boxes* represent stimulus bird cages; *gray bars* represent perches. The arena was subdivided into 13 areas based on line-of-sight and proximity to the stimulus cages

5 presented one female and three males. The experimental trials were conducted in a randomized order. Each trial lasted 3 h and was video recorded from above with a Sony HDR-SR1 digital video camera affixed with a wide-angle lens attachment. One to two trials were conducted each day. The first trial of the day was conducted 1–3 h after the lights came on, and the second trial of the day was conducted 1 h after the first trial ended. The pair was in the chamber for a total of 11–13 days (seven acclimation days and four to six experimental days).

The stimulus birds presented in each trial were selected from a group of 28 adult females and 35 adult males set aside from the breeding colony. During the experimental trials, each focal pair was exposed to six different stimulus males and six different stimulus females in the specified combinations. The same stimulus bird was never presented twice to the same focal pair, and the same 12 stimulus birds were not presented to every pair. We used a random number

Table 1 Number of stimulus birds presented in each experimental trial. Trials were separated into two groups for analysis: (1) "increasing stimulus males" while keeping the number of stimulus females constant: trials 2, 3, and 5; and (2) "increasing stimulus females" while keeping the number of stimulus males constant: trials 1, 3, and 4. These groupings are reflected in the "trials compared" to answer particular questions described in Table 2

Trial	Stimulus females	Stimulus males
1	0	1
2	1	0
3	1	1
4	3	1
5	1	3

table to select stimulus birds and to determine in which arm of the chamber to place each stimulus bird in a given trial. When not in the experimental chamber, stimulus birds were housed in single-sex cages of four to seven individuals.

To score the video recording of each trial, we divided the experimental chamber into 13 regions (Fig. 1) and created a transcript of each focal bird's movements. Each time a bird moved from one region to another, we recorded the new location and time. Videos were scored blind to the trial identity.

To evaluate if the pairs were behaving normally in the experimental chamber (i.e., not overtly stressed and consequently disinterested in mating or courtship activities), we documented the occurrence of pair bonding activities. We recorded copulations, allopreening events, and bouts of nest-building behavior—although pairs did not have a nest box, males would tear the newspaper lining the floor, and proffer the nesting material to the female. To test for the effect of the stimulus birds on pair copulation behavior, we scored pair copulations as (1) within eyesight of a stimulus male, (2) within eyesight of a stimulus female, or (3) out of eyesight of a stimulus bird.

Mate guarding behaviors

We quantified multiple behaviors important to guarding and being guarded. These behaviors can be separated into three categories: (1) "pair behavior" in which we describe general patterns of pair association, (2) "individual behavior" in which we describe an individual's tendency to evade and pursue his/her mate, and (3) "interactions with a potential EPC partner" in which we describe an individual's contact with opposite-sex stimulus birds. Each of these is described in more detail below.

Pair behavior

We gauged pair association with two measures of pair affinity: proximity and eyesight. A pair was considered to be in close proximity if individuals were in the same or adjacent regions of the chamber (Fig. 1). To calculate time within eyesight, we divided the experimental chamber into nine areas based on line-of-sight divisions. For each trial, we calculated the proportion of time the pair spent in close proximity, the proportion of time the pair spent within eyesight of each other, and the average duration of each bout of time the pair spent out of eyesight.

Individual behavior

We assessed an individual's tendency to evade and pursue his/her mate by quantifying leaving and following behavior within one of two specified time periods (see below). Individuals were scored as "leaving" when they left their mate's line-of-sight and as "following" when they restored line-of-sight

after being left by their mate. While "leaving behavior" was measured independently of the mate's behavior, "following behavior" depended on the mate's leaving behavior. In trials where an individual was never left by his/her mate, that individual was excluded from the "following" analysis.

The maximum latency to follow was 1 h and 7 s. Since we felt that a move after such a length of time was not necessarily in response to being left, we grouped "following moves" into two latency groups. We calculated the proportion of leaving moves that an individual followed (1) within 5 s, and (2) within 27 s. We chose 5 s as the "short" latency because it represented the average amount of time between a copulation solicitation and copulation, based on the pair copulations observed in the current study. We chose 27 s as the "long" latency because 90 % of all following moves occurred within this time frame. Since individuals followed most of their mate's movements within 27 s, the long latency gave us a broad picture of following behavior mostly independent of latency. The longer latency also represented the outside limit of what is reported in field studies where habitat features may hinder the simultaneous observation of both pair members (e.g., Kempenaers et al. 1995 [30 s]; Hansen et al. 2009 [15 s]).

Interactions with a potential EPC partner

To quantify an individual's contact with potential EPC partners, we calculated (1) the number of times a focal individual visited an opposite-sex stimulus bird; and (2) the total amount of time a focal bird spent alone with an opposite-sex stimulus bird. "Visits" were calculated by counting the times the focal individual ventured to either the near or far region of the stimulus arm (i.e., within 42 cm of the stimulus cage) (Fig. 1). "Time alone" was calculated by summing the time the focal bird spent in the far region of an arm when the individual's mate was neither in that region nor in the adjacent region (Fig. 1).

For this last set of measurements, scores were reported relative to the focal individual's behavior in that arm in a control trial when the stimulus cage was empty. For example, if a focal female visited the north arm three times in the control trial and ten times in a trial when there was a male stimulus bird in the north arm, the female would be reported as visiting the male stimulus bird +7 times during the experimental trial. If she had spent 20 min alone in the north arm during the control trial and 8 min alone in that arm when a male stimulus was present, she would be reported as spending -12 min alone with the male stimulus bird during the experimental trial. Calculating these measurements relative to the control trial adjusted the scores for any inherent bias in movement or arm preference by birds in the chamber.

Experimental questions

In order to compare male and female guarding behavior, we compared leaving and following behavior between the sexes when the perceived EPC opportunity was the same for both individuals (one stimulus male, one stimulus female) (Table 2, question 1). We then compared the behavior in trials across which the EPC opportunity varied for one pair member but remained constant for the other. When an individual's perceived EPC opportunity increased, a potential cost of mate guarding increased (Table 2, question 2). When an individual's mate's perceived EPC opportunity increased (i.e., when that individual's risk of being cheated on increased), a potential benefit of mate guarding increased (Table 2, question 3). In order to independently examine the active role of the both sexes, we examined guarding behaviors separately from the male and female perspective.

Statistical analysis

We compared leaving and following behavior between the sexes with a paired samples Student's t test (Table 2, question 1). For all measurements of mate guarding behavior, we separated the five experimental trials into two groups for analysis (Table 1). In the first group, the number of stimulus males increased, but the number of stimulus female remained constant; in the second group, the number of stimulus females increased but the number of stimulus males remained constant. Using a repeated-measures (within-pair) ANOVA where pair identity was the repeated measure, we compared the measures of pair and individual behavior (1) as the number of stimulus males increased, and (2) as the number of stimulus females increased (Table 2, questions 2–3).

We tested for an effect of stimulus bird presence on pair copulation behavior with a chi-square goodness-of-fit test comparing the observed locations of copulation events against the expected locations based on a random distribution. All analyses were conducted with SPSS PASW Statistics v17 (Chicago, IL, USA) employing two-tailed tests of probability and an alpha level of 0.05.

Results

Pair bond in the chamber

Most pairs showed clear evidence of a pair bond while in the experimental chamber. Of the 18 experimental pairs, 15 were observed allopreening and over two thirds of the pairs that allopreened also copulated or attempted to nest build (Online Resources 1 and 2). As allopreening is considered a stereotypic sign of pairing in zebra finches (Zann 1996), we excluded the 3 pairs that did not allopreen from subsequent analysis,

reducing the sample size to 15. Although the excluded pairs had been nesting prior to the experiment, their introduction into the experimental chamber may have disrupted either their pair bond or the female's fertility status.

In the 324 h of recorded observation, 9 of the remaining 15 pairs copulated a total of 19 times; 17 copulations (89.4 %) occurred out of eyesight of a stimulus bird; 1 copulation (5.2 %) occurred within eyesight of a stimulus male; and 1 copulation (5.2 %) occurred within eyesight of a stimulus female. Copulations were more likely to occur out of eyesight of a stimulus bird than would be expected from a distribution based solely on the proportion of the experimental chamber within eyesight of stimulus birds ($\chi^2=6.88$, $df=2$, $P=0.032$).

Male vs. female guarding profiles

When EPC opportunity was the same for both pair members (i.e., when the pair was exposed to stimulus birds in an even sex ratio), males and females had similar mate guarding profiles (Table 2, question 1). In trial 3 (one stimulus male, one stimulus female), the focal male and female were equally likely to leave one another ($t_{14}=0.33$, $P=0.743$). There was a nonsignificant tendency for the male to follow a higher proportion of the females' moves than vice versa, both within the short latency (62.6 vs. 32.4 %; $t_{14}=1.74$, $P=0.100$) and the long latency (62.6 vs. 43.8 %; $t_{14}=1.94$, $P=0.073$).

Increasing EPC opportunity

Pair association

Variation in EPC opportunity did not greatly impact general patterns of pair association (Table 2, question 2a). Pairs stayed close together in all trials; pairs spent 72–76 % of the time in close proximity and 79–85 % of the time within eyesight of each other. An average bout out of eyesight lasted 19.2–31.2 s. Variation in the focal female's EPC opportunity (i.e., the number of stimulus males) had no influence on the percent of time a pair spent in close proximity ($F_{2,\ 28}=0.03$, $P=0.968$), the percent of time a pair spent within eyesight ($F_{2,\ 28}=0.15$, $P=0.864$), or the average bout of time a pair spent out of eyesight ($F_{2,\ 28}=0.76$, $P=0.480$). Similarly, variation in the focal male's EPC opportunity (i.e., the number of stimulus females) had no detectable effect on proximity ($F_{2,\ 28}=0.26$, $P=0.772$), eyesight ($F_{2,\ 28}=0.91$, $P=0.415$), or the average bout of time out of eyesight ($F_{2,\ 28}=0.28$, $P=0.757$).

Following behavior

Relative EPC opportunity influenced male, but not female, partner-following behavior (Table 2, question 2b). Male

Table 2 Experimental questions and how these relate to the data analyzed. For questions 2a through 3b, different trials are analyzed according to the female perspective (F) or the male perspective (M) of the same question

Question	Trial(s) compared	Behavior(s)
1. When EPC opportunity is the same for both, are male and female guarding profiles different?	3	Leaving; following
2a. As an individual's EPC opportunity increases, does the pattern of pair association change?	F: 2, 3, 5 M: 1, 3, 4	Proximity; eyesight
2b. As an individual's EPC opportunity increases, does that individual follow their mate less?	F: 2, 3, 5 M: 1, 3, 4	Following
2c. As an individual's EPC opportunity increases, does that individual participation in being guarded less?	F: 2, 3, 5 M: 1, 3, 4	Leaving
3a. As a mate's EPC opportunity increases, do individuals mate guard "more"?	F: 1, 3, 4 M: 2, 3, 5	Leaving; following
3b. As a mate's EPC opportunity increases, do individuals interact less with a potential EPC partner?	F: 1, 3, 4 M: 2, 3, 5	Visits to/time with opp. sex stimulus birds

EPC opportunity (i.e., the number of stimulus females) had an effect on the proportion of the moves that male followed within 5 s (Fig. 2b; $F_{2, 28}$=3.56, P=0.042). Males followed a smaller percentage of his partner's moves as the number of stimulus females increased from zero to three (a priori contrasts $F_{1, 14}$=6.21, P=0.026, partial η^2 (effect size)=0.307). This pattern was not visible in the longer following latency ($F_{2, 24}$=1.91, P=0.167). Female EPC opportunity (i.e., the number of stimulus males) did not appear to influence the proportion of moves that the female followed within the short latency (Fig. 2c; $F_{2, 22}$=0.44, P=0.648) or long latency ($F_{2, 22}$=0.165, P=0.849).

Leaving behavior

Relative EPC opportunity did not greatly influence male or female participation in mate guarding behavior. As their number of potential EPC partners increased, there was little change in male leaving behavior (Fig. 3b; $F_{2, 28}$=0.98, P=0.386) or female leaving behavior (Fig. 3c; $F_{2, 28}$=2.42, P=0.107) (Table 2, question 2c), although there was a nonsignificant tendency for females to leave their partner's eyesight less as the number of stimulus males increased from zero to three (a priori contrasts $F_{1, 14}$=3.42, P=0.086, partial η^2=0.202). This last pattern could indicate that females tend to stay close to their partner male when there are more extra-pair males in the vicinity.
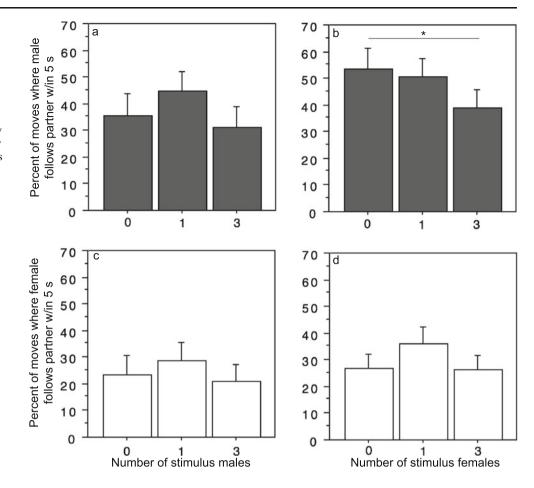
Increasing EPC opportunity for the mate

Leaving and following

Relative cuckoldry risk influenced aspects of male, but not female, mate guarding behavior. From the female

perspective, mate EPC opportunity (i.e., the number of stimulus females) had no noticeable effect on female leaving behavior (Fig. 3d; $F_{2, 28}$=0.15, P=0.857) or female following behavior within the short latency (Fig. 2d; $F_{2, 28}$=1.46, P=0.249) or long latency ($F_{2, 28}$=1.14, P=0.332) (Table 2, question 3a). From the male perspective, cuckoldry risk (i.e., the number of stimulus males) influenced male leaving behavior (Fig 3a; $F_{2, 28}$=3.99, P=0.030). Males left their mate's eyesight fewer times as the number of stimulus males increased from zero to three (a priori contrasts: $F_{1, 14}$=7.45, P=0.016, partial η^2=0.347). However, the number of stimulus males did not appear to influence the proportion of moves that the male followed within short latency (Fig. 2a; $F_{2, 24}$=1.8, P=0.183) or long latency ($F_{2, 24}$=2.11, P=0.143). The absence of a pattern in male following behavior may in part reflect the fact that females had a nonsignificant tendency to leave their males less as the number of stimulus males increased.

Interactions with extra-pair birds

Relative risk of being cuckolded influenced male, but not female, interactions with potential EPC partners (Table 2, question 3b). From the female perspective, male EPC opportunity (i.e., the number of stimulus females) did not have a detectable effect on the number of times the female visited a stimulus male (Fig. 4b; $F_{2, 28}$=0.20, P=0.822) or the total amount of time the female spent alone with a stimulus male ($F_{2, 28}$=0.68, P=0.513). From the male perspective, female EPC opportunity (i.e., the number of stimulus males) had an effect on the focal male's tendency to visit a stimulus female (Fig. 4a; $F_{1.35, 19.0}$=5.03, P=0.028). These data violated the assumption of sphericity (Mauchley's test; W=0.524, P=0.015), so degrees of freedom were corrected using Greenhouse–Geisser estimates of sphericity (ε=0.678). When the

Fig. 2 Following behavior (mean ± SE). Male following behavior (*gray bar*) was influenced by increasing EPC opportunity (**b**) but not by the number of stimulus males available to the female (**a**). Female following behavior (*white bar*) was not affected by an increase in EPC opportunity (**c**) or by the number of stimulus females available to the male (**d**) (*$p<0.05$)

number of stimulus males increased from zero to three, males visited stimulus females fewer times (a priori contrasts: $F_{1, 14}=7.47$, $P=0.016$, partial $\eta^2=0.347$). The number of stimulus males did not greatly influence the male's total time alone with the stimulus female ($F_{2, 28}=0.39$, $P=0.679$).
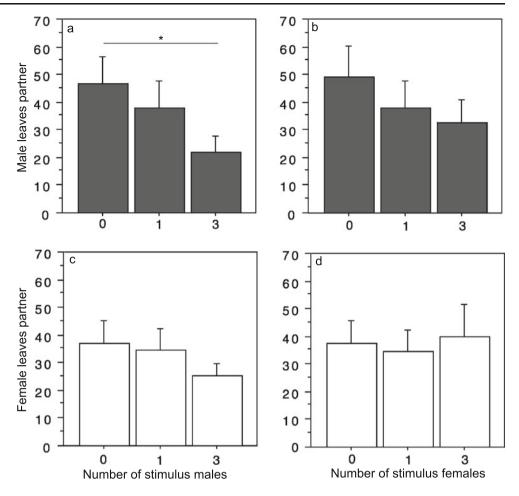
Discussion

Our results indicate that male zebra finches guard females flexibly according to variation in the costs and benefits of engaging in the behavior. Male mate guarding behavior was affected both by the relative threat to paternity and by the relative opportunity to achieve an EPC. While there was not conclusive evidence of flexible female mate guarding or female participation in being guarded, there was a nonsignificant tendency for females to leave their partner male less as the density of extra-pair males increased ($P=0.086$). While the data do not support a claim of flexible female participation, they do indicate that females do not elude their mates *more* as the relative opportunity to achieve an EPC increases. Hence, while males actively alter their partner association behaviors according to the costs and benefits of EPCs, we suggest that females play a stable role in maintaining partner associations regardless of the number of extra-pair males increases in the local area.

Pair behavior

When exposed to stimulus birds in an even sex ratio, pairs in the experimental chamber behaved similarly to pairs breeding in an open aviary (Birkhead et al. 1989). In both situations, males followed a higher proportion of their mate's moves than females, although the trend was not significant in the chamber. In open aviaries, pair copulations are frequently unsuccessful due to interruptions by extra-pair birds (Birkhead et al. 1988; Burley et al. 1994); in this study, the observed location of pair copulations away from stimulus birds suggest that pairs perceived stimulus birds as potentially disruptive. These trends in following and copulation behavior as well as the frequency of pair bonding activities such as allopreening and nest building indicate that pairs were behaving in the experimental chamber in ways that are consistent with pairing in cages and aviaries. As pair behaviors are similar for pairs caged alone, pairs breeding in an open aviary, and pairs in the wild (Birkhead et al. 1988, 1989), we have a reason to believe that patterns in mate guarding within the experimental chamber should reflect patterns in mate guarding in a more natural setting.
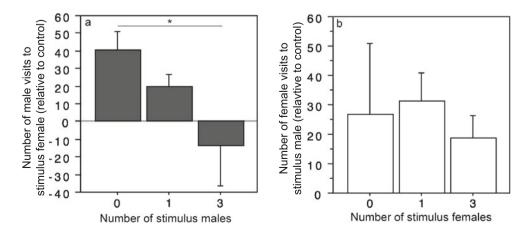
Fig. 3 Leaving behavior (mean ± SE). Male leaving behavior (gray bar) was influenced by the number of stimulus males (**a**), but not by an increasing EPC opportunity (**b**). Female leaving behavior (white bar) was not affected by EPC opportunity (**c**) or number of stimulus females (**d**) (*p<0.05)

Flexible male behavior

As the perceived threat to within-pair paternity increased, males left their mates less and visited the extra-pair stimulus female fewer times. As their own opportunity for an EPC increased, males followed a smaller proportion of their mate's moves within 5 s. Although the change in male behavior did not affect the total amount of time that the pair spent in close proximity or within eyesight of each other, these results suggest that male zebra finches are less inclined to pursue EPCs as the risk of being cuckolded increases and less inclined to mate guard as their opportunity to pursue an EPC increases. These experimental results directly support the idea of a trade-off between mate guarding and pursuing EPFs (e.g., Alatalo et al. 1987) and are consistent with findings from other species that the intensity of male mate guarding fluctuates according to the paternity threat (e.g., Komdeur 2001) and the opportunity to pursue EPCs (e.g., Chuang-Dobbs et al. 2001).

Fig. 4 Visits to a potential EPC partner (mean ± SE relative to control). One opposite-sex stimulus bird presented in all trials. **a** As the number of stimulus males available to the female increased, males (gray bar) visited the stimulus female fewer times. **b** The number of stimulus females had no affect on the number of times the female (white bar) visited the stimulus male (*p<0.05)

There is a great deal of variation in rates of EPF between both species and populations. Ecological explanations for this variation have tended to focus on breeding synchrony (Stutchbury and Morton 1995) and breeding density (Westneat and Sherman 1997; Richardson and Burke 2001; Stewart et al. 2010), but there is conflicting evidence as to the strength and consistency of their impact on observed rates of promiscuity (Bennett and Owens 2002). Flexible mate guarding may account for the discrepancy between the ecological variables that could theoretically increase EPC opportunity and the observed paternity patterns (Petrie and Kempenaers 1998; van Dongen 2008). We have shown that variation in the number and sex ratio of extra-pair birds affects male mate guarding intensity. To the degree that more intense mate guarding limits EPFs, flexible mate guarding may confound simple relationships between the social and ecological environment and patterns in EPF.

Within the experimental arena, male mate guarding behavior seemed to be relatively more responsive to variation in the paternity threat than to variation in male EPC opportunity. Although males followed proportionally fewer moves within 5 s as their EPC opportunity increased, this pattern was not particularly strong when following latency was extended to 27 s; while males were slower to follow mates as their perceived EPC opportunity increased, overall following behavior was unaffected.

In the zebra finch, male mate guarding seems to be structured by the benefits of mate guarding more than by the costs; in other words, for male zebra finches, the fitness costs of losing paternity may be relatively more important than the fitness benefits of pursuing EPFs. These results could imply that for minimally promiscuous species, paternity assurance is more important than pursuing EPCs when the female is fertile. In this context, EPFs may be the result of EPCs occurring when the extra-pair male's mate is not fertile.

Flexible female behavior

There was limited evidence of flexible female participation in being guarded and no discernible evidence that females flexibly guard males. As the perceived opportunity for the female to engage in EPCs increased (i.e., when there were more stimulus males), there was a nonsignificant tendency for females to leave their mates less. While we cannot conclude that females flexibly participate in being guarded, we feel that female participation in male mate guarding warrants further examination. Our experimental design necessitated preventing physical interaction between pair and stimulus birds; this physical separation may have decreased the effect of extra-pair male harassment on female behavior. Females may have participated minimally in being guarded simply because the wire barrier prevented physical harassment by the extra-pair males. Given the limitations of this experimental design, the modest sample size, and the nonsignificant tendency for females to leave their partner's eyesight less as the number of stimulus males increased, female participation in being guarded remains a possibility; we hypothesize that female zebra finches actively stay closer to their partner males as the risks of harassment from other males increases. It is rare for researchers to experimentally explore the active role of the female in mate guarding behaviors, yet we would expect females to experience costs and benefits of participating in such partner association behaviors.

We are confident in concluding that females did not elude their mates *more frequently* as their opportunity to engage in EPC behavior increased. In the zebra finch, a greater opportunity to achieve EPFs does not seem to influence female inclination to engage in EPC behavior. We manipulated female EPC opportunity by varying the number of potential EPC partners, but female zebra finches may perceive EPC opportunity as a function of extra-pair male quality (Houtman 1992); in other words, female behavior in the chamber may have been influenced more by the quality of the stimulus males that were present and less by the overall number of stimulus males. It is possible that a manipulation of the attractiveness of extra-pair males could elicit different responses from focal females than those we report here. Female zebra finches participate in being guarded more when paired to males that have been experimentally manipulated to appear more attractive (Burley et al. 1994). It is possible that females might also alter their participation in being guarded as the attractiveness of extra-pair males varies. Future research could address the relative importance of pair and extra-pair male quality through a manipulation of both pair and stimulus males.

Conclusions

If zebra finch behavior in the chamber is similar to behavior in the wild, it is possible that low levels of EPF are maintained through high levels of male mate guarding and low levels of female interest in pursuing EPFs. If male pursuit of EPFs is highest where there is a low risk of cuckoldry, the paternity threat that an individual male faces may be greatest under conditions of lower breeding synchrony (i.e., when extra-pair males are not guarding their own mates). Female EPC opportunity will not always predict EPF patterns if there are flexible paternity assurance strategies. Further, patterns in mate guarding intensity will not always predict patterns in EPF if a limited measure of mate guarding is employed. Although general measures of pair proximity did not vary in this study, changes in the perceived opportunity for EPCs did alter partner leaving and following behavior.

The theoretical framework underlying the evolution of mate guarding behavior should be expanded to include explicit reference to the active role of the female—here, we showed that females may participate in being guarded and that mate guarding is not solely determined by male behavior. Just as the adaptive function of EPF is explored from the male and female perspective, the adaptive function of mate guarding behavior should also be explored from both perspectives. A broader theoretical framework would allow for the generation of testable predictions about mate guarding intensity as a function of the costs of having an increasingly unfaithful mate and the benefits of pursuing EPFs. When there are reasons to avoid interactions with extra-pair individuals, active participation in being guarded should not be discounted. Only after individually evaluating male and female pursuit and prevention of EPCs can there be clear predictions about the relationships between ecological variables, mate guarding, and patterns in EPF.

Ethical standards   The experiments reported herein comply with the current laws of the USA.

Conflict of interest   The authors declare that they have no conflict of interest.

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