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Affiliative preferences are stable and predict mate choices in both sexes of Japanese quail, *Coturnix japonica*

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Most laboratory studies of mate choice use affiliation as a surrogate measure for actual choice of a partner for copulation. In a series of recent studies using affiliation as a dependent variable, we showed that the tendency of both male and female Japanese quail to affiliate with members of the opposite sex is profoundly affected by seeing them mate with others: female quails show an increased tendency to affiliate with males that they have seen mating; and male quails show a decreased tendency to affiliate with females that they have seen mating. In the present experiments, we examined: (1) the consistency of affiliative preferences of male and female quail for individuals of both the same and opposite sex; and (2) the validity of measures of affiliation as predictors of choice of an individual with whom to mate. We found that: (1) both male and female quail display consistency from trial to trial in their affiliative preferences for members of the opposite but not the same sex, and (2) affiliative preference is a powerful predictor of choice of a partner for copulation in both male and female quail. In Japanese quail, affiliative behaviour is consistent over time and predicts mate choice.

The study of mate choice has been largely motivated by recognition of the potential impact of heritable female preferences on the evolution of male characteristics (Andersson 1994). In the laboratory, the most commonly used method to measure mate choice, what we will refer to here as the 'standard method', involves presenting a 'focal' female with two or more 'target' males confined some distance apart, and comparing the time that the focal female spends near each target male during a single test. This method has been used with fish (e.g. Noonan 1983; Basolo 1990; Schlupp et al. 1994; Dugatkin 1996b; Houde 1997; Howard et al. 1998; Kraak & Bakker 1998), mammals (e.g. Gubernick & Addington 1994) and birds (e.g. Bateson 1982; Swaddle & Cutchill 1994; Fiske & Admunsen 1997; Tobias & Hill 1998; Hill et al. 1999).

Given the theoretical reasons for interest in mate choice, there are problems with using a single test of affiliative preference as a dependent measure in studies of mate choice. First, as Boake (1989) indicated, consistency in female preference sets an upper bound on the heritability of female preference for males (Falconer 1981). Consistent choice can increase the rate of selection for a trait or provide stabilizing selection. On the other hand, if mate choice varies from one occasion to the next, it will have relatively little effect on evolution of traits (Janetos 1980; Wiegmann et al. 1996). The single test of preference used in the standard method does not permit determination of consistency of mate choice (Boake 1989), and the few investigators that have measured consistency of choice (e.g. Poramarcom & Boake 1991; Møller 1994; Godin & Dugatkin 1995; Jennisons et al. 1995) have reported mixed results. Second, and more obviously, the standard method uses affiliative tendency as a dependent variable, whereas affiliative preference assumes biological importance only if it is positively correlated with choice of an individual with whom to mate (Wagner 1998). Reproductive advantage is conferred on those males that reliably get chosen by females as sex partners, not on those males that females prefer to stand near. Unfortunately, the relationship between affiliative preference and mate choice has rarely been measured adequately.

Recently, there has been interest in the possibility that individual experience, particularly individual social experience, can modify heritable female mate preferences. Dugatkin (1996a, b), for example, showed that female Trinidadian guppies, *Poecilia reticulata*, that had observed normally nonpreferred males mate, increased the time they spent affiliating with those males. We provided similar evidence of social influences on the affiliative preferences of both male and female Japanese quail; focal female quail are more likely to affiliate with a previously nonpreferred male after seeing him mate with another female (Galef & White 1998; White & Galef

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1999a), whereas focal male quail are less likely to affiliate with a preferred female after seeing her mate with another male (White & Galef 1999b).

In the present series of experiments, we determined for both male and female quail: (1) whether affiliative preferences are reliable across repeated tests (experiment 1); and (2) whether tests of affiliative preference predict subsequent choice of a partner for copulation in female (experiments 2) and male (experiment 3) quail.

EXPERIMENT 1: RELIABILITY OF AFFILIATIVE PREFERENCE

We determined whether affiliative preferences of individual quail of both sexes are consistent across trials. Because we were interested not only in the reliability of affiliative preferences, but also in the relationship between affiliation and sexual preference, we gave focal subjects the opportunity to affiliate with target individuals of both the same and opposite sex. Differences in the reliability of focal subjects' affiliative responses, as a function of the sex of target subjects, would be consistent with the view that affiliative preferences expressed in the standard method are directly related to sexual behaviour.

Methods

Subjects

We used 21 male and 24 female Japanese quail acquired from a local commercial breeder (Speck's Poultry Farm, Vineland, Ontario) at 48 days of age. After we transported subjects to our laboratory (Hamilton, Ontario), we placed birds in individual, stainless-steel cages, measuring 45.7 × 61.0 cm and 40.6 cm high, housed in a colony room that was illuminated for 16 h/day, with lights on at 0600 hours.

We maintained all birds on ad libitum Mazuri Pheasant Starter 5637 (PMI Feeds, St Louis, Missouri) and water, and for environmental enrichment, twice each week, we gave each bird access to a handful of autoclaved hay.

To allow birds to come into breeding condition, we waited for 30 days before we started experimental work. We considered a female to be ready to breed when she started to lay eggs once every 2 days. To determine whether males would mate when given access to females, we placed each male with a succession of females in breeding condition in one of the end chambers of the apparatus (Fig. 1) that we subsequently used for experiments. We gave each male access to a randomly selected female each day until he either mounted females on 2 successive days or 2 weeks passed without his mounting a female.

After a male had mated with two females in succession, we left him in isolation for 7–10 days, then started the experiment. We first assigned males and females to 32 trios, each consisting of either a single focal female and two target males (eight trios), a single focal male and two target females (eight trios), a focal male and two target males (eight trios), or a focal female and two target females (eight trios). No bird served in both a trio containing a focal male and a trio containing a focal female.

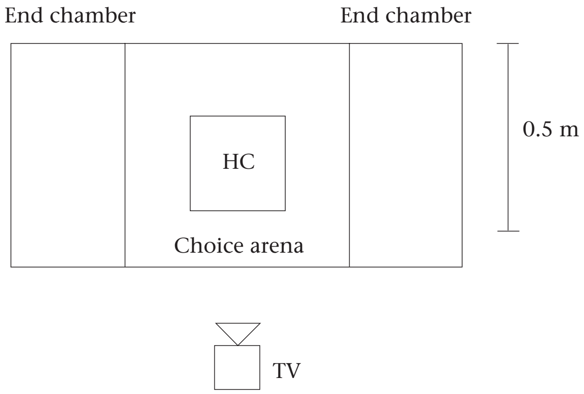

Figure 1. Overhead schematic of the enclosure used in experiments 1, 2 and 3. HC: Holding cage; TV: television camera.

Although it would have been preferable if each animal participated only once in the experiment, practical constraints made it impossible for us to maintain the number of quail needed to achieve that goal. We did, however, ensure that no two focal subjects ever chose between the same pair of target subjects, and no target animals served together as members of more than one trio.

Each focal female and each focal male served as a member of two trios, one containing target subjects of the same sex and one containing target subjects of the opposite sex. We conterbalanced across focal subjects the order in which they served in same-sex and opposite-sex trios.

Apparatus

We conducted all experiments in an enclosure, measuring 121.9 × 61.0 cm and 30.5 cm high, constructed predominantly of painted plywood (Fig. 1). The enclosure had a transparent Plexiglas roof and front wall, and rested on an aluminium tray covered with disposable, absorbant paper pads (Tray Liners, Lilo Products, Hamilton, Ontario). Hardware cloth partitions, placed 30.5 cm from opposite ends of the enclosure, divided it into three compartments.

We also drew a line from top to bottom on the front wall of the enclosure in the middle of the choice arena to enable us to determine, at any time, the end of the enclosure to which a focal subject was closer.

We cut an opening (25.4 × 25.4 cm) in the centre of the roof of the choice arena so that we could raise or lower a transparent Plexiglas holding cage, measuring 25.2 × 25.2 cm and 40.6 cm high (Fig. 1), using a pulley system that we operated from a room adjacent to that housing the enclosure.

A colour CCTV video camera (Panasonic WV-CP412) attached to a videotape recorder (Panasonic AG-1240) and colour video monitor (CT 1331y) faced the transparent Plexiglas front wall of the enclosure and permitted us to observe and record on videotape all behaviour occurring within the enclosure.

Procedure

Each test consisted of three 10-min phases: a pretest, a delay and a post-test.

Pretest. To begin the experiment, we placed a focal subject in the holding cage in the centre of the choice arena and a target subject in each end compartment of the apparatus, and then waited for 1 min to let all subjects settle down. We then started the videotape recorder and raised the holding cage to release the focal subject. In the 10 min after the focal subject took its first step, we recorded the time he or she spent on each side of the choice arena. We refer below to the target subject held on the side of the enclosure where a focal subject spent more than half the 10-min pretest as that focal subject's 'preferred' target subject and the other target subject as that focal subject's 'nonpreferred' target subject.

Delay. At the end of the 10-min pretest, we returned the focal subject to the holding cage and left it there undisturbed for 10 min.

Post-test. We conducted the post-test exactly as we had the pretest, first raising the holding cage, then waiting for the focal subject to take its first step, and afterwards recording, for 10 min, the time that the focal subject spent in each half of the choice arena.

Results and Discussion

Pretest

During the pretest, focal subjects assigned to the four kinds of trios spent an average ± SE of from 6.47 ± 0.30 to 9.03 ± 0.57 min nearer their respective preferred target subjects. There was a significant effect of trio assignment on the time that focal subjects in trios of the four types spent near their respective preferred target subjects (repeated measures ANOVA: $F_{1,13}=21.18$, $P<0.001$). This difference was due to differences between male and female focal subjects in the time that they spent near preferred target subjects. There was no within-subject effect of trio assignment ($F_{1,13}=0.26$, NS) on the time that focal subjects spent near their preferred male and female target subjects.

Post-test

Both male and female focal subjects showed no change between pre- and post-test in time spent with preferred subjects of the opposite sex (matched *t* tests, both t_7s<1.03, NS; Fig. 2), whereas both male and female focal subjects showed a significant change between pre- and post-test in the time that they spent with preferred subjects of the same sex (both matched t_7s>3.13, both Ps<0.05; Fig. 2). Both male and female focal subjects showed a significantly greater change from pre- to post-test in the time they spent with their preferred same-sex target subjects than in the time they spent with their preferred opposite-sex target subjects (both t_7s>2.43, both Ps<0.05). Thus, there was consistency in affiliative preference for same-sex target subjects but not for opposite-sex target subjects, the latter showing what might be considered a regression towards the mean between pre- and post-tests. This difference in consistency of response to same- and opposite-sex target subjects suggests that, in quail, affiliative preference may be related to sexual behaviour.

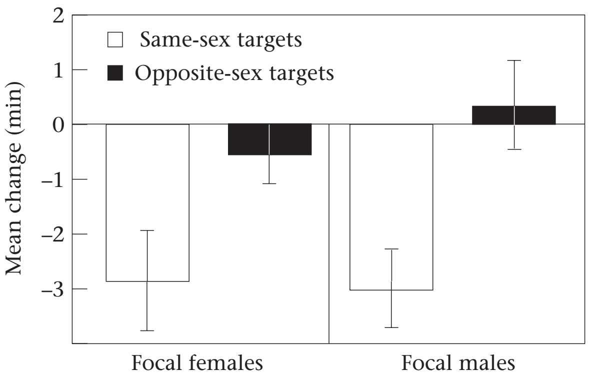

Figure 2. Mean±SE change in time (min) between pre- and post-test that focal male and female subjects spent nearer the male and female target subjects that they had preferred during the pretest.

EXPERIMENT 2: CORRELATIONS BETWEEN AFFILIATIVE PREFERENCE AND MATE CHOICE (FEMALES)

We observed focal females on two occasions while they chose between pairs of males. In the first of these preference tests, we used the same apparatus and procedures that we had used in the pretest of experiment 1 to measure focal females' affiliative preferences between pairs of target males. In the second preference test, we tethered the males a focal female had chosen between in the first preference test at opposite ends of an enclosure, then allowed the focal female to move about the entire enclosure and mate as she would with each tethered male.

We had to tether males because males not only fought when free to do so, but also actively pursued females throughout the enclosure, making it impossible to determine whether females were choosing a male or were being cornered by him.

We hoped to determine whether the measure of affiliative preference that we had used both in experiment 1, and in our previous studies of 'mate choice' in female Japanese quail (Galef & White 1998; White & Galef 1999a), reliably predicted which male a female would actually choose as a mate.

Methods

Subjects

Fifteen female and 10 male experimentally naïve Japanese quail served as subjects. All females were laying eggs and all males had mated with two females in succession in the apparatus (see Methods of experiment 1). We prepared a list of all 45 possible combinations of pairs of the 10 males, then randomly selected 15 of those pairs and assigned one focal female to each.

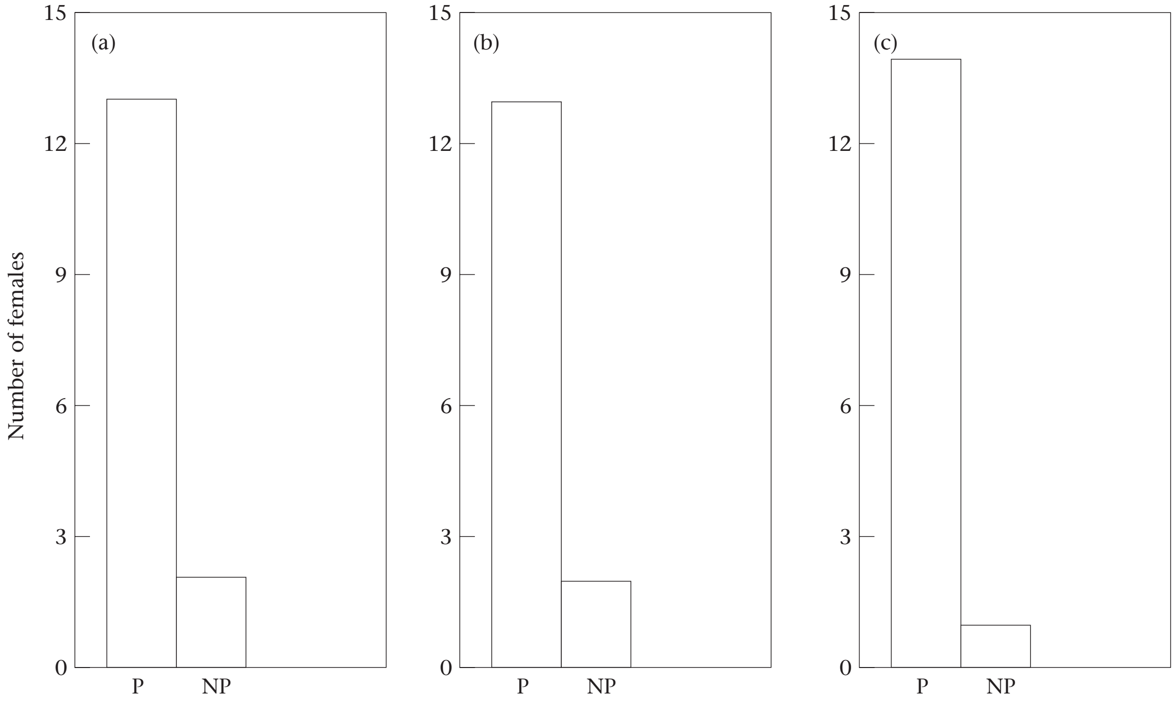

Figure 3. Number of focal females that, during preference test 2, (a) copulated first, (b) copulated last, and (c) copulated most frequently with the target male that they preferred and did not prefer during preference test 1. P: Preferred; NP: not preferred

Apparatus

We used the same apparatus that we had used in experiment 1 during the pretest except that, during both preference tests in the present experiment, target males wore cloth harnesses and were tethered to opposite ends of the enclosure. Each harness, made of cotton cloth weighing 2.5 g, fit over a male's head and around his wings, and could be closed across his chest with a Velcro fastener.

To prevent target males from fighting with one another and from pursuing focal females about the enclosure during the second preference test, we attached a 30-cm piece of twine to the back of each harness and tethered the two males to opposite end walls of the enclosure. Males wore their harnesses and were tethered during both preference tests to minimize disturbance of subjects during the experiment.

Procedure

Each day for 10 min/day, for 14 days before the start of testing, we placed each male in a harness and allowed him to move about the enclosure. At the end of this 2-week period of habituation, we determined whether each male would mate while wearing a harness. For 10 min/day for 5 consecutive days, we placed a different, randomly selected female in one end chamber of the apparatus together with a male in harness. We removed from the experiment any males that did not copulate with females by the fifth day of testing.

Preference test 1 (affiliation). The procedure for the first preference test was the same as that of tests 1 and 2 in experiment 1 except that the two males were wearing their harnesses and were tethered.

Delay. At the end of the first 10-min preference test, we returned the focal female to the Plexiglas holding cage for 5 min.

Preference test 2 (mating). At the end of the 5-min delay, we first removed the partitions separating the end compartments from the choice arena, then released the focal female from the holding cage. During the following 10 min, we recorded all cloacal contacts between each male and the focal female.

Results and Discussion

Preference test 1 (affiliation)

During the first preference test, when focal females could affiliate but not mate with males, focal females spent a mean ± SE of 8.06 ± 0.43 min closer to their respective preferred males.

Preference test 2 (mating)

During the second preference test, each focal female mated one or more times with a male ($\bar{X} \pm \mathrm{SE}=3.4 \pm 0.51$ mounts/female during the 10-min test) and 84.4% of copulations were with males preferred during preference test 1. Both the first and the last copulation of each focal female were significantly more likely to involve the male that female had preferred during preference test 1 (binomial tests: first copulation: $x_{15}=2$, $P<0.01$, Fig. 3a; last copulation: $x_{15}=2$, $P<0.01$, Fig. 3b), and during preference test 2, females copulated significantly more frequently with the males they had preferred than with the males they had not preferred during preference test 1 ($x_{15}=1$, $P=0.005$; Fig. 3c). First mating (Sullivan 1994; Blackwell & Passmore 1996), last mating (Birkhead & Møller 1992), and number of matings (Birkhead & Møller 1992) may all be important determinants of fertilization in birds.

Correlation between preference tests 1 and 2

The correlation between the time focal females spent near their preferred males during preference test 1 and the proportion of copulations that the preferred male obtained during preference test 2 was positive, but not significant (Spearman's rank-order correlation, $r_S=0.34$, $N=15$, $P<0.22$). The nonsignificant result appeared to reflect a severe restriction of range (10 of the 15 focal females copulated exclusively with their preferred males).

The results of experiment 2 suggest that, whatever the parameters of copulation determining reproductive success in Japanese quail, a male with whom a female tends to affiliate is also a male by whom she is likely to be fertilized.

EXPERIMENT 3: CORRELATIONS BETWEEN AFFILIATIVE PREFERENCE AND MATE CHOICE (MALES)

In experiment 1, we found that male Japanese quail, like females of their species, show consistent affiliative preferences when choosing between members of the opposite sex. In experiment 3, we determined whether in male quail, as in females, the affiliative preference of a male predicts his choice of a female with whom to mate.

We did not harness females during the test of male mate preference because female quail, unlike males, do not interact aggressively during brief periods of contact, and males are far more active in their pursuit of females than are females in their pursuit of males.

Methods

Subjects

Fifteen female and 17 male experimentally naïve Japanese quail served as subjects. All had mated repeatedly when placed in the apparatus (see Methods of experiment 1). We prepared a list of all 105 possible combinations of pairs of females, then randomly selected 17 of those pairs and assigned one focal male to each.

Procedure

Preference test 1 (affiliation). The procedure was identical to that of experiment 2 except that a focal male chose between two females in the end enclosures during the 10-min preference test and target females were not wearing harnesses, so there was no need to habituate females to restraint.

Delay. As in experiment 2, at the end of preference test 1, for 5 min, we restrained the focal male in the holding cage in the centre of the choice arena.

Preference test 2 (mating). At the end of the 5-min period of restraint, we removed the partitions separating the choice arena and end compartments, released the focal male from the holding cage, and, for 10 min, recorded all cloacal contacts between the focal male and the two target females.

Results and Discussion

Preference test 1 (affiliation)

Focal males spent an average ± SE of 7.78 ± 0.37 min nearer to their respective preferred females.

Preference test 2 (mating)

On average, each male mated 3.76 ± 0.47 times during the 10-min preference test. Eighty per cent of copulations were with females preferred during preference test 1, significantly more males directed both their first and last matings towards their preferred female than towards their nonpreferred female in preference test 1 (both $x_{17}=3$, $P<0.02$; Fig. 4a,b), and significantly more focal males mated more frequently with their preferred than with their nonpreferred female ($x_{14}=0$, $P<0.001$; Fig. 4c).

Correlation between preference tests 1 and 2

The correlation between the time focal males spent near their preferred females during preference test 1 and the proportion of copulations that the preferred female obtained during preference test 2 was significant (Spearman's rank-order correlation: $r_S=0.49$, $N=17$, $P<0.05$).

For male Japanese quail, as for females of their species, measures of affiliation predict choice of a mating partner with great success.

GENERAL DISCUSSION

The results of experiment 1 indicate that both male and female Japanese quail show consistent tendencies to affiliate with particular members of the opposite, but not of the same, sex. Experiments 2 and 3 show that, for both male and female Japanese quail, affiliative preferences measured using the standard procedure are consistent with the preferences expressed in a situation where mating takes place. Taken together, the results of all three experiments indicate that the measure of affiliative preference used in previous studies of 'mate choice' in quail (Galef & White 1998; White & Galef, 1999a, b) are not only reliable, but also predictive of actual mating behaviour. It would be useful to know whether affiliative choice is consistent and whether affiliation predicts choice of a partner for copulation in other species in

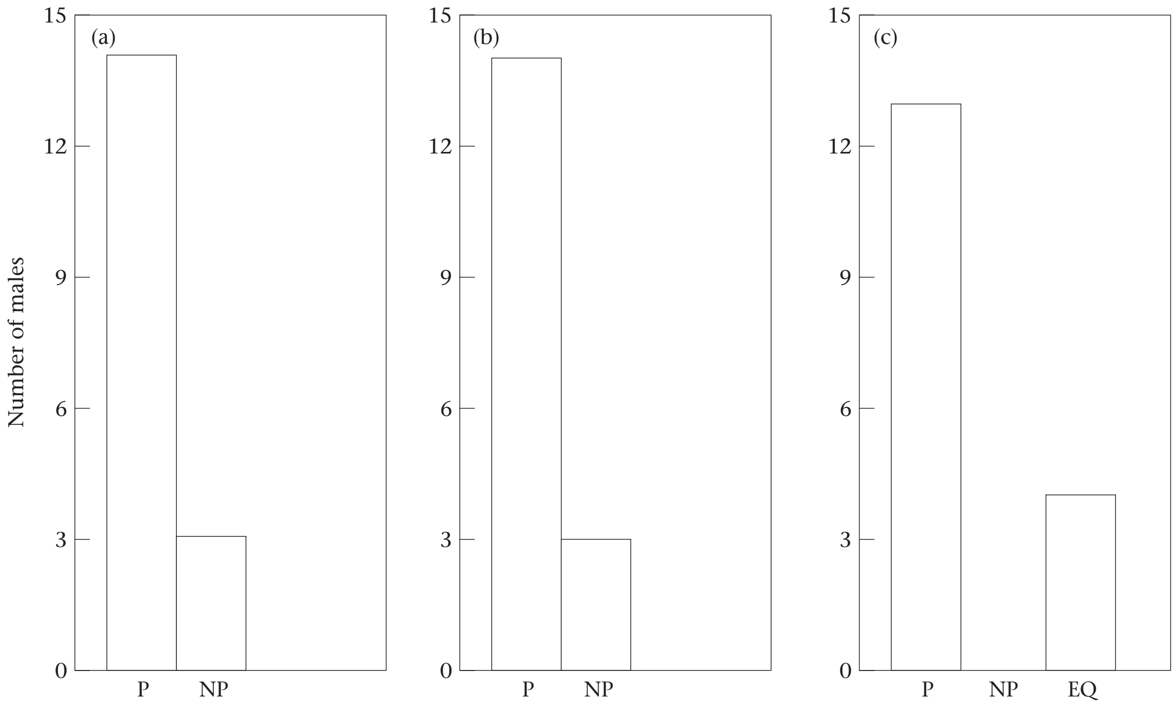

Figure 4. Number of focal males that, during preference test 2, (a) copulated first, (b) copulated last, and (c) copulated more frequently with the target female that they preferred and did not prefer during preference test 1. P: Preferred; NP: not preferred; EQ: equal number of copulations with preferred and nonpreferred female.

which affiliative behaviour has been used as an index of mate preference.

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