



# Familiarity leads to female mate preference for novel males in the guppy, *Poecilia reticulata*

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(Received 11 December 1998; initial acceptance 6 April 1999;  
final acceptance 17 June 1999; MS. number: A8148R)

Guppies are a model vertebrate for studies of sexual selection and life history evolution. None the less, there have been few investigations of the factors responsible for maintaining extreme within-population genetic variation in male coloration. In a laboratory study, we tested the hypothesis that frequency-dependent mate choice contributes to the maintenance of this variation. We attempted to avoid biases inherent in earlier studies of the 'rare male effect' by familiarizing females to males bearing a particular colour pattern and later presenting them with alternate male types, in equal numbers. Females were significantly more likely to mate with males having novel colour patterns than with males having a colour pattern with which they were familiar. This result is consistent with the hypothesis that mate choice is frequency dependent. Other factors such as male and female size were unrelated to mate preference. Implications of the results for theories of sexual selection and the maintenance of variation are discussed.

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Understanding the causes and consequences of genetic variation is one of the key challenges of modern population biology (Lewontin 1974; Kimura 1983; Gillespie 1991). Behavioural mechanisms can play a role in maintaining genetic variation in traits related to survival and reproduction and are therefore an important, but often overlooked, source of variation in organismal fitness. In the simplest scenario, genetic variation within a population is maintained by a balance (equilibrium) between the input of new variation by mutation and elimination of (mostly deleterious) mutations by natural selection. However, population genetic models indicate that some forms of natural selection can actively maintain variation at higher levels than are expected under the simple scenario (reviewed in Charlesworth 1987; Barton & Turelli 1989). Heterozygote advantage, environmental heterogeneity (in which the relative fitnesses of alternative alleles changes in different environments), and frequency-dependent fitness (in which the relative fitnesses of alternative alleles depends upon their frequency within the population) are all forms of selection that can

maintain genetic variation at higher levels than expected under mutation alone. Behavioural mechanisms can lead to the maintenance of variation by one of these forms of selection: for example, a mating preference for rare or novel mates is a form of frequency dependence that can maintain multiple genotypes within a population (Farr 1977, 1980b; Lank et al. 1995).

In guppies, extreme within-population variation for male coloration has been noted (e.g. Haskins et al. 1961; Endler 1978). Mature males display colour patterns characterized by irregular areas of structural (blue, green and purple areas) and pigment-based (yellow, orange, red and black) colours. These colours can appear on the body, caudal fin, or dorsal fin and vary in position and size. This variation has a genetic basis (Winge 1922, 1927; Winge & Ditlevsen 1947; Haskins et al. 1961). It is apparently under selection, as it is associated with variation in mating success (Farr 1980a; Endler 1983; Houde 1988), female mate preference (Kodric-Brown 1985; Breden & Stoner 1987; Houde 1987; Stoner & Breden 1988; Reynolds & Gross 1992; Brooks & Caithness 1995; Endler & Houde 1995) and predation risk (Endler 1978, 1980, 1983). Despite abundant variation in male coloration and strong evidence for selection operating on it, there have been few direct tests of the selective forces that can maintain it (Farr 1977; Endler 1980).

Frequency-dependent selection, in which rare phenotypes are favoured over common ones, is a form of

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selection that can easily maintain large amounts of genetic variation (Crow & Kimura 1970; Cressman 1988; Roff 1992; Judson 1995). In male guppies, frequency-dependent selection on colour patterns has been suggested to occur through a 'rare-male' advantage in mating success. To our knowledge, there have been only two previous direct tests of frequency-dependent mating success in male guppies, both by Farr (1977, 1980b). He examined offspring produced by virgin females allowed to interact with groups of 10 males: nine males had the same colour pattern, and one had a different colour pattern. Each male 'type' bore a sex-linked colour pattern element that could be identified in the offspring of the experimental females. Combining the results of both experiments, 39 out of 111 broods were sired by the rare male, suggesting a mating advantage for males of the rare type.

These experiments, and tests of the 'rare-male effect' in other organisms, have been criticized because of possible confounding factors and biases in the experimental design (Partridge & Hill 1984; Partridge 1988). Partridge (1988) concluded that two experimental biases probably account for many of the reports of a rare-male effect in laboratory experiments that use different male 'types'. One cause of bias occurs if individual females have fixed preferences for different male types, but the preference varies from one female to another. Any 'rare' type in a mixture of males will then garner a disproportionate number of matings, because of the constant frequency of females that prefer that type. For example, if 50% of females prefer type A and 50% prefer B, then either type of male will have disproportionately high mating success when they make up less than 50% of males in a given mating trial. There is some evidence that individual female guppies vary in their mating preferences (Godin & Briggs 1996; Kodric-Brown & Nicoletto 1996).

Partridge & Hill (1984) identified another source of bias that occurs when males of the same type compete with each other more than with males of a different type, so that competition increases with frequency. This problem is most likely when there are behavioural differences between morphs so that interactions between males decrease the mating success of a common morph or facilitate mating success of a rare morph. This intramorph competition can generate a form of frequency-dependent selection, but it is unrelated to a female preference for rare or novel male phenotypes. Both of the above sources of bias can occur if the experimenter varies the frequency of male types present in mating trials (Partridge 1988).

In this study, we tested mating preference of female guppies for males with a novel colour pattern. Because experiments that vary proportions of male types may be subject to the biases described by Partridge & Hill (1984) and Partridge (1988), we developed a method of testing for a rare-male effect that avoids this technique. By using a 'familiarization' period, we exposed females to a 'common' male colour pattern and later presented them with alternative male types in equal numbers in a mating trial. Females in both treatment and control groups were exposed to the same two types of males in their mating trials. However, some females (treatment) had been

exposed to one of the types before the mating trial, whereas other females (controls) had not been previously exposed to either male type. Our predictions were: (1) if fixed female preferences or male-male competition caused the rare-male effect observed in other studies, our protocol should eliminate these effects and eliminate the rare-male advantage; (2) if females do express a preference for novel or rare-male phenotypes, then treatment females should show reduced mating preference for the male morph that was 'familiar' to them. As in Farr's (1977, 1980a, b) experiments, we scored mating success by using sex-linked visible markers.

The goal of the experiment was to determine whether we could reduce female preference for males with a particular colour pattern (M7) by familiarizing females to M7 males. Every female in the experiment was given a choice of mating with an M7 male or a male from a stock population. We then compared mate choice in the two types of females. A reduction in preference for M7 would be indicated if females familiarized to that colour pattern were less likely to mate with M7 males compared to females familiarized to other patterns. From previous studies, we knew M7 males had generally high mating success in trials with females from the same population used here. The rare-male effect, if present, would therefore have to reduce the mating success of a generally successful male morph.

## METHODS

The experimental protocol entailed several steps: mating virgin females to males whose offspring could be identified by a Y-linked colour pattern (M1 males); familiarizing these once-mated females to M7 males or to males with a different colour pattern; presenting females with a choice between an M7 male or a male from her own population during a period when she was known to be receptive to mating (24–48 h after giving birth to her first brood); and determining whether the females familiarized to M7 were less likely to mate with the M7 male in her mating trial, as predicted by the rare-male hypothesis. Each of these steps is described in detail below.

### Experimental Fish

Experimental females were derived from a population in the lower Guanapo River (GP) in Northern Trinidad. Three type of males were used in the experiment: GP, M7 and M1. GP males and females were fourth-generation descendants of wild-caught fish. This population had been maintained by mating pairs of unrelated males and females in each laboratory generation. M1 and M7 males were also derived from wild populations. M7 males bear a distinctive colour pattern that was originally observed in a male from the La Selva River, Trinidad, and all M7 fish are patrilineally descended from this fish, and matrilineally descended from females from the Guanapo and El Cedro (a Guanapo tributary) Rivers. The M7 colour pattern (two or three bright gold vertical bars on the

caudal peduncle, a large black spot at the base of the caudal fin, and orange borders on both the top and bottom of the caudal fin) has shown strict paternal inheritance for six generations in the laboratory. M1 males were derived similarly (the original male was from the El Cedro River) and have a different Y-linked colour pattern (white dorsal fin, diffuse black vertical bar on the caudal peduncle, large orange spots on both the anterior dorsal and the posterior ventral caudal peduncle).

All M7 males have essentially identical colour patterns, as do all M1 males. GP males have quite variable colour patterns, as is typical of natural guppy populations (Endler 1978). A sample of GP and M7 males indicated that they have similar average amounts of orange coloration, as a proportion of body area ( $\bar{X} \pm \text{SE}$ : GP:  $0.04 \pm 0.011$ ,  $N=39$ ; M7:  $0.04 \pm 0.007$ ,  $N=6$ ). M1 males have somewhat larger amounts of orange ( $0.11 \pm 0.006$ ,  $N=6$ ). Estimates of standard errors were lower for M1 and M7 males, as expected. Aspects of male colour pattern (especially orange coloration) have been shown to be important in male mating success and female choice in many populations (reviewed in Houde 1997). However, we controlled for differential effects of colour pattern among groups by using the same M7/GP male pairs in all experimental groups. There were therefore no differences among groups in the colour patterns of males used in the mating trials. However, we did not record the colour patterns of the M1 males used in the 'virginity elimination' step, so there may have been uncontrolled differences in colour patterns among groups at this stage. The very uniform colour patterns displayed by all males of the M1 strain suggest that any such effect would have been minor.

We chose M7 males as the marker males for the mating trials because of the high relative mating success of M7 males in similar mating trials against GP males (unpublished data), and because of the strict paternal inheritance of the pattern. GP males were chosen as the competitor strain for the mating trials because we wanted to present the females with males from their own population as one of their choices. M1 males were chosen as the 'virginity-eliminating' males because, excluding the M7 pattern, the M1 pattern was the most distinctive and most consistently Y-linked colour pattern available.

The experimental fish were produced as follows. Eight pairs of unrelated GP males and females from the F3 generation were mated in standard 10-litre aquaria. Female and male fry were separated at approximately 4 weeks of age (before sexual maturity, and before males had begun to express their colour patterns). Each male was then transferred to its own 10-litre aquarium, and housed there until mature. Females were maintained in 19- or 38-litre aquaria with their sisters until mature. Male fry from the M7 and M1 stocks were isolated from females at 4 weeks of age. M7 males were reared in individual aquaria and M1 male siblings were reared together. We used a random number table to assign aquaria housing GP, M7 and M1 to positions within the laboratory.

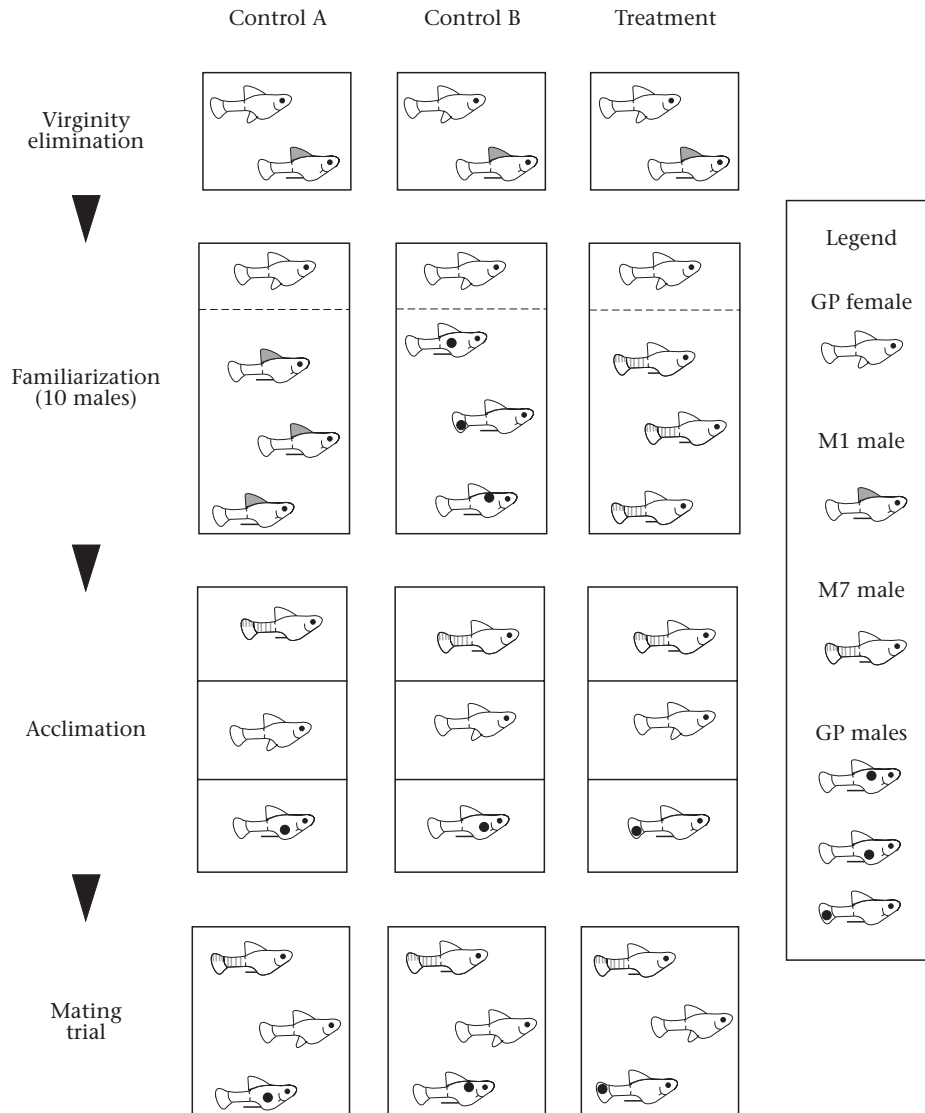
## Familiarization Protocol

To select the females needed for the experiment, we chose four mature females from each of the eight GP families. Two of these four females were then randomly assigned to the treatment group, one to the control A group, and one to control B group. This random assignment (and other random assignments described below) was accomplished by generating lists of pseudorandom numbers using a computer. Each fish was assigned a number and assigned to a group based on that number's first appearance in the pseudorandom number table. We used a different seed for the pseudorandom number generator for each random number table that we used. One additional female was available for the experiment, and we assigned it to one of the groups (control A) by random assignment. Subsequently, one control B female died before completion of the experiment; and another control B female was inadvertently exposed to males before her mating trial and was excluded from the experiment. The number of females used in each group was therefore: treatment,  $N=16$ ; control A,  $N=9$ ; control B,  $N=6$ .

### Experimental overview

The experimental protocol is summarized in Fig. 1. Females in all groups were first mated with a male with the Y-linked M1 pattern (virginity elimination step, see below), then they were 'familiarized' to a group of males, and finally presented with a choice of two males in a mating trial. One of the two males in the mating trial had the M7 pattern, and one was a male from the female's own population (GP). The only difference between the different groups of females was in their exposure to males of different colour patterns before the mating trial. Treatment females were familiarized to a group of M7 males. Control A females were familiarized to a group of males with the M1 colour pattern (note that these males were not the same individuals used in the virginity elimination step). Control B females were exposed to a heterogeneous set of male colour patterns (GP males) in which no particular pattern occurred more than once or twice. Although each control B female would later encounter a GP male in her mating trial, his colour pattern would either not have appeared in her familiarization group, or, if a similar pattern did appear, it would have been rare. We used two different control groups to investigate whether female mate preference is affected by exposure to homogeneous (control A) versus heterogeneous (control B) colour patterns. The prediction of the rare-male hypothesis was therefore that treatment females (familiarized to M7 males) should avoid mating with the M7 male during their mating trials, but control females (familiarized to males with different colour patterns from either male used in their trials) should not.

An important feature of the experiment is that the same set of M7/GP pairs were used in the mating trials for all three groups of females. Differences between female groups were therefore not attributable to differences between males used in the mating trials. The only difference between females in the different groups was in their



**Figure 1.** Diagram of familiarization experiment. Virginitly elimination step: virgin GP females were confined with M1 males for 7 days. Familiarization step: females from the virginitly elimination step were housed in the same aquarium with 10 males, but separated from them by a glass divider. Depending on the experimental group, the female was housed either with M1 males (control A), with GP males (control B), or with M7 males (treatment). Acclimation step: female was moved to an acclimation aquarium on the day she gave birth to her first litter. A GP male was moved to the aquarium immediately to the right (or left) of the female, and an M7 male was moved to an aquarium immediately to the left (or right) of the female. Mating-preference trial: after 24 h of acclimation, the GP and M7 males were placed in the female's aquarium. Males were removed after 24 h.

experience before their mating trials. Each step of the experiment is described in detail below. The step numbers correspond to the parts of Fig. 1.

### Step 1: virginitly elimination

At the beginning of the experiment, all females were sexually mature virgins. Because naive female guppies may show little mate discrimination in their first matings (Houde 1997), our initial step was to mate virgin females to males from the M1 marker stock. In this virginitly elimination step, each female was isolated in a 10-litre aquarium for 1 week with a single adult M1 male. Female guppies can store sperm, so use of males with an identifiable genetic marker was required for this step.

### Step 2: familiarization

The virginitly elimination step was immediately followed by the familiarization step. We moved each female into a 38-litre aquarium containing a central transparent glass divider and a 'baby net' (a divider constructed of course bridal veil) located midway between the glass divider and one end of the aquarium. We placed females between the glass divider and the baby net. Use of a baby net reduces the chances of females cannibalizing their fry. All sides of the aquaria were covered with opaque paper so that the female would be in visual contact with males only in the same aquarium.

One day later, we introduced 10 males into each aquarium on the opposite side of the glass divider from

the female. For treatment females, the 10 males were taken from the M7 stock, and for control A females, the males were taken from the M1 stock. For control B females, the 10 males were taken from the GP stock population. These GP males expressed a wide range of colour patterns with differing combinations of orange and black spots (none of them was similar to patterns of M7 or M1 males). We chose groups of 10 so that similar colour patterns occurred no more than once or twice in any group. Male guppies will court gravid females (Rodd & Sokolowski 1995) and we observed males courting females through the glass divider during this phase (personal observation).

Females remained in familiarization aquaria until they gave birth to their first litter. All fry from this litter had been sired by the M1 male from the virginity elimination step, and were discarded. Birth of the first litter occurred 20–47 days after the females were isolated in the familiarization aquaria ( $\bar{X} \pm SE = 35.5 \pm 6.6$  days). This variation in familiarization time was unavoidable because of the uncertainty in time to parturition in uniparous females and because we wanted to use females in mating trials when they were highly receptive to mating (within a day or two of giving birth; Crow & Liley 1979). Effects of familiarization time on dependent variables are described below (they were all nonsignificant).

### Step 3: acclimation

On the day a female's first litter was born, we removed her from the familiarization aquarium and moved her into the acclimation part of the experiment. She was placed in a 19-litre aquarium. At the same time, an M7 and a GP male (a M7/GP male pair) assigned to this female were introduced into 19-litre aquaria on either side of the female's aquarium. These males were not the same individuals used in the familiarization step.

M7/GP male pairs were chosen by selecting one male offspring from each GP family, and randomly assigning him to one M7 male. We then assigned each male pair to two treatment females and to two control females (one control A female and one control B female). We made this random assignment as described above, except that GP males were never assigned to females from their own family.

The first time a particular M7/GP pair was used, the males were randomly assigned (by coin flipping) to the aquaria to the left or right of the female. Thereafter, males that were on the left in their previous mating trial were put on the right, and vice versa. During acclimation, the males and the female were in visual contact with each other. All male and female fish were between 140 and 160 days of age at the time of the acclimation period.

### Step 4: mating trials and scoring of offspring

After 24 h, we introduced the M7 and GP males into the female's aquarium. We removed the fish from the mating aquarium 24 h later, returning males to their individual aquaria, and the female to an individual 10-litre aquarium supplied with a baby net. All fry produced by females in two subsequent litters were removed

from the female's aquarium immediately after birth, and were reared in 19- or 37-litre aquaria in isolation from other litters. In two cases, fry from one additional litter were also kept because of the relatively small size of previous litters. Male and female fry were separated at approximately 4 weeks of age. Females were discarded, and all males were reared to maturity. At maturity, male offspring were classified as having either the M1 or the M7 sex-linked colour pattern, or neither. Because the only possible sires of the offspring were the M1 male from the virginity elimination step (which would have been produced by stored sperm) and the M7 and GP males from the mating trial, male offspring with neither the M1 nor M7 colour patterns were classified as having been sired by the GP male in the mating trial.

### Morphological Measures

After their last mating trial, we anaesthetized all M7 and GP males and videotaped them on a standard background with a size standard. Using NIH Image software, we made two independent measures of standard length for each male and we calculated total body area and the total area of orange spots on the body. We measured female standard length and mass after the birth of the female's last retained litter, using an electronic balance and calipers.

### Laboratory Conditions

Fish were maintained on a 12:12 h light:dark cycle, at 25–26°C. Females and M1 males were fed brine shrimp and liver paste (a mixture of beef liver and pabulum) every day.

### Data Analyses

We measured female mate preference was in two ways. We scored male mating success (MMS) based on whether or not the female mated with the M7 male in her trial, as indicated by whether she produced any M7 offspring. We also compared the relative reproductive success (RRS) of M7 males and GP males. RRS is a measure of the degree of female preference: the proportion of M7 offspring among those sired during the mating trial. In addition, we calculated the proportion of M7 offspring among all male offspring (including those sired by stored sperm from the M1 male in the virginity elimination step); however, these results were similar to those for RRS, so only RRS is reported, except where noted. RRS provides more information than the 'yes-no' measure of MMS, because multiple matings with the same male can yield higher numbers of offspring from that male. Differences in male fertility will contribute to RRS but, because the same males were used in all treatment and control groups, systematic differences between these groups are not likely to be due to differential male fertility. Male pairs and treatment/control groups were treated as crossed fixed effects in a two-way analysis of variance (ANOVA) of RRS. RRS was arcsine square-root transformed (Sokal & Rohlf

**Table 1.** Mean (SE) number of male offspring sired by M7, GP and M1 males, and mean proportion of male offspring sired by M7 males (relative reproductive success) for each treatment group

	Relative reproductive success of males in each experimental group				
	Control A	Control B	Pooled controls	Treatment	Mean (all groups)
M7	6.33 (1.58)	7.50 (1.94)	6.80 (1.21)	3.75 (1.19)	5.23 (0.87)
GP	3.67 (1.37)	2.83 (1.67)	3.33 (1.04)	9.25 (1.02)	6.39 (0.89)
M1	4.89 (1.30)	6.67 (1.59)	5.60 (1.00)	3.75 (0.97)	4.65 (0.71)
Total male offspring	14.89 (1.11)	17.00 (1.35)	15.73 (0.86)	16.75 (0.83)	16.26 (0.60)
Proportion M7*	0.647 (0.105)	0.688 (0.129)	0.663 (0.080)	0.275 (0.079)	0.463 (0.065)
Proportion M7†	0.400 (0.060)	0.474 (0.110)	0.430 (0.069)	0.220 (0.067)	0.321 (0.051)

Untransformed means are shown.

\*Proportion of all M7 and GP male offspring that were sired by the M7 male.

†Proportion of all M7, GP and M1 male offspring that were sired by the M7 male.

1981). MMS was analysed by log-linear models, using male pairs and treatment/control groups as independent effects.

## RESULTS

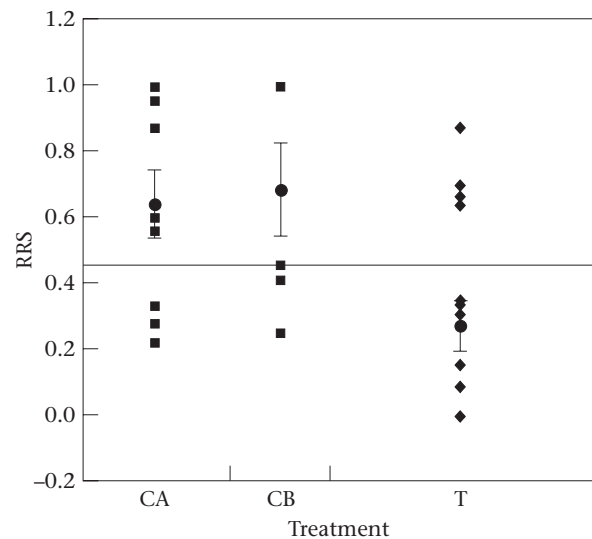
Averaged over all three groups of females, M7 and GP males had comparable mating success and fertility: 26 of 31 females produced offspring sired by GP males and 25 of 31 produced offspring sired by M7 males. Females produced an average  $\pm$  SE of  $6.39 \pm 0.89$  GP and  $5.23 \pm 0.87$  M7 male offspring. All females mated with at least one of the males in her mating trial. Females also produced an average of  $4.65 \pm 0.70$  M1 offspring, indicating that females often used sperm from the virginity-eliminating male even when they subsequently mated with one or both males in their mating trial. The number of M1 offspring did not differ significantly among groups ( $F_{2,29}=1.25$ ,  $P=0.30$ ).

### Relative Reproductive Success

By comparing RRS and MMS in the three groups of females, we tested a prediction of the rare-male hypothesis: treatment females (familiarized to M7 males) avoid mating with M7 males, relative to females in both control groups (familiarized to males other than M7). In the treatment group, 22% of all male offspring were sired by M7 males. In contrast, M7 males sired 40 and 47% of all male offspring in control groups A and B, respectively (Table 1). If M1 offspring are ignored, the difference is even greater, with M7 males making up 28% of the male offspring of treatment females, and 65 and 69% of the offspring of control A and B females, respectively. In the two-way ANOVA of transformed RRS, the difference between treatment and control groups (with controls pooled) was highly significant ( $F_{1,15}=18.01$ ,  $P<0.001$ ). Effects of M7/GP male pairs and interaction effects were not significant ( $F_{7,15}=1.19$ ,  $P=0.36$ ;  $F_{7,15}=1.77$ ,  $P=0.18$ , respectively).

When the two control groups were treated separately, the effect of group on RRS remained significant ( $F_{2,9}=9.70$ ,  $P=0.012$ ). Post hoc tests of this model indicate

that both control groups significantly differed from the treatment group, but did not significantly differ from each other (Fig. 2). Effects of male pairs and interaction effects were not significant ( $F_{5,9}=1.20$   $P=0.38$ ;  $F_{12,9}=1.02$ ,  $P=0.50$ , respectively). Females in both control groups had never seen the M7 colour pattern before, and females in both groups produced more M7 offspring than GP offspring. For control A females, both males in the mating trial were novel. Control B females had seen GP males during familiarization, but males with a pattern similar to the GP male in the mating trial would have been either absent or rare. It therefore appears that females prefer M7 males when both males in the mating trial are novel or rare (controls A and B), and prefer the GP males only



**Figure 2.** Relative reproductive success (RRS, the proportion of male offspring sired by M7) in the treatment, control A, and control B group females. RRS is shown on untransformed scale. However, data analysis was conducted on arcsine square-root transformed data. Post hoc tests were conducted by the Tukey–Kramer means comparison test which gives the absolute difference in means minus the least significant difference, with positive values indicating significant differences. Values of the test for treatment versus control A: 0.11; for treatment versus control B: 0.14; and for control A versus control B:  $-0.47$ . The mean  $\pm$  SE is shown for each group.

**Table 2.** Male mating success (number of females that produced male offspring sired by M7 and GP males) by treatment group

Offspring type	Mating success of males in each treatment group				
	Treatment group of female				
	Control A	Control B	Pooled controls	Treatment	Total
M7	2	3	5	0	5
GP	0	0	0	6	6
Both	7	3	10	10	20
Total	9	6	15	16	31

when they have been familiarized to the M7 pattern (treatment).

We also conducted an analysis to determine whether differences in RRS between groups remained significant when comparing only those females that had actually mated with the M7 male. After removing all females that did not produce M7 offspring, control females produced marginally significantly more M7 offspring than treatment females (66 versus 44%;  $F_{1,23}=4.33$ ,  $P=0.049$ ). This suggests that females in the treatment group that may have mated with the M7 male did so less frequently, or used fewer sperm from these matings, than did the control females.

### Male Mating Success

All control females mated with the M7 males ( $N=15$ ), but only 10 or 16 treatment females did (Table 2). This difference in MMS between treatment and control groups was significant by a likelihood ratio (LR) test ( $\chi^2_1=9.68$ ,  $P=0.002$ ), when the two control groups were pooled. Effect of specific M7/GP pairs was not significant (LR  $\chi^2_7=12.85$ ,  $P=0.08$ ). When control groups A and B were compared separately to the treatment group, each control group differed significantly from the treatment group (control A:  $\chi^2_1=6.388$ ,  $P=0.01$ ; control B:  $\chi^2_1=4.61$ ,  $P=0.03$ ; results of both tests remained significant after correction for multiple tests by the sequential Bonferroni method (Rice 1989). Because all females in both control groups mated with the M7 male, the control groups obviously did not differ significantly from one another, and the comparison yielded a LR  $\chi^2$  value of zero. The fact that M7 males had very high MMS and RRS in both control groups indicates that their poor performance in the treatment group was not due to intrinsically poor mating ability or low female preference.

### Possible Confounding Effects

We performed several tests to investigate possible confounding effects of experimental conditions. Familiarization time for individual females varied from 20 to 47 days, and was marginally significantly associated with total offspring production ( $F_{1,29}=3.61$ ,  $P=0.07$ ). However, neither RRS nor MMS was significantly associated with this variable (RRS:  $F_{1,29}=0.14$ ,  $P=0.71$ ; MMS: LR

$\chi^2_7=0.004$ ,  $P=0.95$ ), and treatment and control females did not differ significantly in average familiarization time ( $F_{1,29}=1.10$ ,  $P=0.30$ ). Treatment and control females did not differ for time to first brood ( $F_{1,29}=1.10$ ,  $P=0.30$ ), for interbrood interval ( $F_{1,29}=0.99$ ,  $P=0.33$ ), or for sex ratio of offspring produced ( $F_{1,29}=0.14$ ,  $P=0.71$ ). The mean sex ratio over all observations was statistically indistinguishable from 1.0 ( $0.984 \pm 0.03$ ,  $t_{31}=0.50$ , two-tailed  $P=0.63$ ).

We were able to test for associations between female family and mate preference, because the 31 females used in the experiment came from eight different families. Female family was not significantly related to RRS ( $F_{7,23}=0.63$ ,  $P=0.72$ ), or to MMS (LR  $\chi^2_7=5.23$ ,  $P=0.63$ ). We also examined the effects of female size (standard length) and the date of the mating trial. Neither of these was significantly associated with RRS nor MMS (results not shown).

We tested for an effect of male experience by conducting a repeated measures ANOVA. There was no effect of the order or number of previous mating trials on the relative mating success of males within a pair ( $F_{3,15}=0.23$ ,  $P>0.87$ , Mauchly sphericity criterion met at  $P>0.60$ ).

### DISCUSSION

Females familiar with M7 males had a significantly lower probability of mating with an M7 male and produced significantly fewer M7 offspring. Despite rather small samples, our results indicate that female guppies show a highly significant alteration of their mate choice preferences in response to being exposed to males with specific colour patterns. This result is consistent with a prediction of the rare-male hypothesis: treatment females (familiarized to M7 males) should avoid mating with M7 males relative to control A and B females (familiarized to males other than M7). Familiarization to a homogenous versus heterogeneous collection of male colour patterns (control A versus control B) had little effect on mate preference. Mate preference was unrelated to other factors, such as female family, female size, and difference in familiarization time. We were able to score paternity only for male offspring, but this should not have biased our results unless females are able to apportion X- and Y-bearing sperm differentially. If females in natural populations behave like our experimental females and express a preference for mates with rare or novel colour patterns, large amounts of genetic variation for male coloration may

be maintained through negative frequency-dependent selection.

Our results are also consistent with those of Farr (1977, 1980b), but we avoided some potential biases inherent in his experimental design. Two potential biases in the previous studies were caused by use of unequal numbers of different male types. In our experiment, there was always exactly one male of each alternative type in the mating trials. The only difference between treatment groups was the previous experience of females. Our results imply that female experience, and not unequal numbers of competing males, caused the rare-male effect observed here. This conclusion does not imply that male-male competition and fixed female preferences are unimportant in guppy mating systems, and thus the relative importance of these phenomena should be investigated. We also cannot rule out the possibility that behavioural differences between the different types of males to which the females were familiarized could have caused differential mate preference in our experimental groups of females. For this effect to account for our results, one would have to argue that groups of M1 and GP males (used in controls) behave similarly to one another, and differently from groups of M7 males (used in treatment). This seems unlikely, but future tests of the rare-male hypothesis should record behaviours of males used in familiarization phases.

Results of two other studies suggest that female choice can be influenced by prior experience. Both studies explored effects of exposing immature female guppies to males with differing colour patterns. Breden et al. (1995) reported that females raised with colourful males display behavioural preferences for colourful males, while females raised with dull males or with no males preferred dull males. Rosenqvist & Houde (1997) found that females raised with variable groups of males preferred males with large amounts of orange coloration, while females raised with homogenous groups of males expressed no mate preferences as adults. In our experiment, juvenile females may have been in visual contact with some adult males because female siblingships were randomly positioned in the laboratory. However, all female siblingships were split among treatment groups, and differences in rearing environment would not have contributed to differences between treatment groups. Furthermore, if adult females responded to familiarization in the same way as the juvenile females in previous experiments, we would predict that treatment and control A females (familiarized to groups of males with homogeneous colour patterns) would have displayed similar preferences (or no preferences) while control B females (exposed to males with varying colour patterns) would display a different pattern of preference. Tables 1 and 2 show that mating preferences of control A females were very similar to control B females and quite dissimilar to treatment females. This contrast between our results and those of experiments using immature females suggests that adult and juvenile females respond differently to a period of familiarization to male colour morphs. Juvenile females may undergo a period of 'imprinting' on the phenotype of conspecific males, perhaps related to

species recognition. This imprinting would then have a very different function than mate preferences in adult females.

The reasons for the expression of a mating preference for novel male colour patterns have not been addressed in this experiment. Several different evolutionary models could potentially account for this preference, and these models need to be addressed in future experiments. For example, females may preferentially mate with novel males because they have been selected for responsiveness to novel visual cues for some reason unrelated to mating per se. If response to novel visual cues leads to a competitive advantage for a limited food resource, then a rare-male effect could be a by-product of selection for efficient foraging. Such a mechanism would be consistent with pleiotropic models of the evolution of female choice (reviewed in Kirkpatrick & Ryan 1991).

Another form of selection for a rare-male preference could occur if local guppy populations are inbred, suffer from inbreeding depression, and have evolved mechanisms for outcrossing. In this scenario, fish occurring in the same pool would be more closely related, on average, than fish occupying different pools. A male immigrating to a new pool, bearing an unfamiliar colour pattern, would then be more distantly related to the resident females than are the resident males. Females that mate with the immigrant male would thus produce offspring that were less inbred. A similar pattern could result if individuals disassortatively mate with respect to MHC haplotype, as has been suggested in mice (Potts et al. 1991; Hedrick 1992), if residents are more likely to share haplotypes than are nonresidents. Various tests of these hypotheses are possible, including the use of molecular markers to measure relatedness in natural populations, laboratory or field measures of inbreeding depression, and studies of mating patterns with respect to kinship or MHC types.

It is difficult to reconcile a preference for novelty per se with 'good genes' or 'runaway' models for the evolution of mate choice, because they do not predict that female preference for alternate types should undergo reversal based on previous experience. It should be noted, however, that other patterns of mate choice in guppies (e.g. preference for amount or brightness of orange colour as in Kodric-Brown 1985, 1989; Houde 1987; Brooks & Caithness 1995) are consistent with these models (reviewed in Houde 1997), and there is no reason to believe that these different evolutionary mechanisms are mutually exclusive. We did not explore the potential for mate choice copying (Dugatkin 1992; Dugatkin & Godin 1992). Because copying increases the opportunity for sexual selection (Wade & Pruett-Jones 1990) it would tend to increase the selective advantage to novel colour patterns. A rare-male effect might be superimposed on other mate choice criteria, or different criteria might operate at different times. Interactions of different mate choice mechanisms should be investigated in future studies.

In summary, our results indicate that preference for novel males can have a strong influence on female mate choice in a laboratory setting. If this type of negative

frequency-dependent selection operates in natural populations, it could maintain the extreme genetic polymorphism in male colour patterns. Field tests of preference for novel colour patterns are now needed to determine whether mate selection based on male colour patterns is frequency dependent.

### Acknowledgments

We thank H. Bryga for help with the design and conduct of the laboratory experiments, A. Houde for making her book available to us before publication, and R. Sawby for preparing Fig. 1. J. Starr isolated the M7 and M1 genetic markers and established the populations that are fixed for those markers. We also thank F. Breden, R. Brooks, H. Bryga, K. Dixon, P. Hedrick, S. Scheiner and the anonymous referees for helpful comments on the manuscript. The government of Trinidad and Tobago kindly granted us permission to export the fish. This work was supported by NSF grant DEB-9419823 to D.N.R. K.A.H. was supported by a National Research Service Award from NIH, F.H.R. by the Center for Population Biology, University of California, Davis, and L.D. by an Undergraduate Research Fellowship from U.C. Riverside. The research presented here was described in Animal Research Protocol No. 9308014-1, approved on 22 February 1994 by the Institutional Animal Care and Use Committee of the University of California, Riverside.

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