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Absence of female conspecifics induces homosexual behaviour in male guppies

KRISTIN L. FIELD* & THOMAS A. WAITE*†
*Department of Evolution, Ecology, and Organismal Biology
†Department of Anthropology, The Ohio State University

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Social environment can have dramatic effects on the expression of species-typical sexual behaviour. Using guppies, *Poecilia reticulata*, we asked how an all-male social environment affects male sexual behaviour. Males were assigned to either single-sex or mixed-sex groups. After 15 weeks, their sexual behaviour was assayed in the presence of three novel individuals of each sex. Males from single-sex groups performed higher rates of sexual displays and sneak copulation attempts towards stimulus males than did males from mixed-sex groups. Males from mixed-sex groups directed the majority of their sexual behaviour towards females using typical heterosexual behaviour. The social environment for each subject was then reversed for 2 weeks, after which subjects in both treatments showed a stronger tendency to display or sneak towards males. These findings reveal that homosexual behaviour is not readily extinguished when females become accessible and that it can be induced even after the ontogeny of heterosexual behaviour. While guppies may rarely occur in all-male groups in nature, these findings highlight the capacity for social environment to shape mating behaviour, which is particularly relevant for captive populations used in behavioural studies or conservation breeding programmes.

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The identification and selection of a potential mate can be critically influenced by social environment. On a short timescale, individuals' responsiveness to social influence can lead to nonindependent mate choice (Dugatkin 1996; Freeberg 2000; Galef & White 2000). On a longer timescale, social environment and 'learned sexuality' can have dramatic effects on the expression of species-typical sexual behaviour (Woodson 2002). Early social environment may also shape sexual behaviour in subtler ways. For example, the range of male phenotypes to which female guppies, *Poecilia reticulata*, are exposed when immature may affect choosiness and preference for extravagant male traits later in life (Rosenqvist & Houde 1997).

Much of the classical work on effects of early social experience on mate choice has focused on sexual imprinting in avian systems, in which young birds learn characteristics of their parents' phenotype and later use this information to select mates (Hess 1973; Immelmann 1975; Bateson 1978; Clayton 1994). Social interactions prior to breeding as well as interactions with mates can modify the effect of early social experience (e.g. Kruijt & Meeuwissen 1993; Nagle & Kreutzer 1997), consistent with the model that sexual imprinting consists of an early acquisition phase and a later consolidation phase (Bischof 1994). There has been considerable attention given to the proximate mechanisms of imprinting (e.g. Bolhuis 1991), as well as the conditions and traits that promote imprinting (e.g. Witte & Sawka 2003). Based on their extensive review, ten Cate & Vos (1999) argue that sexual imprinting in birds has the potential to affect evolutionary processes such as speciation, hybridization, sexual selection and coevolution between brood parasites and hosts. Thus, imprinting has provided a rich basis for inquiry, at least in birds. In many mammals and a limited number of fish, imprinting-like processes have been identified, although these systems tend to be less understood (reviewed in Colgan 1983).

Our study investigated the effects of early social environment, characterized by a lack of females, on later sexual behaviour in male guppies, *Poecilia reticulata*. This work was prompted by our earlier observation of

Correspondence: K. L. Field, Department of Evolution, Ecology, and Organismal Biology, The Ohio State University, 318 W. 12th Avenue, Columbus, OH 43210, U.S.A. (email: field.23@osu.edu).

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homosexual behaviour by males in all-male social conditions in our laboratory, an observation also noted by Liley (1966) and Evans & Magurran (1999). Although this earlier work clearly documents the occurrence of male–male sexual behaviour in all-male groups, it does not specifically test the hypothesis that the absence of conspecific females causes homosexual behaviour. Our experiment manipulated sex ratio in small groups of guppies to determine whether presence or absence of females promotes homosexual behaviour in males. Because the Trinidadian guppy is an important model system for studies of female choice and evolutionary trade-offs resulting from competing pressures of natural and sexual selection (reviewed by Houde 1997), it should be particularly important to understand the conditions affecting expression of sexual behaviour in this species.

Our experiment was designed to determine: (1) whether male guppies would direct sexual behaviour towards other males when housed in single-sex tanks and (2) whether males would adjust their sexual behaviour in response to a change in housing conditions (i.e. sex ratio). With regard to effects of changed housing conditions, we asked specifically: (1) whether males that had been housed in mixed-sex tanks would start courting males if females were removed and (2) whether males that had been housed in single-sex tanks would immediately direct sexual behaviour towards females when they were made available. Beyond extending our knowledge of how social environment facilitates the development of typical heterosexual behaviour, our results may have important implications for the use of captive populations for experiments or conservation programmes.

METHODS

Study Animals

Guppies are group-living with a promiscuous mating system, in which the polymorphic, colourful males contribute only sperm. Males spend much of their time engaged in sexual behaviour (Endler 1987). They use two alternative mating tactics, the sigmoid display and the sneak (referred to as ‘thrust’ in some sources) (Liley 1966). The display, in which a male curves his body in an S shape and quivers within a female’s visual field, elicits copulation from receptive females, which presumably select males based on their individual colour patterns and display behaviour (Houde 1997). The sneak occurs when a male swims alongside a female and attempts to thrust his gonopodium into her reproductive tract, which can result in fertilization without female cooperation. The identity of the female to which a male displays is usually apparent to a human observer due to close proximity of the male to the female and the orientation of the male relative to the female’s head. The female target of a sneak is virtually always unambiguous because the male must swim up beside a female and swing his gonopodium forward and upward to contact her urogenital pore. Even when a male is unsuccessful, his target is evident by proximity and his orientation. Copulation is rarely observed in laboratory tanks, although sigmoid displays and sneaks are extremely common and individual males switch between the two behaviours frequently. These behaviours are unique to sexual behaviour. In contrast, agonistic behaviour consists of chases, nips, and a parallel swimming display that is readily distinguished from the sexual display (Liley 1966; Magurran & Seghers 1991). We use the term ‘display’ to indicate the sexual sigmoid display.

Although some reports include ‘biting’ at the female’s urogenital pore as part of the male sexual repertoire (Baerends et al. 1955), others have failed to note this behaviour (Liley 1966) or observed it too rarely to include it in analysis (Rodd & Sokolowski 1995). We did not record ‘bites’ because they occur rarely in this population. Furthermore, they are difficult to distinguish from a sneak, since a male will often have his gonopodium positioned forward as he moves his snout towards a female’s vent to bite. Since a female often darts away when she detects a male approaching her vent, it is difficult to tell whether a male is attempting a sneak or a bite. Any bites that were performed with a flexed gonopodium would have been counted as sneaks in our study.

General Maintenance of Study Animals

We used guppies descended from individuals originally captured in 1997 from the Paria River, Trinidad and established in our laboratory in 1998. Adults were housed in breeding tanks. Offspring were removed from these tanks weekly and placed into juvenile tanks. At the onset of sexual maturation (Houde 1997; Rodd & Reznick 1997), individuals were removed from the juvenile tanks and placed into either an experimental tank or a breeding tank, depending on our needs.

Fish were maintained under a 12:12 h light:dark cycle, using full-spectrum bulbs (GE Sunshine, 15 W, General Electric, Cleveland, Ohio, U.S.A.) positioned over the tanks and standard fluorescent ceiling fixtures. Tanks contained gravel, an aerated sponge filter, a heater, and, in the breeding tanks, java moss, *Vesicularia dubyana*, and ranged in temperature from 23 to 26°C. Tanks were cleaned and approximately 20% of the water was replaced weekly. We fed the fish commercial flake food daily and supplemental live food (*Daphnia*, *Artemia*, or *Enchytraeus buchholzi*) occasionally. This maintenance regime and the following experimental protocol were approved by the Institutional Laboratory Animal Care and Use Committee (No. 98A0094).

Overview of Experimental Design

In this experiment, we established replicates of mixed-sex and single-sex groups for 104–106 days. We monitored male sexual behaviour in these initial housing groups with all-occurrence sampling over 10 weeks and with focal-male sampling during the final week of this phase. Subjects were then removed from their housing groups and tested for sexual response to unfamiliar individuals during a 10-min free-swim test. We then reversed the housing conditions, such that subjects with mixed-sex early experience were placed in single-sex groups and vice versa. During the 2-week reversed housing phase, we monitored sexual behaviour. In a second free-swim test, subjects were again tested with a new set of unfamiliar individuals.

Group establishment

We established groups of 10 individuals, either five males and five females or 10 males. Maturing males were randomly assigned to one of two treatment groups, mixed-sex or single-sex. Maturing females were selected to be at least as large as males ($\bar{X} \pm$ SE standard length: females: 16.3 ± 0.39 mm; males: 13.6 ± 0.15 mm). All individuals had been reared in juvenile tanks, had not reproduced, and had not observed adults engaging in sexual behaviour. Furthermore, we did not observe sexual behaviour being performed by the young males at the time they were placed into the experiment. We ran six replicate tanks (37.8 litres) for each treatment. The tanks were set up and maintained in the manner described above. Due to limited availability of simultaneously maturing fish, replicates were established over 4 weeks (group establishment stage). Six replicates of each treatment were thus run sequentially, but replicates were always started in pairs (one of each treatment). During the group establishment stage (28–31 days), individuals were added such that group size was equalized in the two treatments. The first replicates were begun on 19 July 1999 and the last ones were completed on 3 September 2000. Replicates took 152 to 154 days to complete, from the beginning of group establishment to the final test.

Phase 1: initial housing

Once groups had been established, they were left for 35–38 days during which the youngest fish in the group was able to mature fully. Over the next 10 weeks, we monitored sigmoid displays by conducting all-occurrence sampling of each replicate tank for 30 min twice a day on a weekly basis. Due to scheduling constraints, sampling times were not standardized, but conducted during the first or second part of the day. The first observations occurred between 1002 and 1342 hours and the second ones between 1535 and 2006 hours. The purpose of these observations was to determine whether males in single-sex and mixed-sex tanks were displaying at similar rates. To gain information about individual males, we also conducted focal-male sampling during the ninth week. Individual males, which could be identified by unique colour patterns, were observed for 10 min per day for 6 consecutive days.

Free-swim test 1

After group establishment and the initial housing phase, males were tested for effects of the social conditions in which they were housed. This test was designed so we could observe each subject's sexual behaviour under standardized conditions in which he could interact freely with unfamiliar individuals of both sexes. Each male was tested in a randomly selected order such that males from each treatment alternated in order. The novel testing tank (20.3 × 40 × 25.4 cm) was filled 21.6 cm high and equipped with an air stone and heater. We placed gravel on the bottom and rested a 15-W full-spectrum strip light above the tank. Our intention was to make the test tank conditions as similar as possible to the males' housing tank conditions. We observed guppies from behind a blind. Prior to introduction of the subject, three males and three females from our breeding tanks were placed into the test tank. Subject males had no experience with any of the individuals from the breeding tanks. The subject and stimulus individuals were allowed to acclimate for 15 min, or until at least four individuals were active.

Following the acclimation period, we observed the subject during a 10-min test period. When he displayed, the sex of the nearest individual was recorded. When he appeared to be equidistant between two individuals of different sexes, the display was categorized as 'unknown'. We defined a sneak copulation attempt as any occasion when the subject thrust his gonopodium towards another fish's anal region while the two fish were within one-half of a body length of each other. We recorded the sex of the target animal for each sneak attempt. Following the test, the subject was returned to his housing tank. The stimulus fish were exchanged for other stimulus individuals. Each subject was tested with a unique group of stimulus fish.

Phase 2: reversed housing

After all subjects had been tested in the free-swim test, we randomly chose half of the single-sex treatment males and placed them in mixed-sex tanks. The mixed-sex treatment males were moved into single-sex tanks. In other words, males that had experienced all-male conditions were placed in mixed-sex tanks and vice versa. The subjects spent 2 weeks in these 'reversed conditions'. Every male was observed in its housing tank for 10 min per day, using focal sampling, for the final 5 consecutive days of this phase.

Free-swim test 2

Following 2 weeks in the reversed conditions, we conducted a second free-swim test in the same manner as described above for free-swim test 1.

Data and Analysis

Statistical analyses were conducted using SPSS (2000, v. 10.0.7). We lost one replicate of the mixed-sex treatment to disease during week 7 of the all-occurrence sampling. Thus, we were able to analyse the results from five replicates for the mixed-sex treatment and six replicates for the single-sex treatment. As described below, most analyses used repeated measures multivariate analysis of variance (RM MANOVA).

Behaviour in housing tanks

We monitored male behaviour using all-occurrence sampling during the initial housing phase. Males could interact either with males and females or just males, depending on whether they were housed in a mixed-sex or single-sex tank, respectively. We compared the behaviour of males in their housing tanks using RM MANOVA. Our model specified treatment (mixed- or single-sex) as the between-subjects factor, and week (10 weeks) and time of day (early or late) as within-subjects factors. Owing to missing observations for 2 weeks for one replicate of the single-sex treatment, we were only able to use five replicates for each treatment.

The focal male observations made at the end of phase 1 and phase 2 were used to describe individual variation in display behaviour between the subjects in the housing tanks. Since we did not conduct all-occurrence sampling during phase 2, we also used focal-male sampling to estimate display rate during the reversal of social conditions. We used Mann–Whitney *U* tests to determine whether the average display rate observed during focal observations differed between mixed-sex and single-sex tanks.

Behaviour in free-swim tests

We compared results of the two free-swim tests to determine whether initial social conditions affected male sexual behaviour and whether reversal of conditions caused a change in behaviour. The tests allowed subjects to interact with unfamiliar individuals of both sexes in a novel environment. Again, we used RM MANOVA to compare the behaviour of males from mixed-sex ($N = 18$) and single-sex treatments ($N = 23$). This model allowed us to examine the effect of reversed conditions on male behaviour. Counts of displays and of sneaks directed towards males and towards females were transformed (square root). Displays that could not be categorized unambiguously as male- or female-directed were excluded (65 of 785 observed displays = 8%). The model specified treatment and replicate as between-subjects factors and free-swim test (1 and 2) as the within-subjects factor; all four behavioural measures (male-directed displays, female-directed displays, male-directed sneaks, female-directed sneaks) were analysed in this single model.

We also analysed the free-swim data to determine whether the proportion of male-directed sexual behaviour differed between mixed-sex and single-sex treatments and between free-swim tests 1 and 2. Analysis of the proportion of sexual behaviour directed towards males provided a simple evaluation of whether differences in absolute number of displays and sneaks used in the above analysis truly represented a stronger preference for males. We used only those males that performed at least one display and one sneak in both free-swim tests (10 males from the mixed-sex treatment and five males from the single-sex treatment). The proportions of displays and sneaks directed towards males were transformed (arcsine square root). We used the same RM MANOVA model described above for the count data, except that we had two measures (proportion of male-directed displays and proportion of male-directed sneaks) for this analysis.

RESULTS

Behaviour in Housing Tanks

Phase 1

The number of displays per 30-min, all-occurrence sampling period during the initial housing phase was converted to per capita displays per minute, such that we had an estimate of display rate for each replicate tank measured weekly, in the morning and afternoon, over 10 weeks. Subjects in the mixed-sex treatment performed a mean ± SE of 0.76 ± 0.13 displays per male per min, which was not significantly different from the display rate of males in the single-sex treatment (0.80 ± 0.13; RM MANOVA: $F_{1,8} = 0.053$, $P = 0.82$, power = 0.06). Display rate varied across weeks ($F_{7.35,58.8} = 2.99$, $P = 0.008$, Huynh–Feldt corrected with resulting noninteger degrees of freedom), but not with time of day ($F_{1,8} = 3.956$, $P = 0.082$). There was a two-way interaction between week and time of day ($F_{9,72} = 2.057$, $P = 0.045$, Huynh–Feldt corrected) and a three-way interaction between week, time of day and treatment ($F_{9,72} = 2.031$, $P = 0.048$, Huynh–Feldt corrected). Thus, our data suggest that males displayed at a slightly higher rate in the afternoon ($\overline{X}$ ± SE: 0.81 ± 0.09 displays per male per min) than in the morning (0.75 ± 0.10). Furthermore, display rates varied, but not in a generalizable pattern, from week to week (range of weekly values: 0.63–0.95 displays per min per male; combined means for treatments and times), which interacted with time of day and treatment.

We also estimated display rate by averaging each subject's rate over 6 days of focal male observations and then averaging the mean rate across subjects within a treatment. During the last week of the phase 1 initial housing, males in the mixed-sex treatment performed 1.13 ± 0.27 displays/min ($N = 18$ males) and males in the single-sex treatment performed 1.41 ± 0.19 displays/min ($N = 23$). There was wide variation in display rate between subject males (Fig. 1), and the difference between mixed-sex and same-sex treatments was not significant (Mann–Whitney *U* test: $U = 11$, $N_{\text{single}} = 6$ replicates, $N_{\text{mixed}} = 5$ replicates, $P = 0.54$).

Phase 2

During the final week of reversed housing, subjects in the mixed-sex treatment (now housed with males only) displayed 0.98 ± 0.24 times/min ($N = 18$ males). Single-sex treatment males, which had been housed with females for over 1 week, displayed 1.31 ± 0.21 times/min ($N = 23$ males). When examined at the level of the replicate, there was no statistical difference between display rates in mixed-sex versus single-sex replicates (Mann–Whitney *U* test: $U = 9$, $N_{\text{single}} = 6$ replicates, $N_{\text{mixed}} = 5$ replicates, $P = 0.33$). We recorded the sex of the target of displays for these single-sex treatment males, which were now housed with females, for five of the replicates. Although males in the single-sex treatment displayed frequently, only 3.7% of the observed displays were directed towards females (37 of 993 displays; excluding nine ambiguous cases; see above).

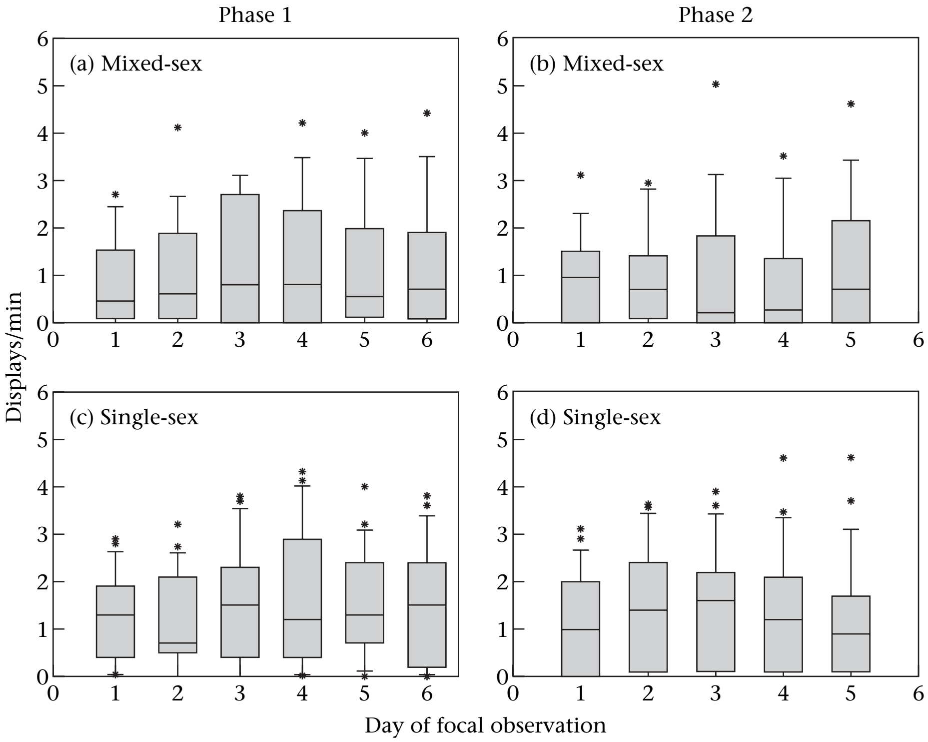

Figure 1. Box plots indicating variation in display rate for males in mixed- (a, b) and single-sex (c, d) treatments during 6 days of focal male observation during phase 1 (a, c) and 5 days of focal male observation during phase 2 (b, d). Boxes show 25th and 75th percentiles with the median indicated as a horizontal line within the box. Whiskers indicate 10th and 90th percentiles. Outliers are shown as solid dots.

Behaviour in Free-swim Tests

Subjects from the two treatments showed significant differences in their sexual interactions with the unfamiliar individuals in the free-swim test (overall treatment effect: $F_{4,27} = 24.314$, $P < 0.001$; for each measure: male-directed displays: $F_{1,30} = 10.973$, $P = 0.002$; female-directed displays: $F_{1,30} = 23.938$, $P < 0.001$; male-directed sneaks: $F_{1,30} = 11.864$, $P = 0.002$; female-directed sneaks: $F_{1,30} = 77.674$, $P < 0.001$). Subjects from the mixed-sex treatment directed displays and sneaks towards females more frequently than did subjects from the single-sex treatment. Conversely, subjects in the single-sex treatment directed sexual behaviour towards males more often than did subjects in the mixed-sex treatment (Fig. 2). For male-directed displays and female-directed sneaks a significant amount of variation could be attributed to the replicate to which the subject belonged (overall replicate effect, Pillai's trace: $F_{20,120} = 1.974$, $P = 0.013$; for each measure: male-directed displays: $F_{5,30} = 3.938$, $P = 0.007$; female-directed displays: $F_{5,30} = 1.173$, $P = 0.35$; male-directed sneaks: $F_{5,30} = 0.984$, $P = 0.44$; female-directed sneaks: $F_{5,30} = 6.029$, $P = 0.001$). A significant interaction between treatment and replicate emerged only for female-directed sneaks ($F_{4,30} = 7.133$, $P < 0.001$).

Overall, there was no detectable effect of the phase 2 reversed housing conditions ($F_{4,27} = 1.855$, $P = 0.15$). However, a closer examination of specific behaviours reveals that the rate of male-directed displays and sneaks increased after phase 2 (male-directed displays: $F_{1,30} = 6.453$, $P = 0.016$; male-directed sneaks: $F_{1,30} = 4.045$, $P = 0.053$), whereas the rate of female-directed displays and sneaks remained similar (female-directed displays: $F_{1,30} = 0.335$, $P = 0.57$; female-directed sneaks: $F_{1,30} = 0.576$, $P = 0.45$; Fig. 2). There were no interactions between the reversal and between-subjects factors.

When we examined the subset of males that used both the display and sneak tactics during free-swim tests, we found that males ($N = 10$) from the mixed-sex treatment directed a smaller proportion of their sexual behaviour towards males than did males ($N = 5$) from the single-sex treatment ($F_{2,7} = 61.967$, $P < 0.001$; for each measure: proportion of displays directed towards males: $F_{1,8} = 39.266$, $P < 0.001$; proportion of sneaks directed towards males: $F_{1,8} = 4.274$, $P < 0.001$; Fig. 3). Furthermore, there was a detectable effect of the reversed social conditions ($F_{1,8} = 4.758$, $P = 0.050$; for each measure: proportion of displays directed towards males: $F_{1,8} = 5.911$, $P = 0.041$; proportion of sneaks directed towards males: $F_{1,8} = 8.537$, $P = 0.019$). Males in both treatments tended to increase the proportion of male-directed sexual behaviour following the reversal of housing conditions. The increase in proportion of male-directed displays performed by mixed-sex treatment subjects during free-swim test 2 compared to free-swim test 1 approached significance when tested with a two-tailed Wilcoxon matched-pairs signed-ranks test ($T = 32$, $N = 8$, $P = 0.055$). The effect of the reversal on male-directed

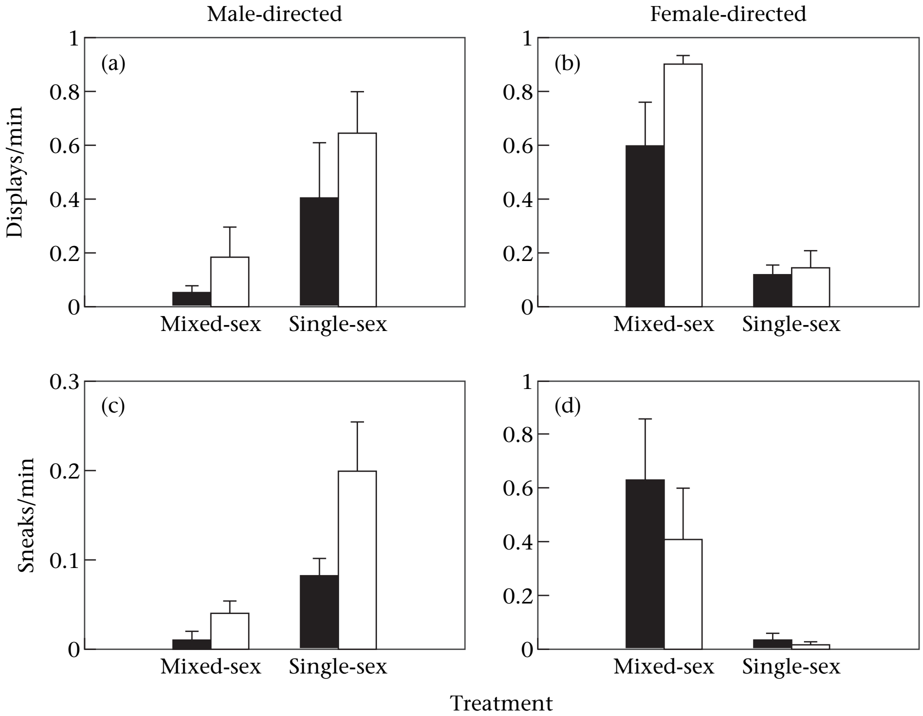

Figure 2. Mean + SE number of displays (a, b) and sneaks (c, d) directed towards males (a, c) and females (b, d) during free-swim tests 1 (■) and 2 (□). *N* = 18 males in five replicates for mixed-sex; *N* = 23 males in six replicates for single-sex. Note differences in scaling of *Y* axes.

sneaks for these subjects, as well as on both behaviours for single-sex treatment subjects, could not be compared statistically in this way because of initially small sample sizes used for this part of the analysis, compounded by a number of males whose behaviour did not change between tests. However, we describe the observed patterns. Four of 10 mixed-sex treatment males increased the proportion of sneaks directed towards males, while the remaining six subjects performed sneaks only towards females in both free-swim tests. Of the five single-sex treatment males, three subjects increased the proportion of male-directed displays, one subject continued to display exclusively to males, and one subject decreased his proportion of male-directed displays from 1.0 to 0.89. Three of these subjects attempted male-directed sneaks exclusively for both tests. One single-sex subject increased his proportion of male-directed sneaks from 0.4 to 1.0, while another one decreased his proportion from 1.0 to 0.5.

Thus, both analysis of the rate of engaging in sexual behaviour and analysis of the proportion of male-directed sexual behaviour revealed that single-sex treatment males engaged in homosexual behaviour more frequently than the mixed-sex treatment males. Results from the proportion-based analysis should be interpreted conservatively because of the small sample size and the problems of estimating proportions with a limited number of events. However, these results were concordant with the larger analysis based on absolute number of behavioural events and suggest differences between treatments not only in the number of displays or sneaks but also in the allocation of male- or female-directed sexual behaviour.

DISCUSSION

We found that naïve male guppies housed in single-sex tanks for over 15 weeks developed male-directed sexual behaviour, which persisted even when females became accessible. When female-experienced males were deprived of females for 2 weeks, they showed a tendency to direct sexual behaviour towards males when tested in a mixed-sex group. Early environment not only shaped mating behaviour, but did so in a way that depended on the type of environment experienced.

A possible explanation for the single-sex treatment subjects' persistence of male-directed behaviour is that they still had access to males during phase 2. Furthermore, the subjects were familiar with these males and had previous experience courting them and being courted by them. Thus, single-sex treatment males were given a choice of sexual targets during the phase 2 reversed conditions. It is possible that if these males had been placed individually in groups of females, they would have readily engaged in female-directed sexual behaviour.

In contrast, the mixed-sex treatment subjects were forced to stop engaging in sexual behaviour, which accounts for the majority of male activity, or to court males. The tendency to copy other individuals' behaviour, which has been shown in guppies in the contexts of mate choice (Dugatkin 1996) and foraging (Laland & Williams 1998), may have facilitated the acquisition of homosexual behaviour by the mixed-sex treatment subjects. These subjects were grouped with stimulus males that actively engaged in male-directed sexual behaviour. Presumably, had the mixed-sex treatment males been housed

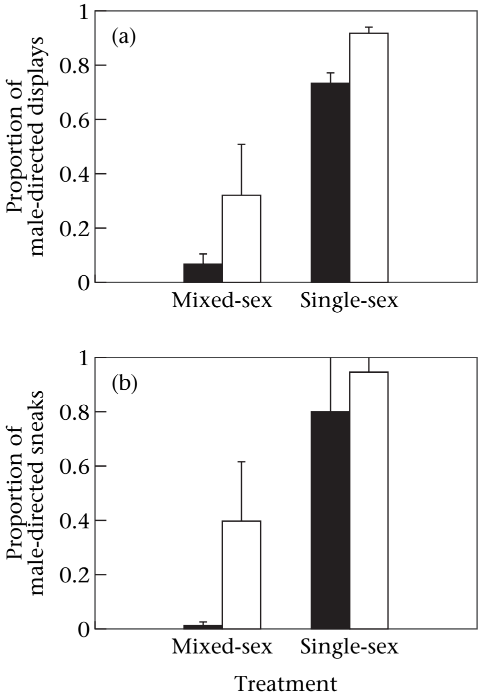

Figure 3. Mean + SE proportion of sexual behaviour directed towards males using subset of subjects that both displayed (a) and sneaked (b) in both free-swim tests (■: free-swim test 1; □: free-swim test 2; $\overline{X}$+SE; mixed-sex treatment: $N = 10$ males in four replicates; single-sex treatment: $N = 5$ males in three replicates).

continuously in mixed-sex tanks, they would have continued to direct their sexual behaviour towards females.

We cannot eliminate the possibility that the persistence of male-directed sexual behaviour in the single-sex treatment subjects was caused by fear of females, which have different coloration from and can be much larger than males. We did not measure length of the stimulus individuals in the free-swim tests. However, since we wanted to use sexually mature females (population range for minimum standard length of gravid, wild-caught females: 15.1–17.4 mm; Reznick 1996), we chose females that appeared to be at least as large as the largest subjects and which were typically clearly larger than all subjects. After the final free-swim test, subjects measured 16.4 ± 1.4 mm standard length, and the sizes of the subjects in mixed-sex and single-sex treatments were similar (t test: $t_{44} = -1.09$, $P = 0.281$). Although the relative difference between subject and stimulus female length was unknown, the size of the single-sex subjects did not predict the number of female-directed displays performed (linear regression: $F_{1,21} = 1.419$, $r^2 = 0.06$, $P = 0.247$), which would have been expected if smaller males were particularly fearful of larger females.

Furthermore, males did not appear to be afraid of females during the tests and when housed for 2 weeks with them in phase 2. The subjects did not show typical alarm response (Brown & Godin 1999) when initially introduced to females in the free-swim 1 test or when approached by them during phase 2 focal observations. It is possible that responses we did not measure, such as latency to approach or to court, may have reflected subtler effects of male fearfulness of females. Further experimentation would be necessary to distinguish between lack of motivation to court females versus wariness of them.

Alternatively, it is possible that the single-sex treatment subjects did not recognize the females as potential mates. Experience with females may be necessary for such recognition to develop. Successful experience with females is correlated with better discrimination of males with female-like plumage in pied flycatchers, *Ficedula hypoleuca* (Slagsvold & Saetre 1991). Experience with females, however, may not be enough. Male Japanese quail, *Coturnix japonica*, having previous copulatory experience with females do not respond differently to male and female stimuli until after they experience repeated visual exposure to other males (Domjan & Ravert 1991).

In a series of experiments on heterospecific discrimination and behavioural isolating mechanisms, Liley (1966) found that male guppies frequently resort to male–male courtship when housed with heterospecific females (*Poecilia picta*). When males are housed with both conspecific and heterospecific females (*P. parae*), it appears that positive feedback from female guppies, specifically receptive behaviour, facilitates male species discrimination and preference for conspecific females. From these experiments, Liley proposed that males learn, perhaps during a 'critical period', to direct courtship towards the class of individuals that provides the most 'positive reinforcement', which may be males in the absence of conspecific females.

Our findings that the mixed-sex treatment subjects did engage in some male–male courtship when housed without females, even after extensive experience with females, suggests that males do have flexibility to direct these signals to targets other than to those learned to be appropriate earlier in life. However, these female-experienced males may have been induced to court males through 'positive reinforcement' similar to that described by Liley (1966). Because males spend so much of their time performing sexual behaviour and consequently interacting with other individuals, the mixed-sex subjects may have been motivated by the stimulation from these interactions, even though they were with other males.

For the single-sex subjects, it is possible that the free-swim tests and the 2-week phase 2 period were not long enough for sufficient reinforcement from receptive females, which may have altered their preferences. For example, female canaries modify their preferences for song type after breeding with males having nonpreferred song types (Nagle & Kreutzer 1997), and male cowbirds that imprint on canaries later learn to direct song almost entirely to conspecific females after experience with them, particularly when grouped with older, experienced males (Freeberg et al. 1995). In our experiment, it is unlikely that the subjects would have had much or any exposure to receptive females. Females are receptive to male displays for 3–4 days following parturition, typically every 3–4 weeks (Liley 1966; Houde 1997). We did not control for female receptivity, as we used stimulus fish from our stock breeding tanks. We did not see females responding positively to male displays (gliding or copulating) during

Table 1. Sex ratios in three Trinidadian pool-riffle structured streams (sampled March–April 2000)

Stream	Quare II*	El Cedro*	Ramdeen
Mean pool sex ratio†	0.70	0.49	0.58
Number of pools sampled	61	25	17
Range of sex ratios	0–2.33	0–1.33	0–0.92
Number of male-biased pools	11	2	0
Number of all-male pools	1‡	1‡	0

Census includes mature males and females that were at least 14 mm standard length, SL, at Quare and Ramdeen (≥16 mm SL at El Cedro; size at maturity estimated by population-specific life history characteristics; D. Reznick, personal communication). Male-biased pools had fewer than 17 individuals, whereas female-biased pools contained 1–416 individuals.

*Data collected with D. Reznick & C. Ghalambor.

†Male:female.

‡Single male present.

the tests or focal observations, and it is likely that the females were not receptive, or at least not motivated to mate with these particular males. In mixed-sex stock tanks, lack of female receptivity, characterized by ignoring or evading males, does not prevent the males from displaying to or attempting sneak copulations with females (Liley 1966, personal observation). Since we did not perform formal observations of the stimulus fish, we cannot eliminate the possibility that particular stimulus individuals increased or decreased subjects' responsiveness. However, by using a novel combination of stimulus fish for each subject, we minimized the chance of a treatment bias. In terms of modifying preferences with breeding experience, the free-swim tests and phase 2 environments containing nonreceptive females and receptive males probably promoted the retention of the single-sex treatment subjects' homosexual behaviour.

These findings strongly suggest that a mixed-sex environment is necessary for the development of typical heterosexual behaviour. In Trinidad, the pool-riffle structured streams in which guppies live tend to have female-biased sex ratios (Haskins et al. 1961; Seghers 1973; Godin 1995; Rodd & Reznick 1997). Male-biased sex ratios do occur, but infrequently (Table 1). Sex ratios also fluctuate temporally. The Paria River, from which our laboratory population originated, showed a female-biased sex ratio of 0.66 (male:female) during one year, and a male-biased sex ratio (1.14) 2 years later (Seghers 1973). However, it appears that the social ecology, with respect to sex ratio, has been stable enough in the species' evolutionary history to provide reliable social cues necessary for the development of normal sexual behaviour in natural populations. Presumably, it is the low probability that males will spend any length of time in all male groups in the wild that allows for learned discrimination to work reliably and for homosexual behaviour to be easily induced in laboratory social environments.

There are few studies available to assess the generality of our results and many studies that suggest effects of early environment on later sexuality are often specific to species, sex and/or context. For example, male rats and, typically, male rams do not show continued sexual interest in other males once females become available (Hård & Larsson 1968; Perkins & Fitzgerald 1997). Yet, reciprocally cross-fostered male, but not female, sheep and goats develop strong, lasting sexual and affiliation preferences for heterospecifics (Kendrick et al. 1998). Slagsvold et al. (2002) found that cross-fostering in natural populations does not affect pied flycatchers, but does affect reproductive success of some blue tits, *Parus caeruleus*, and the majority of great tits, *P. major*.

Social influence on sexual behaviour is an interesting and complex problem, which can influence the evolution of a population (e.g. Laland 1994). Our results suggest that the social environment in which animals are housed could influence their behaviour in ways that could be problematic for sexual selection experiments as well as for conservation breeding programmes. Reproductive behavioural modifications caused by social environment deserve more attention, particularly as habitat alteration and loss increase the number of captive populations and alter the social structure of wild populations.

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