

## Intra-sexual preferences for familiar fish in male guppies

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Male guppies *Poecilia reticulata* were observed to develop shoaling preferences for familiar males over a 12 day period, at which time they showed a clear preference for familiar over non-familiar males.

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Animal groups represent non-random assemblages of individuals, frequently assorted by species, body length, parasite load and familiarity (Krause & Ruxton, 2002). The importance of familiarity in structuring social interactions has received much attention in recent years (Griffiths, 2003). Associations between familiar individuals can be beneficial, for example by maximizing antipredator responses through greater shoal cohesion and co-ordinated antipredator behaviour (Chivers *et al.*, 1995), reducing aggression, and increasing food intake, possibly due to reduced competition (Utne-Palm & Hart, 2000). Furthermore, repeated interactions between familiar individuals are thought to be essential for the evolution of reciprocal altruism (Dugatkin, 1997).

The guppy *Poecilia reticulata* Peters has been a key model species in investigating the evolution of group living (Seghers, 1974), including the role of familiarity in structuring social interactions (Griffiths & Magurran, 1997*a, b*, 1998). The guppy is a viviparous fish with a non-territorial and non-resource-based mating system (Liley & Seghers, 1975). Previous investigations of familiarity in guppies have concentrated on females, observing preferences for familiar individuals under both laboratory (Griffiths & Magurran, 1997*a*) and field conditions (Griffiths & Magurran, 1998). Males in contrast are thought to trade-off the antipredator benefits of shoaling against increased access to females, spending less time shoaling and moving between shoals more frequently than females (Croft *et al.*, 2003).

The advantages of associating with familiar conspecifics of the same sex, however, may not be restricted to females. Male guppies have the ability to recognize familiar individuals in the context of mate choice, preferring to mate

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with novel females (Kelley *et al.*, 1999). This preference is reinforced by females preferring to mate with novel males (Hughes *et al.*, 1999). A social preference for familiar individuals, however, has not previously been documented between male guppies. For example, in an investigation of wild caught guppy shoals, Griffiths & Magurran (1998) found that male guppies showed no preference for associating with members of their own shoal (defined as being familiar), whereas females did. To investigate if male guppies are capable of distinguishing between familiar males and to quantify the importance of familiarity in structuring male–male associations, the methodology described by Griffiths & Magurran (1997a) was adapted. Griffiths & Magurran (1997a) previously used the methodology to investigate the development of familiarity in female guppies thus making the current results on males directly comparable with the opposite sex. Furthermore, the duration of time taken for familiarity to develop in male guppies was quantified.

Initially 100 males were collected from 15 pools in the Arima River in the northern mountain range of Trinidad during May 2002. The fish were held together in a holding pool (outdoor concrete pool:  $196 \times 265$  cm at a water depth of 8 cm and temperature of  $27^\circ\text{C}$ ) for a period of 48 h after capture to allow complete mixing of fish. Thirty-six males of matching body length (total length,  $L_T$ , mean  $\pm$  S.E. =  $21.4 \pm 0.9$  mm) were removed from the holding pool and assigned to six groups at random (each group containing six males). Each group was housed in its own aquarium ( $15 \times 15 \times 30$  cm, water depth = 10 cm and temperature =  $25^\circ\text{C}$ ). Fish within groups were designated as familiar and between groups as unfamiliar. The fish were kept on a photoperiod of 12L : 12D and fed twice per day on 'Champion'<sup>TM</sup> (Champion Pet Foods, Exton, PA, U.S.A.) dry fish flakes.

It has been shown that familiarity takes 12 days to develop in female guppies (Griffiths & Magurran, 1997a). Accordingly, the current experiment ran for a period of 12 days and tested guppies for shoaling preferences based on familiarity on days 1, 8 and 12. To assess shoaling preferences, a binary choice test was used whereby individual test fish were given a choice between shoaling with two stimulus shoals, one composed of four familiar fish and the second composed of four unfamiliar fish. Stimulus fish were presented to the test fish in two transparent cylinders (diameter: 7 cm) positioned 12 cm from either end of the experimental aquarium ( $70 \times 29.5 \times 30$  cm, water depth = 10 cm, temperature =  $25^\circ\text{C}$ ). The stimulus cylinders were perforated to allow visual and olfactory cues to be exchanged. The allocation of the stimulus fish to the cylinders was randomized to control for side preferences by test fish. Test fish were released into the experimental aquarium *via* a release cylinder (diameter: 5 cm) located in the centre of the test tank. The test fish remained in the release cylinder for a settling-in time of 15 min after which time the cylinder was raised using a remote pulley mechanism to release the test fish. On release the observer recorded the total time spent by the test fish within defined association zones (8 cm perimeter) of the preferred and non-preferred stimulus fish over a 10 min period. The distance used to define the association zone represents about four body lengths, and is within the interindividual distance of fish within the same shoal (Pitcher *et al.*, 1983). Two different fish from each group were tested per day and the order that groups were tested in was randomized.

A control experiment was conducted following the procedure described above whereby test fish were given a choice between associating with familiar and unfamiliar fish only after 12 days of association. This was done to control for the effect of repeated exposure to the experimental treatment on the development of familiarity.

A repeated measures ANOVA was used to analyse the development of familiarity in the male groups, whereby familiarity and group were included as between subject factors, and day of testing the within subject factor. The percentage time spent shoaling was compared to 50% of the total time spent shoaling, *i.e.* the expected time assuming no preference. All percentages were arcsine-transformed prior to analysis (Sokal & Rohlf, 1995). Since there was no significant difference in the percentage time spent shoaling by the two test males from each group (one-way ANOVA, day 1: d.f. = 1 and 10,  $P=0.96$ ; day 8: d.f. = 1 and 10,  $P=0.12$ ; day 12: d.f. = 1 and 10,  $P=0.18$ ), data from both males from each group were included in the analysis.

Overall familiarity was found to be a significant factor (Table I) with males preferring to shoal with familiar individuals. A significant interaction was found between familiarity and day (Table I) with the preference for familiar fish increasing with time [Fig. 1(a)].

Following Griffiths & Magurran (1997a) the control experiment was conducted on day 12, as this appeared to be the critical point for the development of familiarity [Fig. 1(a)]. Once again no significant difference was found in the percentage time spent shoaling by the two test males from each group (one-way ANOVA, d.f. = 1 and 10,  $P=0.52$ ), thus data from both males were included in the analysis. A significant preference for shoaling with familiar fish was observed in the control group on day 12 [one-way ANOVA, d.f. = 1 and 10,  $P<0.01$ ; Fig. 1(b)]. No significant difference was observed between the experiment (day 12) and control in the mean percentage time spent shoaling with familiar fish (independent *t*-test,  $n=12$ ,  $P>0.05$ ).

TABLE I. Repeated measures ANOVA of guppy shoaling behaviour. Factors included into the model were familiarity, day of testing and group. Shoaling behaviour was measured as the percentage of time spent with stimulus fish in the choice tests

| Source                  | d.f. | Mean square | <i>F</i> | <i>P</i> |
|-------------------------|------|-------------|----------|----------|
| Within subject effects  |      |             |          |          |
| Day                     | 2    | 672.07      | 3.57     | 0.04     |
| Day × familiarity       | 2    | 672.07      | 3.57     | 0.04     |
| Day × group             | 10   | 144.43      | 0.77     | 0.67     |
| Error                   | 24   |             |          |          |
| Between subject effects |      |             |          |          |
| Familiarity             | 1    | 1379.64     | 24.04    | <0.01    |
| Group                   | 5    | 140.28      | 2.44     | 0.88     |
| Familiarity × group     | 5    | 140.28      | 2.44     | 0.17     |
| Error                   | 12   | 57.48       |          |          |

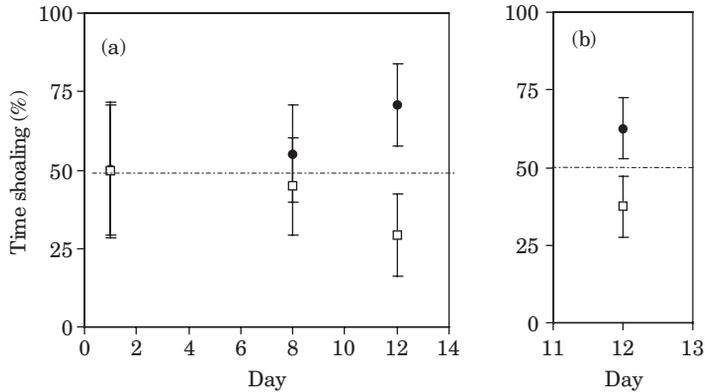


FIG. 1. The mean  $\pm$  s.d. percentage time male guppies spent shoaling with familiar (●) and unfamiliar (□) conspecifics. (a) When the choice was presented repeatedly on days 1, 8 and 12 over a 12 day period. (b) When the choice was only presented on day 12, thus not exposing the fish to the trial conditions on the other days. ---, the expected time spent shoaling assuming no preference (50%).

Male guppies developed preferences for familiar over non-familiar individuals after 12 days of association. The current findings are in accordance with previous observations by Griffiths & Magurran (1997a) who found that familiarity took 12 days to acquire in female guppies. The results of the control experiment illustrate that the acquisition of familiarity after 12 days was not a consequence of repeated exposure to the experimental procedure. The current results, however, are in contrast to previous work by Griffiths & Magurran (1998) who reported that male guppies captured in free-ranging shoals in the Tacarigua River (northern mountain range of Trinidad) showed no preference for males from a shoal they had been captured in over males from other shoals. Given the highly dynamic nature of guppy shoaling behaviour whereby both males and females (but particularly males; Croft *et al.*, 2003) exchange between shoals on a short time scale (s or min), the above experiment by Griffiths & Magurran (1998) may not have provided males with sufficient time for acquiring familiarity. Further work investigating the context in which preferences for familiar fish are important in the wild will be most interesting. For example, familiar recognition may be constrained by population size (Griffiths & Magurran, 1997b) and furthermore, the potential effects of other factors on familiarity such as environmental complexity remain largely unknown (Griffiths, 2003). Future field work examining pair-wise interactions across shoals within a population, by individually marking fish and recording their social interactions (*e.g.* Ward *et al.*, 2002) may help resolve the importance of familiarity in structuring male–male interactions in the wild.

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