

## Sexual selection and competitive mating success of males guppies (*Poecilia reticulata*) from four Trinidad populations

P.H. Luyten and N.R. Liley

Department of Zoology, University of British Columbia, 6270 University Boulevard, Vancouver, BC Canada, V6T 2A9

Received May 2, 1990 / Accepted December 26, 1990

**Summary.** Luyten and Liley (1985) obtained evidence that in populations of Trinidad guppies from two clear headwater streams, where *Rivulus hartii* is the major aquatic predator, selection has favored visual aspects of male mating behavior – display activity and conspicuous coloration. Males in two lowland turbid stream populations, with numerous aquatic predators, were found to rely more heavily upon non-display components of courtship. In this study males of the four populations were placed in competition in clear water for mating with females of the same populations. Males of two populations were allowed to compete in turbid water. Mating success was determined on the basis of the frequency of insemination and the proportion of sperm attributable to males of the competing populations. The sperm of competing males were identified by radioisotope labeling and autoradiographic techniques. Males from headwater populations were more successful than lowland males in mating with headwater females in clear water. There was no difference in mating success of headwater and lowland males in competition for lowland females in clear water, but in turbid water lowland males were more successful in inseminating lowland females. Males were more successful in mating with females of their own population when the two headwater population were in competition. The results are consistent with the hypothesis that conspicuous coloration and high frequency and duration of display are adaptations to clear water conditions, and that non-display tactics are adapted to turbid water. Evidence of intrapopulation preferences suggest that male traits and female sexual responses in the guppy have evolved in parallel as predicted by sexual selection theory.

### Introduction

The Trinidad guppy, *Poecilia reticulata*, (Poeciliidae) offers a unique opportunity to investigate selection pressures affecting the morphological and behavioral charac-

teristics of a single species. Correlations between behavioral and morphological characteristics of guppy populations and physical and biotic variables have stimulated a number of field and laboratory studies. Considerable attention has been directed to the possible role of predation in shaping the morphology (Liley and Seghers 1975), predator avoidance behavior (Seghers 1973, 1974a, b; Farr 1975), and life history characteristics of the guppy (Reznick and Endler 1982). Field and laboratory data indicate that male color patterns are the result of a compromise between selection due to predation for crypsis and sexual selection favoring conspicuous coloration (Haskins et al. 1961; Endler 1978, 1980, 1983, 1987).

Sexual selection appears to be mediated through female choice rather than intermale competition. Although there are several studies of male-male competition in the laboratory (Gandolfi 1971; Ballin 1973; Farr 1976, 1977, 1980a, b; Gorlick 1976), there is little evidence of intermale competition in nature (personal observation) or in an artificial laboratory stream (Crow 1981; see also Endler 1983). Accordingly, interest has centered upon the role of female choice, or epigamic, sexual selection in the evolution of sexual display and color pattern in the male guppy (Bischoff et al. 1985; Breden and Stoner 1987; Farr 1980a; Endler 1978, 1983; Houde 1987, 1988; Houde and Endler 1990; Kodric-Brown 1985, 1989; Stoner and Breden 1988).

The *sigmoid display* is the most distinctive feature of the male's courtship behavior, which serves to "show off" the color pattern in a highly conspicuous fashion. Display may be followed by a *mating attempt* in which the male attempts to insert his gonopodium into the female's genital pore. For the mating attempt to be successful, the female must cooperate with the male (Baerends et al. 1955; Liley 1966).

Alternatively, a male may inseminate a female by means of a *gonopodial thrust*, an action which involves neither male display nor female cooperation. In a gonopodial thrust the male approaches the female from behind and thrusts his gonopodium towards the female's

genital pore. Most thrusts do not result in contact, but there is evidence that a small proportion of thrusts that contact the female also result in insemination (Liley 1966; Farr 1980a).

Liley (1966) suggested that the existence of alternative insemination tactics may permit individuals to occupy a variety of habitats ranging from clear water, where display is effective, to localities where the water is turbid and gonopodial thrusting provides a more reliable means of sperm transfer. Predation may also affect the relative importance of the two modes of insemination: Luyten and Liley (1985) proposed that in clear headstream waters, in which the major aquatic predator is *Rivulus* (a small fish that attacks only small guppies, Liley and Seghers 1975), sexual selection has favored the evolution of visual components of courtship, including conspicuous coloration, large size, and courtship display. In turbid lowland streams with numerous aquatic predators, selection has resulted in a greater emphasis upon non-visual components, i.e., a reduction in size, coloration, and courtship displays and an increased dependence upon the alternative insemination tactic, i.e., gonopodial thrusting.

A series of field and laboratory studies provide support for this hypothesis: Luyten and Liley (1985) found that male guppies in two clear mountain "headstreams" performed more frequent sigmoid displays and displays of longer duration than males in a turbid "lowland" river and an intermediate "midstream" river. Males in the turbid lowland river were found to exhibit higher frequencies of gonopodial thrusts than males in the other three rivers. Phenotypic variation in dependence upon visual and non-visual elements of courtship may also occur. Endler (1987) demonstrated a decrease in visually conspicuous behavioral elements of male guppy courtship in the presence of predators or under high light intensities.

This study provides a further test of the hypothesis that population differences in male courtship are the result of sexual selection operating under different conditions of predation and turbidity. Males from four populations were placed in competition for mating with female under clear water conditions. Males from two of the populations were also allowed to compete in turbid water. It was not possible to measure reproductive success directly, i.e., the production of progeny of different males; instead, mating success was determined on the basis of number of inseminations and sperm contributed by males of the different populations. Sperm were identified using radioisotope labelling and autoradiographic techniques.

The hypothesis outlined above led to the following predictions:

1. Under clear water conditions, males from a headstream population with their bright coloration and more vigorous display activity would be more successful in mating than males from turbid and predator-rich midstream or lowland rivers, when placed in competition for females of both populations.
2. If heavy predation and turbid water have resulted in selection for less conspicuous appearance and mating

behavior, then in turbid water the converse would apply, i.e., males from predator-rich, turbid streams would be more successful than those from headstreams.

3. There should be little or no difference in mating success of midstream and lowland males, with similar courtship adaptations, when placed in competition for females of both populations. Similarly, there would be no differences in mating success between males of the two headstream populations in competition for females of both populations.

## Methods

*The study populations.* Mating competition experiments were carried out with wild-caught guppies from four locations: two headstream populations, the Naranjo and Paria rivers, which flow south and north, respectively, off the Northern Range of Trinidad; a lowland river, the Guayamare; and an intermediate midstream, the Lower (L.) Aripo, 10 km downstream of its major tributary, the Naranjo (locations in Luyten and Liley 1985). Except for a few hours after heavy rain, the Naranjo and Paria rivers remain crystal clear. The Guayamare is persistently turbid, even after a period of several days without rain. In a series of readings from May to November 1978, a white "secchi" disk could no longer be distinguished at a mean depth of  $5.4 \text{ cm} \pm 0.465 \text{ SE}$ . The Lower Aripo is intermediate between headstream and lowland rivers: the water may remain turbid for several days after heavy rain but becomes clear in a dry period.

The only major fish predator in the Naranjo and Paria rivers is *Rivulus hartii* (Cyprinodontidae). In the L. Aripo and Guayamare, there are a number of cichlid and characid predators (Seghers 1973; Liley and Seghers 1975; Endler 1983).

*Labelling of sperm with radioactive tracer.* The radioisotopes thymidine- $^3\text{H}$  and thymidine- $^{14}\text{C}$  are incorporated into the DNA of cells (Reichard and Esteborn 1951). Billard (1966) and Crowley (1968) documented incorporation of thymidine- $\text{H}^3$  into guppy sperm and subsequent identification of labelled sperm using standard autoradiographic methods.

Preliminary studies revealed that 21–28 days after an injection of the isotopes, 70–80% of sperm taken from male guppies were labelled. Furthermore, it was established that labelled sperm could be successfully recovered from females mated with injected males for a period of 7 days. Neither the injection nor the isotope were found to have a significant effect on the courtship behavior of isotope-injected males as compared with saline-injected and non-injected males.

In the mating experiments described below, males were anaesthetized with 1:600 MS 222 (Tricaine methane sulphonate – Sandoz) and stripped of sperm by firmly stroking the abdominal region with a blunt probe. In the first experiment, 2.0  $\mu\text{l}$  of thymidine solution (methyl  $\text{C}^{14}$ ; 1.0 Ci/l; New England Nuclear) was injected into the abdominal cavity using a Hamilton syringe fitted with a no. 30 needle. In the second experiment, males received 2.0  $\mu\text{l}$  of thymidine- $\text{H}^3$  (1.0 Ci/l; New England Nuclear). In each case, the total dose at each injection was 2.0  $\mu\text{Ci}$ . Unlabelled males were injected with 2.0  $\mu\text{l}$  of saline (0.6% NaCl).

*Recovery of labelled sperm from females.* Females were anaesthetized with MS 222 and placed under a dissecting microscope. Micropipettes drawn from 2.5-mm diameter glass tubing were used to take oviduct samples from the females. The micropipette was first partially filled with distilled water and then inserted into the female's genital opening. The distilled water was flushed 4 times from pipette to pipette before emptying onto a glass slide. The procedure was repeated 3 times for each female. The sample was spread over approximately two-thirds of the slide and allowed to dry in the air.

*Staining and autoradiography.* The dry smears were stained in Mayer's haemotoxylin and eosin, dehydrated in alcohol, and allowed to dry. Each slide was then dipped in liquid Kodak NTB-3 Nuclear Emulsion (diluted 1:1 with distilled water) and placed in a vertical position to dry. The dry slides were placed in slide boxes containing a small amount of anhydrous CaSO<sub>4</sub> (Drierite), sealed, and stored at room temperature. After 14 days, slides were developed in Kodak D19 developer, followed by Kodak Fixer and Edwal Hypoeliminitor.

*Assessment of sperm-smears.* Smears were examined under a light microscope for the presence of sperm. When sperm were present, the smear was scanned for a region in which individual sperm could be identified and "scored" (in some areas the density of sperm was too high to allow identification of individual sperm). A sperm was considered labelled if there was one or more black grains in the emulsion overlying it. Beginning at the left side of a micrometer grid, labelled and unlabelled sperm were tallied until a total of 100 sperm had been assessed, moving the grid to the right as many times as necessary. This procedure was repeated in two other areas of the same preparation, giving a total of 300 sperm.

#### Mating experiments

*Experiment 1: Mating competition in clear water.* Four competitive mating trials were conducted in clear water. In each trial, males from two of the four populations were placed with females from the same two populations. Males of one of the populations were injected with isotope. In a replicate of each trial, the population receiving the isotope in the first trial was injected with saline, and vice versa. Males were allowed to compete for mating for 7 days, starting 25 days after the initial injection of the males.

Limiting the experimental mating period to 7 days was a compromise between the period over which high levels of labelled sperm could be expected to be present in isotope injected males, and the period of time after insemination over which sperm remain

accessible by oviduct flushing. Pilot data suggested that maximum levels of labelled sperm were present in male ejaculate from day 22 to 32 after the first of two injections. On the other hand, sperm may be readily recovered from the oviduct for up to 7 days following an insemination (Clark and Aronson 1951; Liley 1966). Few if any sperm are present in smears taken after 10 days or more, as the sperm become embedded in cells lining the ovarian lumen (Jalabert and Billard 1969). In order to minimize the contribution of pre-experimental inseminations to the sperm smear, females were isolated from males 15 days before the mating trial after first flushing the oviduct with saline to remove sperm remaining free in the oviduct and ovarian lumen.

Mating competition experiments were conducted in an outdoor concrete pool (270 × 200 × 43 cm deep) at the Asa Wright Nature Centre (Simla), Trinidad. The pool received a continuous flow of clear water from a local spring. Water depth was maintained at 24 cm. Each replicate of each trial was conducted in one of six cages placed in the pool. The cages (122 cm long × 61 cm wide × 43 cm high) consisted of a wood frame covered with a plastic mosquito screen.

The combinations of populations examined in each of four trials are listed in Table 1. Trial 1 is used as an example of the procedure for all trials:

*Trial 1.* Naranjo and Guayamare populations.

*Day 0:* Twenty-five Naranjo (N) males and 30 Guayamare (G) males were stripped and injected with thymidine-C<sup>14</sup> and saline, respectively, and placed in separate holding tanks. In a replicate begun on the same day, Guayamare males received the thymidine and the Naranjo fish received saline.

*Day 5:* Stripping and injection were repeated.

*Day 10:* Thirty-five N females and 35 G females were clipped for later identification. Two posterior rays (6, 7) of the anal fin of N females and the 2 anterior rays (1, 2) of G females were clipped. The oviduct of each female was flushed with saline to remove free sperm from previous inseminations. Females were then placed together, but isolated from males, in a cage. The replicate group females were given the same treatment, except the rays clipped were reversed. The cages were checked regularly for young and these were removed.

**Table 1.** Mating competition in clear water: total number of females inseminated by males of each population including females mated with both populations; female mated with males of only one population. Asterisks difference significant,  $P < 0.05$ ,  $\chi^2$

Females	n	Total inseminated	Number of females inseminated by:		Inseminations attributable to males of one population only:	
			Male Naranjo	Male Guayamare	Male Naranjo	Male Guayamare
Trial 1						
Naranjo (headstream)	60	28	26	17	11*	2
Guayamare (lowland)	59	26	20	20	6	6
Trial 2						
			L.Aripo	Naranjo	L.Aripo	Naranjo
Naranjo (headstream)	60	25	19	24	1*	6
L. Aripo (midstream)	60	29	25	26	3	4
Trial 3						
			L.Aripo	Guayamare	L.Aripo	Guayamare
L. Aripo (midstream)	59	21	16	18	3	5
Guayamare (lowland)	58	27	21	23	4	6
Trial 4						
			Naranjo	Paria	Naranjo	Paria
Naranjo (headstream)	60	32	32	23	9*	0
Paria (headstream)	60	28	19	26	2*	9

**Table 2.** Mating competition in turbid water: total number of females inseminated by males of each population, including females mated with males of both populations; females mated with males of only one population. *Asterisks* difference significant  $P < 0.05$ ,  $\chi^2$

Females	n	Total inseminated	Number of females inseminated by males of:		Inseminations attributed to males of one population:	
			Naranjo	Guayamare	Naranjo	Guayamare
Naranjo (headstream)	59	25	20	23	3	5
Guayamare (lowland)	60	22	9*	20	2*	13

*Day 24:* The number of females of each stock was reduced to 30.

*Day 25:* The start of the mating period: 20 N males and 20 G males were added to the 30 females of each stock already present in a cage. Sperm samples taken from the remaining isotope-injected N males on day 28 provided an estimate of the proportion of sperm labelled at the middle of the mating period. Sperm smears taken on day 28 from the remaining saline injected G males provided a measure of naturally occurring background radiation.

*Day 32:* End of the mating period: oviduct samples were taken from all females.

*Experiment 2: mating competition in turbid water.* Males of one headstream population, Naranjo, and one lowland population, Guayamare, were allowed to compete for mating under turbid water conditions. Guppies from Naranjo and Guayamare rivers were shipped to the University of British Columbia, Vancouver. Stocks were housed in tanks of 43- and 61-l capacity with gravel substrates and planted with *Vallisneria* sp. and *Lemna* sp. All tanks were illuminated by 30- to 40-watt fluorescent tubes (13L:11D) and maintained at 23–26° C. Tank water was filtered by subgravel or charcoal filters. One-third of the water in each tank was regularly replaced with dechlorinated tap water. All fish were fed with a commercially prepared dried fish food, Tetra-min, occasionally supplemented with brine shrimp or *Tubifex* worms.

The mating experiment was carried out in two stream tanks. Each tank consisted of an oval channel of plexiglass 25 cm wide, 15 cm deep. Mating experiments were conducted in the straight (100 cm) sections of the channels. Each tank held approximately 500 l of water and was aerated using air stones. Water in the tanks was made turbid by adding fine sediment from the bed of a local stream. A barely perceptible current maintained by a submersible pump helped to keep the fine sediment in suspension. Turbidity was checked twice a day during the experimental period: the distance at which a white disk 10 cm in diameter became no longer visible was maintained at  $6 \pm 2$  cm. This was done by removing water from the experimental tanks and adding fresh turbid water.

The experimental design and procedures were similar to those in Experiment 1 with the following modifications. Tritiated thymidine was used instead of the  $C^{14}$  isotope. Each fish was injected 3 times with 2  $\mu$ Cu in 2  $\mu$ l on days 0, 5, and 10. (The third injection was added in an attempt to increase the period over which high levels of labelling would be maintained.) Females were marked by injecting a small amount of trypan blue under the skin of the upper or lower caudal peduncle. Turbid water was added to the experimental tanks on day 22, 3 days before males and females were combined in the experimental sections of the channels and allowed to interact freely.

#### *Measures of mating success and mate choice*

*Attribution of inseminations to males of competing population.* For many of the females that mated, it was possible to attribute the insemination to a male(s) of one population only; for others, the proportion of labelled and unlabelled sperm (corrected for background radiation, see the following) indicated that females had been inseminated by one or more males of both competing populations. Therefore, for each trial the mating success of males of one

population was assessed in terms of (1) the total number of females inseminated by males of that population (this includes females also inseminated by males of the other population) and (2) females inseminated by males of only one population (a subset of females in (1)).

Identification of females mating with males of only one population was as follows: If a smear contained over 81.6% (experiment 1) or 79.3% (experiment 2) labelled sperm, it was attributed to a male(s) from the isotope injected population. If the smear contained over 94.6% (experiment 1) or 94.3% (experiment 2) unlabelled sperm, it was attributed to a male(s) from the saline injected population. These criteria are based on corrections for (a) background radiation: on average just over 5% of sperm of saline injected males "appeared" to be labelled, and (b) the mean proportion of labelled sperm present in sperm stripped from isotope injected control males (corrected for background radiation) = 76.2%,  $n=40$  (experiment 1) and 73.9%,  $n=20$  (experiment 2).

The number of inseminations by males in each replicate were combined and compared using the  $\chi^2$  test,  $\alpha=0.05$  (Snedecor and Cochran 1967).

*The proportion of sperm contributed by males of competing populations.* The sperm sampling techniques did not allow a reliable estimate of the actual number of inseminations achieved by an individual female with males of each population. An overall assessment of the relative success of males of competing populations is provided by comparing the number of sperm (out of 300 scored) attributed to males of the competing populations (Wilcoxon matched-pairs signed-rank test,  $\alpha=0.05$ , Snedecor and Cochran 1967).

## Results

Almost 45% of females in both experiments showed evidence of recent insemination (Tables 1 and 2). The similarity in the number of females mated in each trial (mean = 26.7, range 21–32) suggests that it is unlikely that major differences in sexual motivation were responsible for differences in mate preference between and within trials.

In a majority of mated females (64%), the proportion of labelled and unlabelled sperm indicates they had been inseminated by one or more males of both competing populations (Tables 1 and 2). In the remaining females, insemination could be attributed to males of one population only (Tables 1 and 2).

#### *Experiment 1: mating competition in clear water*

Naranjo (headstream) males were more successful in mating with Naranjo females than either Guayamare (lowland) (trial 1) or L. Aripo (midstream) males (trial 2). Although suggestive, the differences are not significant when the total number of females inseminated

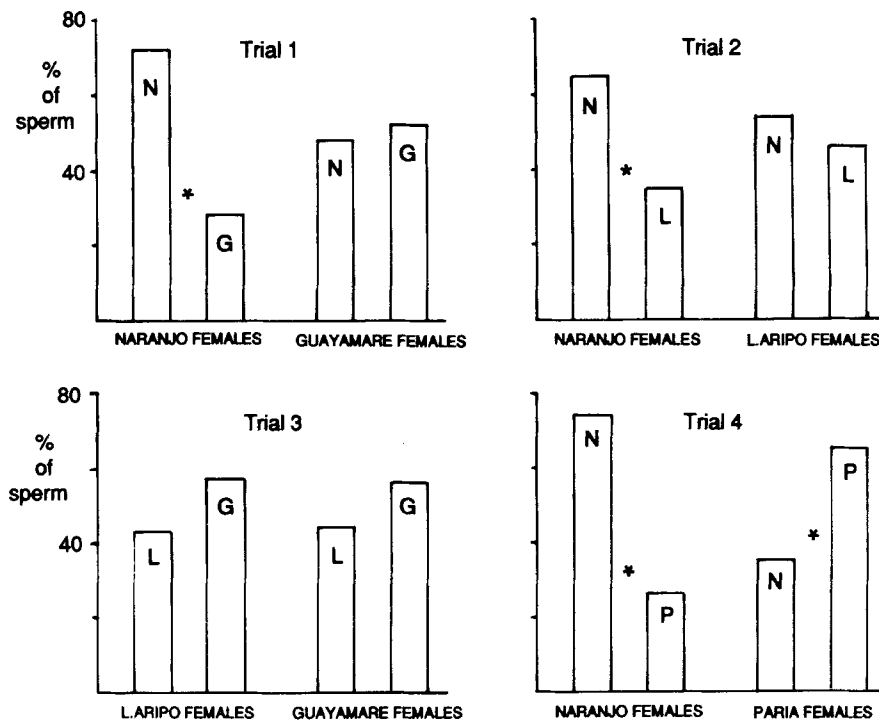


Fig. 1. Mating competition in clear water: proportion (%) of sperm recovered from females attributed to males of the competing populations in four separate trials (see Table 1). Asterisks difference significant  $P < 0.05$ , Wilcoxon Test: N=Naranjo; P=Paria; G=Guayamare; L=Lower Aripo

by competing males are compared (Table 1). However, in both trials if the numbers of inseminations attributable to males of only one population are compared, the differences are significant ( $P < 0.05$ ). There were no differences in the number of Guayamare females (trial 1) and L. Aripo females (trial 2) inseminated by males of competing populations.

There were no differences in the number of females inseminated when Guayamare and L. Aripo males competed for mating with Guayamare and L. Aripo females (trial 3, Table 1). In trial 4, Naranjo females were more frequently inseminated by Naranjo males than by competing Paria males, but only in the numbers of females for which insemination could be attributed to males of only one population is the difference significant. Conversely, Paria females were inseminated more often by Paria males: the difference in the numbers of females inseminated by males of only one population is significant.

In comparing the overall mating success as indicated by the proportion of sperm from competing males, Naranjo males contributed more sperm to Naranjo females than did Guayamare males in trial 1 (Fig. 1) and L. Aripo males in trial 2 (Fig. 2). There were no significant differences in the amount of sperm contributed to Guayamare females by Naranjo and Guayamare males (trial 1). Similarly, there were no significant differences in numbers of sperm from Naranjo and L. Aripo males recovered from L. Aripo females (trial 2).

There were no significant differences in the numbers of sperm contributed to L. Aripo and Guayamare females by males of those two rivers (trial 3). In trial 4, Naranjo females had more sperm contributed to them by Naranjo males than Paria males. Conversely, Paria females carried more sperm from Paria males than Naranjo males.

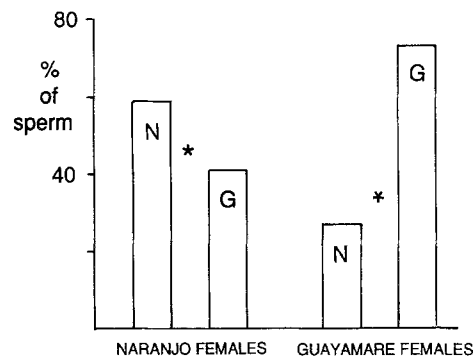


Fig. 2. Mating competition in turbid water: proportion (%) of sperm recovered from females attributed to males of two competing populations, Naranjo and Guayamare. Asterisks difference significant  $P < 0.05$ , Wilcoxon Test

#### Experiment 2: mating competition in turbid water

Guayamare males were more successful than Naranjo males in mating with Guayamare females in turbid water, both in terms of inseminations attributable to one population and total numbers of sperm (Table 2; Fig. 2). Among the Naranjo females, there was no significant difference in the number of inseminations attributable to Guayamare and Naranjo males. Naranjo males did, however, contribute significantly more sperm to Naranjo females than did Guayamare males.

#### Discussion

Female guppies remain sexually receptive for several days following parturition (Liley 1966) and may cooperate in mating with several males over that period. In

this study, almost 45% of females were found to have mated during the experimental mating period. Although the sampling technique did not allow a precise estimate of the number of inseminations achieved by an individual female, it was clear from the proportion of sperm found to be labelled that 62% of the females had mated with males of both competing populations.

The total numbers of females inseminated by males of competing populations, including those mated with males of both populations (Tables 1 and 2), provide suggestive evidence (not statistically significant) of non-random mating in several of the trials. Our inability to estimate the actual number of inseminations achieved by males of competing populations evidently obscures the degree of selectivity in mating. Differences in mating success of males of competing populations are indicated more clearly in estimates of the proportions of sperm attributable to males of the two populations. Furthermore, when females mated with a male(s) of only one population are considered (Tables 1 and 2), there are significant differences in the numbers of females inseminated by competing males. Females mating with males of only one population may have been more selective in their responses, perhaps because of motivational differences: a female close to the end of her period of receptivity may have a higher response threshold and show a higher level of discrimination than a female at peak receptivity.

Underlying the hypothesis that female choice has resulted in the evolution of conspicuous coloration and courtship of the male guppy is the assumption that the capacity of a male to evoke sexual responses from a female is directly related to both the conspicuousness and the vigor of the male's display. It is difficult to separate completely the two aspects of the display.

Farr (1980a), working with laboratory strains of guppies, could find little evidence of an effect of color and pattern brightness on reproductive success and concluded that display rate is the major determinant of male success. Farr proposed that male coloration serves as a tie-breaker among males having similar display frequencies. However, experimental studies by Breden and Stoner (1987), Endler (1983), Houde (1987, 1988), Houde and Endler (1990), Kodric-Brown (1985, 1989), Long and Houde (1989), and Stoner and Breden (1988) demonstrate clearly the importance of male coloration in the responses of females in choice situations. In addition, Endler (1983) emphasizes the importance of the background – environmental illumination and substrate characteristics – in determining the conspicuousness of male color patterns.

In the first experiment reported here, the water was clear and only partially shaded for much of the day. The mating cages provided a uniform, fine-grained, grey screen background. Under these conditions, Naranjo (headstream) males were more successful than Guayamare (lowland) males in mating with Naranjo females. This result is consistent with the prediction that headstream males would be more successful than midstream and lowland males in competition for headstream females in clear water. However, contrary to expectation,

Naranjo males were no more successful than Guayamare and L. Aripo (midstream) males in mating with Guayamare and L. Aripo females, respectively.

These findings suggest that whereas greater display activity and more conspicuous appearance may enhance the ability of headstream males to achieve mating with their own females, these characteristics are less effective in eliciting sexual responses in midstream or lowland females.

Increasing the turbidity of the water effectively reversed the outcome: in turbid water, Guayamare males were considerably more successful than Naranjo males in mating with Guayamare females (proportion of sperm contributed by Guayamare males: 52% in clear water, 72% in turbid water,  $P \leq 0.025$ , Mann-Whitney *U*-test). Naranjo males were still more successful with their own females in turbid conditions as measured by the proportion of sperm contributed by males of the two populations, but the difference between Guayamare and Naranjo males was reduced considerably compared with the outcome in clear water: the mean number of sperm contributed to Naranjo females by Naranjo males in clear water was 72%, compared with 58% under turbid conditions ( $P \leq 0.04$ ).

Was the increased success of Guayamare males in turbid water due to less reliance by those males upon visual signals and to a greater dependence upon gonopodial thrusting as the mechanism of insemination? We have no way of answering that question. The increased success of Guayamare males could reflect a greater frequency of gonopodial thrusting or it could be that other differences, for example in schooling and in orientation behavior, or a response to female pheromones (to be discussed hereafter) increase the mating success of lowland males in turbid water.

Our experimental findings confirm the importance of visual components in the courtship of headstream fish, but at the same time these findings indicate that what we have assumed to be the more conspicuous display does not itself ensure mating success with midstream or lowland males. Indeed, the results call into question the assumption that the capacity to attract a mate, and hence the basis for epigamic sexual selection, is simply a product of the conspicuousness of the coloration and amount of display. Instead, female responsiveness appears to be "adaptively tuned" to the stimulus normally provided by males of their own populations. For example, in clear water the more active and conspicuous Naranjo (headstream) males were as successful as lowland males in mating with lowland females; in turbid water, lowland Guayamare males were considerably more successful than Naranjo in mating with their own females, but had less success with Naranjo females. Evidently, the greater conspicuousness of Naranjo males overrides to some extent the intrapopulation preference of Guayamare females in clear water, but any "advantage" is lost in turbid water where Guayamare males are relatively more successful, even with Naranjo females.

A correlation between male traits and female preference is most clearly indicated by the interpopulation discrimination observed when the two headstream popula-

tions, Paria and Naranjo, were allowed to compete: males of both populations were more successful with females of their own populations. This result is contrary to our prediction that males from populations from rivers with roughly similar conditions and exhibiting similar courtship strategies would not differ significantly in mating success with females of either population. Note, however, this prediction was supported when L. Aripo and Guayamare males were allowed to compete.

The basis for these interpopulation discriminations is unknown. Females may respond to differences in courtship not detected by us. Alternatively, in spite of the remarkable variety of male color patterns, females may respond more readily to the color patterns of their own populations. Males of the Paria and Naranjo populations rely on quite different color schemes to achieve a conspicuous appearance: Naranjo males are characterized by highly distinctive large black markings emphasized by surrounding iridescent white or blue structural colors; Paria males typically have relatively large areas of intense red-orange patches of carotenoid pigmentation separated by distinct black bars or patches; the black, structural and carotenoid markings of L. Aripo and Guayamare are generally far less bold than in headstream males (see color plates in Endler 1983: a. Lower Aripo, b. Aripo – low predation sample similar to Naranjo, c. Paria).

Houde (1987, 1988), Long and Houde (1989), and Kodric-Brown (1985) have demonstrated the importance of orange pigment in the color patterns of male guppies as a cue for female mate choice. In a comparison of mating responses of females of several Trinidad guppy populations (including Paria), Houde (1988) and Houde and Endler (1990) found that the degree of female sexual response is correlated with the population average area of orange pigmentation.

Although the work cited above has emphasized the importance of orange pigmentation in evoking female responses, it is clear that other components of the coloration pattern may play a major role. Orange pigmentation is not well developed in Naranjo males, and Houde and Endler (1990) obtained low levels of sexual response to males with large areas of orange in females of three populations with males with small areas of orange. In addition, differences in male-female interaction may favor the non-display insemination tactic in some populations more than others. Females are not known to actively cooperate with gonopodial thrusting males. Nevertheless, certain features of the behavior of lowland and midstream males may facilitate thrusting behavior. Lowland and midstream fish tend to form schools and maintain shorter inter-individual distances (Seghers 1973, 1974a, b; Luyten and Liley 1985). Although schooling is primarily a predator avoidance behavior, it almost certainly facilitates male-female encounters and interactions, including gonopodial thrusting in clear water, and is likely to be a major factor in turbid water where visual display is less effective.

Emphasis in this and other studies cited has been placed upon female choice. However, there is some evidence that mate selection does not depend entirely upon

female choice. Ballin (1973) and Crow (1981) demonstrated in a series of mate-choice experiments a tendency for males to direct more courtship to females of their own populations. The basis for this male preference was not clear. One possibility is that males responded to minor morphological differences or to subtle female responses to their courtship. Population specific pheromones may also play a role. Snyder (1978) working with a headstream (Paria) and a lowland (Caparo) population demonstrated that males of both populations showed a greater sexual response to ovariectomized (pheromonally inert) females in the presence of chemical cues derived from females of their own populations.

### *Sexual selection and speciation*

The results of the present study support the hypothesis that population differences in coloration and mating behavior are adaptations to different physical and biotic conditions in the streams occupied by the four populations examined. The findings also provide support for Houde and Endler's (1990) proposal that male traits and female preferences in the guppy appear to have evolved in parallel, as predicted by several models of sexual selection.

Mayr (1963) and Dobzhansky (1951) have argued that geographic variation in species characteristics is the inevitable consequence of geographic variation in the environment and is the essential first step in speciation. West-Eberhard (1983) considered the possible role of sexual selection in this evolutionary process and stressed that under sexual selection, social signals are likely to diverge in isolated populations independently of any requirements for species recognition. Populations whose signals have diverged in this way are likely to be pre-adapted for species recognition by the prior acquisition of specific markers and need only to be selected to distinguish them.

Investigations of Trinidad guppy populations provide support for the Mayr (1963) and Dobzhansky (1951) hypothesis: guppies from geographically separated populations vary in a wide range of ecological, morphological, and behavioral characteristics that can, in turn, be correlated with variation in the environment. In particular, mating behavior and mate selection show a divergence among populations which, although not sufficient to maintain reproductive isolation (the populations will interbreed freely in the laboratory), appear to represent an early phase in speciation and confirm that population recognition may arise at an early stage in evolutionary divergence in the absence of selection for reproductive isolation.

*Acknowledgements.* We would like to thank Dr. J. Price, Dr. J.S. Kenny, Mrs. Reanty Hosein, and Mr. Mahase Ramlal for their hospitality and help during our stay in Trinidad. Dr. T. Baird kindly read the manuscript and made numerous comments and suggestions. The work was supported by a National Science and Engineering Research Council of Canada Operating Grant to N.R.L.

## References

- Baerends GP, Brouwer R, Waterbolk HT (1955) Ethological studies on *Lebistes reticulatus* (Peters). *Behaviour* 8:249–334
- Ballin PJ (1973) Geographic variation of courtship behaviour of the guppy, *Poecilia reticulata*. M Sc Thesis, University of British Columbia, Vancouver, pp 1–135
- Billard R (1966) Contribution a l'étude de la reproduction chez le Poisson téléostéen *Lebistes reticulatus*, au moyen de l'insemination artificielle. Ph D Thesis, University of Lyon, pp 1–83
- Bischoff RJ, Gould JL, Rubenstein DI (1985) Tail size and female choice in the guppy (*Poecilia reticulata*). *Behav Ecol Sociobiol* 17:253–255
- Breden F, Stoner G (1987) Male predation risk determines female preferences in the Trinidad guppy. *Nature* 329:831–833
- Clark E, Aronson LR (1951) Sexual behaviour in the guppy, *Lebistes reticulatus* (Peters). *Zoologica* 36:49–66
- Crow RT (1981) Behavioural adaptations to stream velocity in the guppy, *Poecilia reticulata*. M Sc Thesis, University of British Columbia, Vancouver, pp 1–106
- Crowley TJ (1968) The radioactive labelling of poeciliid spermatazoa. M Sc Thesis, University of Calgary
- Dobzhansky T (1951) *Genetics and the origin of species*, 3rd edn. Columbia University Press, New York
- Endler JA (1978) A predator's view of animal color patterns. *Evol Biol* 11:319–364
- Endler JA (1980) Natural selection on color patterns in *Poecilia reticulata*. *Evolution* 34:76–91
- Endler JA (1983) Natural and sexual selection on color patterns in poeciliid fishes. *Environ Biol Fishes* 9:173–190
- Endler JA (1987) Predation, light intensity and courtship behaviour in *Poecilia reticulata* (Pisces: Poeciliidae). *Anim Behav* 35:1376
- Farr JA (1975) The role of predation in the evolution of social behaviour of natural populations of the guppy, *Poecilia reticulata* (Pisces: Poeciliidae). *Evolution* 29:151–158
- Farr JA (1976) Social facilitation of male sexual behaviour, intra-sexual competition and sexual selection in the guppy, *Poecilia reticulata* (Pisces: Poeciliidae). *Evolution* 30:707–717
- Farr JA (1977) Mate rarity or novelty, female choice behaviour and sexual selection in the guppy, *Poecilia reticulata* Peters (Pisces: Poeciliidae). *Evolution* 31:162–168
- Farr JA (1980a) Social behaviour pattern as determinants of reproductive success in guppy, *Poecilia reticulata* Peters (Pisces: Poeciliidae) – an experimental study of the effects of intermale competition, female choice, and sexual selection. *Behaviour* 74:38–91
- Farr JA (1980b) The effects of sexual experience and female receptivity on courtship-rape decisions in male guppies, *Poecilia reticulata* (Pisces: Poeciliidae). *Anim Behav* 28:1195–1201
- Gandolfi G (1971) Sexual selection in relation to the social status of males in *Poecilia reticulata* (Teleostei: Poeciliidae). *Boll Zool* 38:35–48
- Gorlick DL (1976) Dominance hierarchies and factors influencing dominance in the guppy *Poecilia reticulata* (Peters). *Anim Behav* 24:336–346
- Haskins CP, Haskins EF, McLaughlin JJA, Hewitt RE (1961) Polymorphism and population structure in *Lebistes reticulatus*. An ecological study. In: Blair WF (ed) *Vertebrate speciation*. University of Texas Press, Austin, pp 320–395
- Houde AE (1987) Male choice based upon naturally occurring color-pattern variation in a guppy population. *Evolution* 41:1–10
- Houde AE (1988) Genetic difference in female choice between two guppy populations. *Anim Behav* 36:510–516
- Houde AE, Endler JA (1990) Correlated evolution of female mating preferences and male color patterns in the guppy *Poecilia reticulata*. *Science* 248:1405–1408
- Jalabert B, Billard R (1969) Étude ultrastructurale du site de Conservation des spermatozoides dans l'ovaire de *Poecilia reticulata* (Poisson Téléostéen). *Biol Anim Biochem Biophys* 9:273–280
- Kodric-Brown A (1985) Female preference and sexual selection for male coloration in the guppy (*Poecilia reticulata*). *Behav Ecol Sociobiol* 17:199–205
- Kodric-Brown A (1989) Dietary carotenoids and male mating success in the guppy: an environmental component to female choice. *Behav Ecol Sociobiol* 25:393–401
- Liley NR (1966) Ethological isolating mechanisms in four sympatric species of poeciliid fishes. *Behaviour (Suppl)* 13:1–197
- Liley NR, Seghers BH (1975) Factors affecting the morphology and behaviour of guppies in Trinidad. In: Baerends GP, Beer C, Manning A (eds) *Function and evolution in behaviour*. Clarendon Press, Oxford, pp 92–118
- Long KD, Houde AE (1989) Orange spots as a visual cue for female mate choice in the guppy (*Poecilia reticulata*). *Ethology* 82:316–324
- Luyten PH, Liley NR (1985) Geographic variation in the sexual behaviour of the guppy, *Poecilia reticulata* (Peters). *Behaviour* 95:164–179
- Mayr E (1963) *Animal species and evolution*. Harvard University Press, Cambridge
- Reznick D, Endler JA (1982) The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). *Evolution* 36:160–177
- Reichard P, Esteborn B (1951) Utilization of desoxyribosides in the synthesis of polynucleotides. *J Biol Chem* 188:839
- Seghers BH (1973) An analysis of geographic variation of the anti-predator adaptations of the guppy, *Poecilia reticulata*. Ph D Thesis, University of British Columbia, Vancouver, pp 1–273
- Seghers BH (1974a) Schooling behavior in the guppy (*Poecilia reticulata*): an evolutionary response to predation. *Evolution* 28:486–489
- Seghers BH (1974b) Geographic variation in the response of guppies (*Poecilia reticulata*) to aerial predators. *Oecologia* 14:93–98
- Snedecor GW, Cochran WG (1967) *Statistical methods*. Iowa State University Press, Ames
- Snyder BE (1978) A comparison of responses to the sexual pheromones produced by two allopatric races of the guppy, (*Poecilia reticulata*, Peters). B Sc Thesis, University of British Columbia, Vancouver, pp 1–57
- Stoner G, Breden F (1988) Phenotypic differentiation in female preference related to geographic variation in male predation risk in the Trinidad guppy (*Poecilia reticulata*). *Behav Ecol Sociobiol* 22:285–291
- West-Eberhard MJ (1983) Sexual selection, social competition, and speciation. *Q Rev Biol* 58:155–183