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Fertilized female quail avoid conspecific males: female tactics when potential benefits of new sexual encounters are reduced

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In a pair of experiments, we determined whether recently fertilized Japanese quail, *Coturnix japonica*, with less to gain from new sexual encounters than females without sperm in their sperm-storage tubules, would reduce their probability of sexual harassment by avoiding conspecific males (experiment 1) and affiliating with conspecific females (experiment 2). We found that after copulating with a male, female quail that were laying fertilized eggs spent less time near males and more time near females than female quail laying unfertilized eggs. Furthermore, females that were denied access to males for 14 days (i.e. those with empty sperm-storage tubules) and females that failed to lay fertile eggs after copulation behaved similarly towards conspecific males and females. We interpret these results as indicating that the cost to female quail of interacting with males of their species has led to the evolution of female tactics to avoid male harassment when females' eggs are successfully fertilized and potential reproductive benefits of additional sexual encounters are reduced.

Although mating provides obvious reproductive benefits, it can also be costly, increasing risk of predation (Mougeot & Bretagnolle 2000), decreasing time spent foraging (Stone 1995), and leading to acquisition of sexually transmitted diseases (Sheldon 1993). Most relevant to the experiments reported here, females of some species (e.g. elephant seals, *Mirounga angustirostris*: Le Boeuf & Mesnick 1991; Ross's geese, *Chen rossi*: Dunn et al. 1999; fallow deer, *Dama dama*: Clutton-Brock & McComb 1993) also risk injury during sexual encounters with conspecific males.

In Japanese quail, *Coturnix japonica*, the subject species in the present experiments, courting males chase and peck at females, seize feathers at the back of a female's head and often drag the female around by her head feathers before jumping onto her back to achieve cloacal contact (Mills et al. 1997; Persaud & Galef 2003). Such behaviours, which are costly to females and induce them to mate immediately, have been termed 'sexual harassment', and are considered a form of male sexual coercion (Clutton-Brock & Parker 1995).

Sexual harassment of females by males has led to the evolution of female tactics to mitigate potential damage inflicted by coercive males (reviewed in Smuts & Smuts 1993). Here, we are concerned with two such female tactics: (1) avoidance of males and (2) aggregation with other females.

To avoid male attention, females of some species simply keep males at a distance. For example, egg-laying female dragonflies, *Libellula pulchella*, make rapid 'escape flights' away from an approaching male (McMillan 2000). Females of other species, Japanese quail among them (Persaud & Galef 2003), form groups, apparently as a selfish herd defence against harassing males (McComb & Clutton-Brock 1994). In the laboratory, female Japanese quail generally do not affiliate with other females, but will aggregate in the presence of a male, presumably to reduce their probabilities of being the target of a male's advances (Persaud & Galef 2003).

For a female in need of gametes to fertilize her eggs, the benefits of mating are considerable. However, once a female has secured gametes from one male, the benefits to fertilized females of additional matings are reduced. Although females with sperm in their sperm-storage tubules might be able to increase their fitness by obtaining copulations from additional males (e.g. Foerster et al. 2003), the benefits of such matings will surely be less than the benefits to unfertilized females of obtaining sperm. Consequently, the cost-to-benefit ratios of encounters with males should be greater in fertilized than in unfertilized females, and fertilized females should be more likely to avoid contact with males than unfertilized

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females. If male avoidance and female aggregation are, in fact, tactics used by female quail to avoid male harassment, and if females are sensitive to the relative costs and benefits of mating, females might be expected to avoid males and affiliate with other females more frequently when fertilized than when unfertilized.

Japanese quail are particularly suitable subjects for studies of effects of fertilization on females' responses to male advances because female quail can store and utilize sperm for as long as 12 days after insemination (Birkhead & Møller 1992). Consequently, for several days after being successfully inseminated, the costs to a female of mating with other males remain high, while the reproductive benefits of such matings are substantially reduced.

In the two experiments described below, we examined the tendencies of females that had or had not been successfully fertilized to both avoid conspecific males (experiment 1) and aggregate with conspecific females (experiment 2). Evidence that fertilized females are more likely to avoid males and to aggregate with females would be consistent with interpretations of these behaviours as female tactics to reduce costs associated with mating.

EXPERIMENT 1: MALE AVOIDANCE

Experiment 1 was divided in two parts. In part 1, we tested females' responses to males after females were paired with a male and had laid either fertilized or unfertilized eggs. Obviously, females that laid fertilized eggs had been successfully fertilized and those that laid unfertilized eggs had not. Because this procedure resulted in females assigning themselves to 'fertilized' and 'unfertilized' conditions, the results of part 1 were essentially correlational. Therefore, in the second part of experiment 1, we tested the responses of 'unmated' females that had been denied contact with males for at least 14 days before testing. This 2-week period of isolation ensured that females had no utilizable sperm left in their sperm-storage tubules (Birkhead & Møller 1992). Although it would have been desirable to have an additional group of females that we had forced to become fertilized before we tested their responses to males, we could find no way to force females to accept male sperm without introducing serious confounds.

If female Japanese quail are less willing to incur costs of mating when fertilized than when in need of male gametes, mated females whose eggs have been fertilized should spend less time affiliating with males than mated females whose eggs have not been fertilized.

Methods

Part 1: mated females

Subjects. Twenty-eight female and 28 male Japanese quail, obtained from Speck's Poultry Farm (Vineland, Ontario, Canada) when 49 days of age, served as subjects in the first part of the experiment. Throughout the experiment, each bird resided in an individual, commercial, quail-breeding cage (Berry Hill Ltd, St Thomas, Ontario), measuring 51 × 25.5 × 22.5 cm, that was housed in a single temperature- and humidity-controlled colony room illuminated on a 16:8 h light:dark cycle. All subjects had ad libitum access to both water and food (Mazuri Pheasant Starter, PMI Feeds, St Louis, Missouri, U.S.A.).

Once females started to lay one egg per day (when approximately 70 days of age) we considered them sexually mature and ready to use to test males for sexual maturity. To conduct such a test, we placed each male with a different female in a randomly selected end compartment of the experimental apparatus (Fig. 1) each day for 5 min/day until he copulated on two consecutive days. We then left all females undisturbed in their home cages for 14 days to ensure that no sperm remained in females' sperm-storage vesicles.

Apparatus. The experiment took place in an enclosure constructed of transparent Plexiglas, painted plywood and half-inch (1.27-cm) hardware cloth that rested on an aluminium tray lined with absorbent paper pads (Tray liners, Lilo Products, Hamilton, Ontario). The enclosure, described in greater detail in White & Galef (2000), consisted of a central compartment, measuring 58 × 65 × 40 cm, separated by hardware-cloth partitions from two end chambers, each measuring 46 × 65 × 40 cm. A line drawn vertically at the midpoint of the Plexiglas front wall of the central compartment facilitated locating a subject within it.

We centred a plywood ancillary cage, measuring 43 × 43 × 40 cm, behind the central compartment and cut a window (34 × 24 cm) between the ancillary cage and central compartment. This window could be fitted with both transparent and opaque Plexiglas barriers. Removal of the opaque barrier permitted occupants of the central compartment and ancillary cage to see one another.

A holding cage constructed of transparent Plexiglas, measuring 25 × 25 × 50 cm, placed at the midpoint of

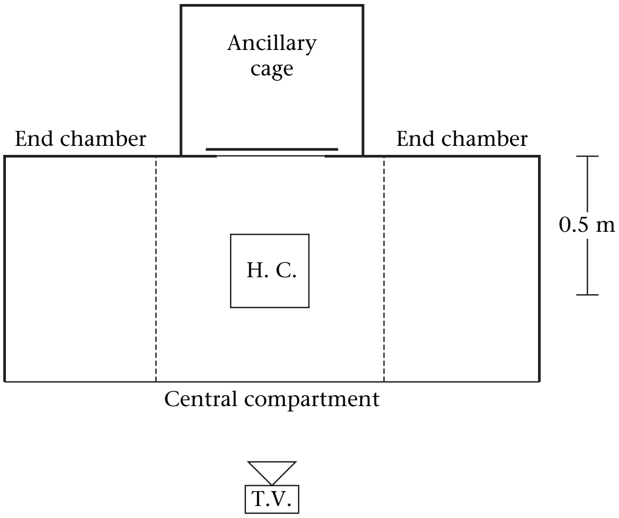

Figure 1. Experimental apparatus used in experiments 1 and 2. Dashed lines: hardware-cloth partitions; heavy lines: opaque walls; light lines: transparent Plexiglas; T.V.: closed-circuit television; H. C.: holding cage. The opaque barrier separating the ancillary cage from the central compartment was in place except during the choice phase of experiment 2.

the central compartment, could be raised by a rope and pulley system to release a restrained subject to move freely about the central compartment.

Procedure. Each trial consisted of a 10-min 'copulation' phase and a 10-min 'choice' phase. During both phases, we left the opaque Plexiglas barrier in place between the ancillary cage and central compartment.

To begin the copulation phase, we placed a female and a male in the central compartment of the apparatus and allowed the pair to court and mate for 10 min. We monitored the subjects on closed-circuit television to ensure that the male copulated and we recorded the interactions of subjects for later analysis. We then returned both subjects to their respective home cages and left them undisturbed for 3 days before carrying out the choice phase.

To begin the choice phase, we placed a female in the holding cage and a male that was unfamiliar to that female in a randomly selected end chamber of the apparatus. We then raised the holding cage to free the female, and for 10 min after a female had taken her first step following her release from the holding cage, we monitored her movements about the central compartment. We determined the amount of time the female spent closer to each end chamber, one containing a male, and the other empty.

For 12 days following a female's participation in the copulation phase, we collected her eggs each day. We labelled the eggs using a felt-tipped pen and then placed them in an incubator that rotated eggs once every 2 h and maintained them at 37.5 °C (Hova-Bator Incubator, 2362N, G.Q.F. Mfg; Savannah, Georgia, U.S.A.). After 5 days of incubation, an experimenter opened each egg to see whether it contained a blood spot indicating fertilization (Adkins-Regan 1995).

Part 2: unmated females

Subjects. Fourteen female and 14 male Japanese quail obtained, housed and tested for sexual maturity as described for mated females in part 1, served as subjects.

Apparatus. The apparatus was the same as that used in part 1.

Procedure. All females remained undisturbed in their individual home cages for 14 days before testing. We then gave them a 10-min choice test identical to that given to females in part 1.

Results and Discussion

During the copulation phase in part 1, all males mounted and achieved one or more cloacal contacts with their female partners, indicating ejaculation (Mills et al. 1997). Of the 28 mated females, 11 laid fertilized eggs on at least the day before, the day of and the day after the choice test ('fertilized' females) and 17 ('unfertilized' females) laid only unfertilized eggs.

We found a significant effect of condition on the time that females spent closer to the end chamber containing the male during the choice phase (one-way ANOVA: $F_{2,39} = 3.32$, $P = 0.04$; Fig. 2). Fertilized females spent significantly less of the choice phase closer to the end chamber containing the male than did either unfertilized (Fisher's PLSD test: $P = 0.02$) or unmated females ($P = 0.04$). Unfertilized and unmated females did not differ from one another. Furthermore, fertilized females spent significantly less than half the choice phase closer to the male (one-sample t test: $t_{10} = -2.49$, $P = 0.03$), whereas unfertilized and unmated females showed no tendency to avoid the male (one-sample t tests: $t_{16} = 0.69$, $P = 0.05$; $t_{13} = 0.38$, $P = 0.78$, respectively).

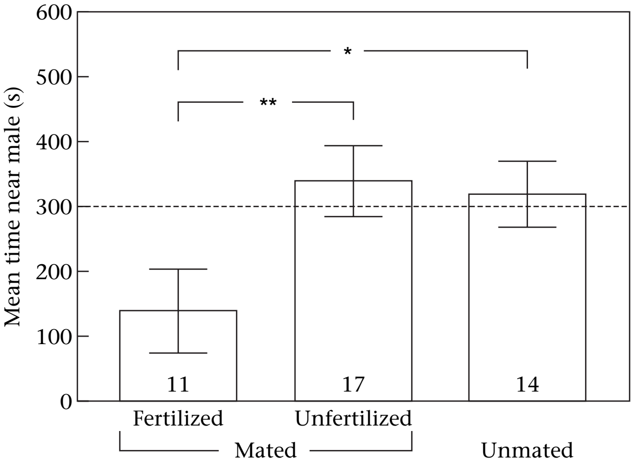

Figure 2. Mean ± SE time (s) that fertilized and unfertilized focal females (part 1) and unmated females (part 2) spent on the side of the central compartment closer to males during the choice phase of experiment 1. The dashed line indicates behaviour expected based on the hypothesis that females are indifferent to the presence of a male. Numbers within histograms = N/condition. *$P < 0.05$; **$P < 0.02$.

The data are consistent with the hypothesis that female quail are more likely to avoid males when they have sperm in their storage vesicles and the potential benefits of mating are consequently relatively low.

EXPERIMENT 2: FEMALE AGGREGATION

Experiment 2, like experiment 1, consisted of two parts. In part 1, we tested females' responses to males after females were paired with a male and had laid either fertilized or unfertilized eggs. In part 2, we examined the behaviour of unmated females.

We have found previously that female quail tend to affiliate when in the presence of a male (Persaud & Galef 2003), suggesting that such affiliation is a tactic females use to reduce the probability of male sexual harassment. If females congregate to reduce sexual harassment by males and they are sensitive to the relative costs and benefits of contact with males, then females with utilizable sperm in their sperm-storage vesicles should be more likely than unfertilized females to aggregate in the presence of a male.

Methods

Part 1: mated females

Subjects. Twenty-four male and 24 female subjects, obtained, housed and tested for sexual maturity as described in experiment 1, served as subjects in this experiment. During the first (‘copulation’) phase of the experiment, each female was paired with a randomly selected male. During the second (‘choice’) phase, male and female subjects were randomly assigned to trios each consisting of a ‘focal’ female, a ‘target’ female and a ‘stimulus’ male.

Each female served twice in the second phase of the experiment, once as a focal female and once as a target female, and each served as a focal female before serving as a target female. No pair of females served together in more than one trio.

All males served as stimulus males only after participating in the copulation phase of the experiment and no male interacted in both the copulation and choice phases with the same focal female.

Apparatus. The apparatus was that used in experiment 1 except that, during the choice phase of the experiment, the opaque partition separating the ancillary cage from the central compartment was removed, allowing the focal female in the central compartment and the stimulus male in the ancillary cage to see one another.

Procedure. The procedure was identical to that of experiment 1 except for the subjects present in the apparatus during the choice phase. In the present experiment, we placed a stimulus male in the ancillary cage and a target female (rather than a male) in a randomly selected end chamber of the apparatus. Thus, during the choice phase, focal female subjects chose between an empty end compartment and one containing a target female while a male was in view.

Collection and incubation of eggs. Collection and incubation of eggs were carried out as in experiment 1.

Part 2: unmated females

Subjects. Eleven female and 11 male Japanese quail obtained, housed and tested for sexual maturity as described for experiment 1, served as subjects.

Apparatus. The apparatus was that used in part 1.

Procedure. We treated unmated female subjects in part 2 exactly as we treated the mated females in part 1 except that unmated females did not participate in a copulation phase and spent 14 days in their home cages without contact with males before testing in the choice phase. Unmated females then participated in a choice phase identical to that experienced by females in part 1.

Results and Discussion

During the copulation phase of part 1, all males were observed to mount and achieve cloacal contacts with their female partners, indicating ejaculation. Nine of the 24 mated females laid fertilized eggs on at least the day before, the day of and the day after the choice test (‘fertilized’ females) and 15 females laid only unfertilized eggs (‘unfertilized’ females).

We found a significant effect of condition on the time that focal female subjects spent closer to the end chamber containing the target female during the choice phase (one-way ANOVA: $F_{2,32} = 4.76$, $P = 0.02$; Fig. 3). Fertilized mated focal females spent significantly more time closer to the end chamber containing the target female than did members of either of the other two groups (Fisher’s PLSD tests: $P = 0.04$ and $P = 0.005$, respectively). Unfertilized and unmated females did not differ from one another ($P = 0.25$). Furthermore, fertilized females spent significantly more than half of the choice phase closer to the end chamber containing the target female (one-sample t test: $t_8 = 3.40$, $P = 0.009$), whereas unfertilized ($t_{14} = -0.08$, $P = 0.94$) and unmated ($t_{11} = -0.95$, $P = 0.36$) females showed no tendency to affiliate with another female during the choice test.

The finding that fertilized females in the presence of a male were more likely to aggregate with others of their sex than were unfertilized females is consistent with the hypothesis that female Japanese quail use affiliation with other females as a tactic to avoid sexual harassment by males. When females were not fertilized, and the benefits of mating were relatively high, females were indifferent to the presence of other females. When females had been fertilized and the benefits of mating were relatively low, they congregated with other females, reducing the probability that a male would interact with them.

It might be argued that the differences in behaviour shown by fertilized and unfertilized females in both experiments may have reflected differences in their experiences with males during the copulation phases of each experiment. To determine whether there were systematic differences in the copulatory behaviour of males paired with females subsequently laying fertilized and unfertilized eggs, an experimenter unaware of the fertilization state of females scored videotapes of each of 33 randomly selected 10-min copulation phases for ‘attempted’ and

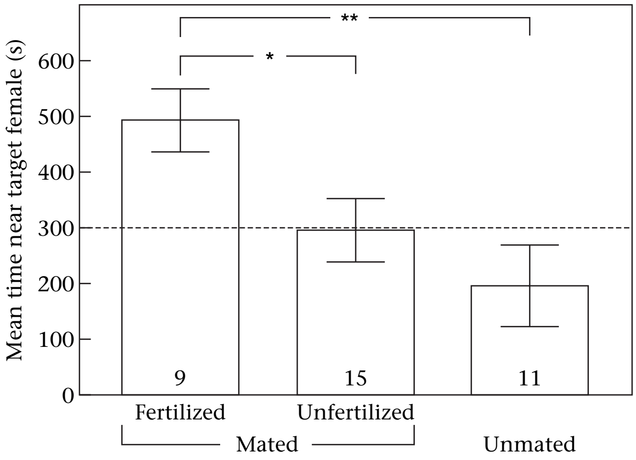

Figure 3. Mean ± SE time (s) that fertilized and unfertilized females (part 1) and unmated females (part 2) spent on the side of the central compartment closer to the end chamber containing a female during the choice phase of experiment 2. The dashed line indicates behaviour expected based on the hypothesis that females are indifferent to the presence of a target female. Numbers within histograms = N/condition. *$P < 0.04$; **$P < 0.01$.

'successful' copulations by males. Successful copulations are identified by a distinct pause in behaviour following mounting of a female by a male during which sperm transfer occurs (Mills et al. 1997). Those mountings after which no distinct pause could be discerned were classified as attempted copulations since it was uncertain whether sperm transfer had occurred.

We found no difference in the number of successful (Student's *t* test: $t_{31} = 0.42$, $P = 0.68$; Fig. 4) or attempted ($t_{31} = 0.62$, $P = 0.54$; Fig. 4) copulations and no difference in the number of both types of copulation considered together ($t_{31} = 0.72$, $P = 0.48$; Fig. 4) experienced by the 10 fertilized and 23 unfertilized females whose copulation phases we examined. We are currently analysing the behaviours of females to determine whether there are any that predict the success of males in fertilizing their eggs.

GENERAL DISCUSSION

Taken together, the results of the present experiments indicate that female Japanese quail use tactics that reduce their probability of interaction with males when the potential benefits of such interaction are relatively low. Although the results of part 1 of both experiments are open to the interpretation that some female quail (i.e. 'unfertilized' females) both avoid males and resist fertilization, the results of part 2 of both experiments suggest that, to the contrary, successful fertilization of a female quail alters her subsequent behaviour towards males. Although subjects in part 1 of both experiments assigned themselves to 'fertilized' and 'unfertilized' conditions, when we assigned females to the 'unmated' condition in part 2 of both experiments, these 'unmated' females behaved as did the 'unfertilized' females in part 1. Consequently, differences in the behaviour of fertilized and unfertilized females towards males in part 1 of both experiments cannot be attributed simply to differences in their tendencies to retain or not retain sperm.

Japanese quail are not unique in showing changes in responses to males after mating. For example, contrary to the behaviours shown by Japanese quail in the present studies after copulating, female orang-utans, *Pongo pygmaeus abelii*, affiliate with male 'friends' who protect them from sexual harassment and feticide (Fox 2002). Female prairie voles, *Microtus ochrogaster*, affiliate less with other females after copulation and during pregnancy, and this decrease in affiliative behaviour is even more common when males are present (Bowler et al. 2002). Such differences across species in responses to mating and conception probably reflect differences in the relative costs and benefits of interaction with conspecifics of both sexes.

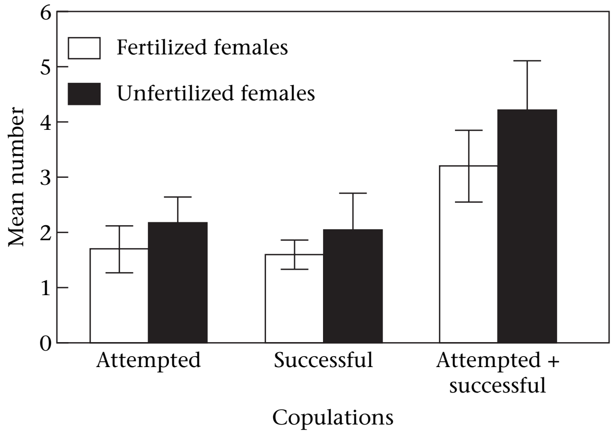

Figure 4. Mean ± SE number of attempted copulations and successful copulations (separately and combined) that fertilized and unfertilized females experienced during the copulation phase of experiments 1 and 2.

The present experiments do not address the question of how female Japanese quail come to behave differently when laying fertilized and unfertilized eggs. Because mating in Japanese quail frequently fails to result in fertilization of females (Adkins-Regan 1995), female quail cannot use the physical act of copulation to determine that their eggs have been fertilized. Whether the changes in the behaviour of fertilized females towards males described here were a direct response to the presence of utilizable sperm in the female's reproductive tract, a consequence of having copulated and not ejected sperm from the cloaca, or the result of some other cause remains to be determined. Birkhead & Møller (1992) have provided data suggesting that female quail may be able to control release of sperm from storage tubules, implying that females are sensitive to the presence of sperm in the reproductive tract.

Although, in the present experiments, we have focused on potential benefits of avoiding males and affiliating with females when potential gains from additional sexual encounters are reduced, there are also potential costs to such behaviours. For example, by avoiding males, females lose any protection that males may offer from harassment by other males (Linklater et al. 1999; Fox 2002; Foerster et al. 2003). Affiliation with female conspecifics may increase competition for resources (Cassini 2000) and risk of disease (Campagna et al. 1992). However, results of the present experiments suggest that, despite such potential costs, female Japanese quail, which are open to physical harassment by males, find it advantageous to avoid males and to aggregate with conspecific females after fertilization has reduced the potential benefits of additional sexual encounters.

Acknowledgments

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