

Multiple paternity and kin recognition mechanisms in a guppy population

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Abstract

Help directed toward kin (nepotism) is an important example of social behaviour. Such helping behaviour requires a mechanism to distinguish kin from nonkin. The prevailing kin recognition hypothesis is that when familiarity is a reliable cue of relatedness, other mechanisms of recognition will not evolve. However, when familiarity is an unreliable cue of relatedness, kin recognition by phenotype matching is instead predicted to evolve. Here we use genetic markers to show that guppies (*Poecilia reticulata*) from a population in a tributary of the Paria River in Trinidad are characterized by a high degree of multiple mating with 95% of broods having more than one sire and some dams having offspring sired by six males. These levels of multiple mating are the highest reported among live-bearing fishes. The mean relatedness of brood-mates was 0.36 (as compared to 0.5 for full-siblings). Therefore, familiarity does not seem to be a reliable mechanism to assess full-sibling relatedness. Using two-choice behavioural trials, we found that juveniles from this population use both phenotype matching and familiarity to distinguish kin from nonkin. However, we did not find strong evidence that the guppies use these mechanisms to form shoals of related individuals as adults, which is similar to results from other guppy populations in Trinidad. The use of both familiarity and phenotype matching is discussed in the context of the Paria River guppy population's mating system and ecology. Overall, these data provide support for the kin recognition hypothesis and increase our understanding of the evolution of kin recognition systems.

Keywords: altruism, guppy, kin recognition, multiple paternity, phenotype matching, social behaviour

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Introduction

The evolution of altruistic behaviour has intrigued biologists for decades, and in many species such behaviour has evolved through kin selection (Hamilton 1964; Wilson 1975; Trivers 1985). However, the discovery of widespread promiscuity in animal mating systems has challenged the evolutionary importance of kin selection (Burke *et al.* 1989; Jennions & Petrie 2000). This challenge would be resolved if promiscuous species evolved a kin recognition mechanism to discriminate between kin and nonkin. Two mechanisms of direct kin recognition are familiarity and phenotype matching (Holmes & Sherman 1982; Sherman *et al.* 1997; Hauber & Sherman 2001). When kin recognition by familiarity is used, individuals learn phenotypic cues of

conspecifics encountered during early development and 'remember' these specific individuals as kin. When kin recognition by phenotype matching is used, individuals instead learn the phenotypic cues of their rearing associates (or its own cues) and use these cues to form a 'kin template.' Individuals later compare phenotypic cues of putative kin to the template and, based on the similarity of the cue to the template, determine the degree of relatedness of the individual (Holmes & Sherman 1982). So, in phenotype matching, specific individuals are not remembered as kin. Self-referencing is the special case of phenotype matching where individuals use their own cues to form their kin template. The prevailing kin recognition hypothesis is that phenotype matching should evolve only when familiarity is an unreliable cue of genetic relatedness, as can be the case in promiscuous mating systems (Holmes & Sherman 1982; Sherman *et al.* 1997; Hauber & Sherman 2001). Here we genetically dissect mating patterns in a

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population of the guppy (*Poecilia reticulata*) characterized by low predation. We then detail the kin recognition mechanisms used by individuals within the population. We compare our data to results from a previous study of a population characterized by high predation to test for the predicted association between promiscuity and kin recognition by phenotype matching.

The guppy is a live-bearing fish with internal fertilization and a nonresource-based promiscuous mating system (Houde 1997; Magurran 2005). Northern Trinidad, where the guppy is most commonly studied, is a mountainous region with waterfalls that restrict movement of aquatic organisms from downstream to upstream populations. Waterfalls not only exclude larger guppy predators from upstream locales, but also restrict gene flow from downstream to upstream populations, which has resulted in genetic differentiation and variation in guppy behaviour within short geographical distances (Reznick & Endler 1982; Crispo *et al.* 2005). The level of predation risk, evaluated on the basis of what predators are present, is one ecological variable used to explain variation in guppy characters, including life history (Reznick & Endler 1982) and mate choice (Breden & Stoner 1987). Guppies from both high predation and low predation populations tend to have strong site fidelity (Reznick *et al.* 1996; Croft *et al.* 2003), but in low predation populations, mortality is as much as 2.5 times lower than in high predation populations, which greatly increases the likelihood of overlapping generations in low predation populations vs. high predation populations (Reznick *et al.* 1996). Consequently, in populations with low predation, the probability of meeting a sibling from a different brood is high and phenotype matching should be a more reliable mechanism of kin recognition than familiarity. Conversely, in populations with high predation, the probability of meeting a sibling from a different brood is low, and, barring multiple mating, familiarity should be a reliable mechanism of kin recognition (Holmes 1986). Indeed, a study on the Lower Tacarigua River, which is characterized by high predation and low multiple mating (Reznick *et al.* 1996; Evans & Magurran 2001) showed that guppies use familiarity and not phenotype matching to recognize kin (Griffiths & Magurran 1999).

In contrast, tributaries of the Paria River are characterized by low predation risk to guppies (Reznick *et al.* 1996) and a laboratory study of descendents from the Paria River showed that broods are sired by multiple males, with the most successful male siring only about two-thirds of the brood (Pitcher *et al.* 2003; also see Kelly *et al.* 1999). In this case, familiarity reliably indicates a level of kinship of only half-siblings ($R = 0.25$), but it does not provide a reliable indicator of the actual level of relatedness (i.e. a brood-mate could be either 0.25 or 0.5 related). In addition, because individuals in tributaries of the Paria River typically are found in small pools, it is likely that individuals will come

into contact with unfamiliar paternal half-siblings and possibly unfamiliar half- or full-siblings from their mothers' other broods. Thus, phenotype matching would be required to distinguish between full- and half-sibling brood-mates and to distinguish between unfamiliar kin and unrelated individuals.

Here we test for kin recognition by phenotype matching and familiarity mechanisms in a population from a tributary of the Paria River. First, we determine the level of multiple mating within a natural population from the Paria River tributary using microsatellite paternity analysis to assess the mean relatedness of brood-mates, and hence the accuracy of kin recognition by phenotype matching rather than recognition by familiarity. Second, we perform two-choice behavioural experiments to observe the preference of juvenile guppies for potential shoaling partners of different levels of either relatedness or familiarity. Our expectation was that these guppies would be able to use phenotype matching to recognize kin. Finally, we test one application of kin recognition in the wild, that is, if adult shoals are structured on the basis of kinship. We did this test in part to compare to a previous study that found the relatedness within shoals was not significantly different from relatedness within the entire population for two Trinidadian rivers where guppies are subject to high predation (Russell *et al.* 2004).

Materials and methods

Multiple mating and paternity assignment

In May 2005, gravid female guppies were collected from a tributary of the Paria River in northern Trinidad and isolated in individual tanks until they gave birth. Newborn guppies and females were then euthanized. Fin clips from females and entire body tissue of juveniles were preserved in 95% ethanol within 24 h of parturition.

The parentage of each juvenile was determined using microsatellite DNA analysis at three loci (*Pre1*, *Pre13*, *Pre15*; allele frequencies are shown in Fig. 1 and locus heterozygosities are shown in Table 1; primer sequences are published in Paterson *et al.* 2005). First, DNA was isolated from the females as well as the juveniles using a proteinase K digestion (Neff *et al.* 2000). We then used a Whatman-Biometra T1 Thermocycler to amplify the microsatellites with the following programme: 60 s at 92 °C; 15 cycles of 30 s at 92 °C, 30 s at 60 °C, and 30 s at 72 °C; and 34 cycles of 30 s at 92 °C, 30 s at 55 °C and 30 s at 72 °C. Each 10 µL polymerase chain reaction (PCR) contained ~75 ng of total DNA, 3 mM MgCl₂, 1× PCR buffer (Fisher), 0.25 mM of each deoxynucleotide (Fisher), 0.25 U *Taq* DNA polymerase (Fisher) and 0.25 µM of each forward and reverse primer (Invitrogen Life Technologies), and the forward primer was fluorescently labelled (Beckman Coulter). PCR products

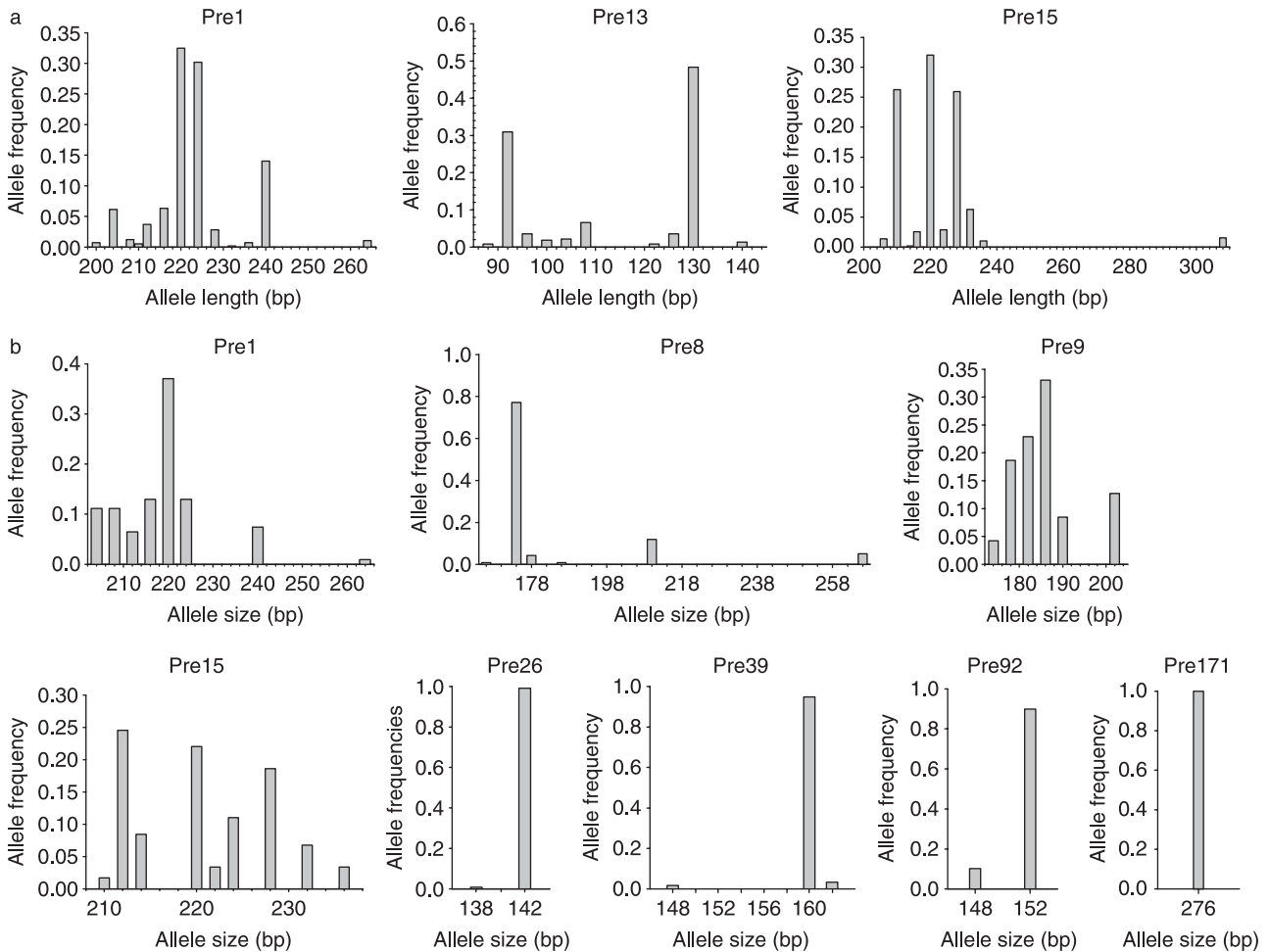


Fig. 1 Allele frequency distributions for (a) three microsatellite loci used in the multiple mating and paternity assignment analysis, and (b) eight microsatellite loci used in the adult relatedness in shoals analysis.

Table 1 Summary statistics for 10 microsatellite loci in parentage and adult shoal structure analyses, including number of individuals scored (*n*), number of alleles, observed heterozygosity (H_O), and expected heterozygosity (H_E)

Locus	Analysis	<i>n</i>	No. of alleles	H_O	H_E
Pre1	Parentage	282	13	0.45*	0.77
	Shoal structure	56	8	0.32*	0.78
Pre8	Shoal structure	59	6	0.27	0.26
Pre9	Shoal structure	59	6	0.80	0.83
Pre13	Parentage	294	10	0.53	0.67
Pre15	Parentage	295	10	0.81	0.76
	Shoal structure	59	9	0.85	0.84
Pre26	Shoal structure	59	2	0.02	0.02
Pre39	Shoal structure	59	3	0.10	0.10
Pre92	Shoal structure	59	2	0.20	0.18
Pre171	Shoal structure	59	1	0.00	0.00

*denotes a significant deviation from Hardy–Weinberg equilibrium ($P < 0.001$; Raymond & Rousset 1995).

were run following standard protocol for the CEQ 8000 Genetic Analysis System (Beckman Coulter). Offspring were assigned to parents using COLONY version 1.2, a parentage assignment program that reconstructs putative sires based on a maximum-likelihood method (Wang 2004). For this analysis, we set the typing error rate at the suggested 0.025 (Wang 2004). Average relatedness was then determined for each brood by determining the mean of all pairwise relatedness comparisons within a brood.

Juvenile behavioural trials and kin recognition

Guppies used in the behavioural trials were descendants of individuals caught in a tributary of the Paria River. For the duration of the study, guppies were kept in tanks containing a bottom layer of neutral-colour gravel with water temperature maintained at 24–26 °C and on a 12 h:12 h light–dark cycle to simulate natural tropical conditions (Houde 1997). A single male was mated to either one or

two (a 'mating triad') virgin females over the course of 7 days. Guppies within a brood born from the same mother were thus full-siblings, and guppies born from the other mother within a mating triad were paternal half-siblings.

Newborn guppies were isolated within 24 h of birth and were reared in visual and chemical isolation until they were large enough to be marked by tail clipping (mean = 33.6 days, range = 22–48 days). After the isolation period, six or eight guppies – three or four from each of two kin groups – were anaesthetized (using 15 mg MS-222 in 50 mL water) and marked according to kin group by cutting and removing either the top third or bottom third of the guppy's caudal fin. Following Griffiths & Magurran (1997), the fish were then combined into a single rearing tank for 12–15 days before the trials began. Because guppies have been shown to shoal preferentially with tank-mates after a period of 12 days (Griffiths & Magurran 1997), guppies in these rearing tanks were assumed to be familiar with one another. Thus, each rearing tank contained familiar full-siblings and familiar half-siblings or familiar nonkin.

Four types of dichotomous choice trials were performed: full-sibling vs. unrelated, full-sibling vs. half-sibling, half-sibling vs. unrelated (where both choices were either familiar or unfamiliar), and familiar vs. unfamiliar (where both choices were either full-siblings or unrelated). In a trial, a focal fish was presented with pairs of 'stimulus' guppies on either side of a test tank differing either in the level of relatedness or familiarity (but not both), and given the choice of associating with either group. Each focal fish was also used as 'stimulus' fish in either one or two trials.

The test arena was a tank (19 cm × 34 cm × 20 cm, with water depth of 15 cm) divided into three compartments by two transparent, porous plastic sheets that allowed visual and chemical communication between compartments (Griffiths & Magurran 1999). The centre compartment, which contained the focal fish, was 18 cm in length, and was further divided into two peripheral 'association zones' 5 cm in length, and a centre 'neutral zone' of 8 cm. The outer compartments were 8 cm in length, and housed a pair of guppies corresponding to one treatment of relatedness or familiarity.

A focal fish was allowed 15–30 min to settle and explore the arena before the trial began. Each trial lasted 15 min. Both the number of times that the focal fish switched from associating with one group to the other group, and the time spent within each association zone were recorded. During a trial, if the focal fish was entirely within the association zone, or had its gill slits within this region with its head orientated towards the barrier, it was said to be associating with the fish on that side. If the focal fish did not associate with both groups, or if any of the fish in the test arena displayed courtship behaviour (e.g. a characteristic sigmoid display) during the course of the trial, the trial was discarded from further analysis. Guppies are considered juveniles

until about age 70 days when, for example, males start producing sperm (Evans *et al.* 2002). Thus, because all our guppies were younger than 70 days of age, we considered them a priori to be juveniles. Fish were used only once as a focal fish. Water changes were performed in the test tank between trials to remove any olfactory cues from previous trials.

We compared the percentage of time spent associating with the familiar (or more related) stimulus to the percentage of time spent with the unfamiliar (or less related) stimulus using Wilcoxon signed-rank tests. This nonparametric test was used because the data were not normally distributed. The significance level was set at $\alpha = 0.05$ and tests were performed using *srss* (version 14.0).

Adult relatedness in shoals

In December 2006, we captured entire shoals using seine nets from two pools within the tributary of the Paria River. The two pools were connected by a 3 m stream. There were no guppies caught by seine net or otherwise observed within 25 m upstream or downstream of the pools. Fish were euthanized by an overdose of clove oil and preserved in 95% ethanol for subsequent genetic analysis.

The genotypes of each guppy caught were determined at eight microsatellite loci (*Pre1*, *Pre8*, *Pre9*, *Pre15*, *Pre26*, *Pre39*, *Pre92*, *Pre171*; allele frequencies are shown in Fig. 1; primers are published in Becher *et al.* 2002 and Paterson *et al.* 2005). We followed the DNA extraction and PCR protocol described above but with minor modification: an initial step of 94 °C for 3 min, and then 35 cycles of 30 s at 94 °C, 30 s at 53 °C (for *Pre39* and *Pre171*; 56 °C for *Pre92*, 60 °C for *Pre1*, 62 °C for *Pre15* and *Pre28*; and 65 °C for *Pre8* and *Pre9*), 30 s at 72 °C and a final elongation at 72 °C for 7 min. This PCR protocol provided equivalent or better amplification than the previous protocol. PCR product was run following the standard protocol for the CEQ 8000 Genetic Analysis System. Relatedness values among individuals were calculated using *ML-RELATE* (Kalinowski *et al.* 2006). To test the hypothesis that guppy shoals are composed of relatives, for each shoal we determined the mean relatedness of pairs within the shoal (within-shoal relatedness) and compared this to the mean relatedness of pairs where one individual is in the shoal and one individual is outside the shoal (outside-shoal relatedness) using a paired *t*-test.

Results

Multiple mating and paternity assignment

We genotyped 23 broods from females that were inseminated in natural populations, of which parentage was successfully assigned to 22 broods. For one brood, the COLONY program

Table 2 Multiple mating for 22 broods of guppies from a tributary of the Paria River in Trinidad. Brood size (*n*), mean relatedness of brood-mates (*R*), and the percentage of the brood sired by up to six putative males as calculated by COLONY version 1.2 (Wang 2004) are provided

Family	Brood size (<i>n</i>)	Relatedness (<i>R</i>)	Sire 1 (%)	Sire 2 (%)	Sire 3 (%)	Sire 4 (%)	Sire 5 (%)	Sire 6 (%)
1	8	0.44	88	12				
2	32	0.30	31	28	19	12	6	3
3	20	0.36	55	40	5			
4	15	0.37	67	27	7			
5	13	0.29	31	31	15	15	8	
6	10	0.38	70	30				
7	11	0.34	45	45	9			
8	7	0.36	57	43				
9	9	0.30	33	33	22	11		
10	8	0.44	88	12				
11	4	0.38	75	25				
12	3	0.50	100					
13	11	0.30	36	27	27	9		
14	17	0.32	41	35	18	6		
15	7	0.38	71	29				
16	6	0.37	67	33				
17	12	0.29	33	25	16	16	8	
18	11	0.37	64	36				
19	8	0.37	62	38				
20	5	0.40	80	20				
21	7	0.32	43	43	14			
22	40	0.31	40	22	12	10	10	5

Note: The percentage of the brood fertilized by each sire may not sum to 100% due to rounding error.

failed to resolve paternity presumably because there were multiple, equally probable solutions. The average brood size for the 23 broods was 12.9 individuals (range = 3–40). We detected an average of 3 sires per brood (range = 1–6, $n = 22$). The average relatedness within a brood was 0.36 (range = 0.29–0.5; Table 2). Brood size and number of sires were positively correlated ($r = 0.79$, $n = 22$, $P < 0.001$). Similar results were found when the locus that deviated from expected Hardy–Weinberg equilibrium proportions was omitted (data not shown; see Table 1 for Hardy–Weinberg results).

Juvenile behavioural trials and kin recognition

A total of 144 recognition trials were conducted to distinguish between the preference for familiar or related fish as shoal-mates. Although all fish used in the trials appeared to be immature based on colouration (Evans *et al.* 2002), courtship behaviour occurred in 13 trials, and these were discarded from further analysis. An additional seven trials were discarded because the focal fish had not sampled both pairs (i.e. did not cross the centre line), and five trials were discarded because a stimulus fish escaped from its side of the tank. Fifty-nine trials tested the preference for full-siblings vs. unrelated individuals, 17 tested the preference for full-siblings vs. half-siblings, 13 tested the

preference for half-siblings vs. unrelated individuals, and 30 tested the preference for familiar vs. unfamiliar individuals.

Focal fish had no preference for full-siblings over unrelated individuals when the focal fish was familiar with both stimulus groups (mean \pm SE full-siblings = $53.4 \pm 5.6\%$; unrelated = $46.6 \pm 5.6\%$; $Z = 0.57$, $n = 22$, $P = 0.57$). However, when both stimulus groups were unfamiliar with the focal fish, the focal fish had a significant preference for full-siblings vs. unrelated individuals as shoaling partners (mean \pm SE full-siblings = $58.8 \pm 4.2\%$; unrelated = $41.2 \pm 4.2\%$; $Z = 2.02$, $n = 37$, $P = 0.043$). This preference for full-siblings remained significant when the full-sibling vs. unrelated tests were pooled ($Z = 2.02$, $n = 59$, $P = 0.043$; Fig. 2). There was a trend for focal fish to prefer familiar fish to unfamiliar fish as shoaling partners when both stimulus groups were full-siblings of the focal fish (mean \pm SE familiar = $60.0 \pm 7.5\%$; unrelated = $40.0 \pm 7.5\%$; $Z = 1.48$, $n = 14$, $P = 0.14$), and when both stimulus groups were both unrelated to the focal fish (mean \pm SE familiar = $59.7 \pm 6.1\%$; unrelated = $40.3 \pm 6.1\%$; $Z = 1.40$, $n = 16$, $P = 0.16$). This preference for familiar fish as shoaling partners was significant when the data were pooled and both stimulus groups were either full-siblings or unrelated to the focal fish ($Z = 1.96$, $n = 30$, $P = 0.049$; Fig. 2). Focal fish also preferred full-siblings over half-siblings (12 out of 17 trials; $Z = 0.781$, $n = 17$, $P = 0.44$) and half-siblings over unrelated individuals (nine out of 13

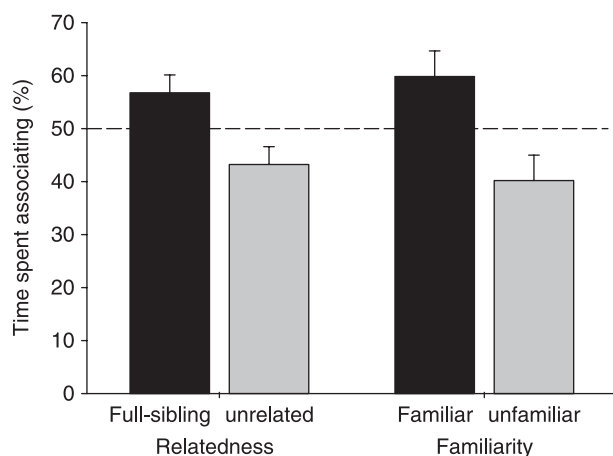


Fig. 2 Relative association times \pm SE of juvenile guppies from a tributary in the Paria River in Trinidad for full-siblings over unrelated individuals ($n = 59$), or familiar individuals over unfamiliar individuals ($n = 30$). The dashed line represents the expectation of 50% association time with either stimulus group. Association time was calculated as time spent associating with one stimulus shoal as a percentage of the total time spent associating with either stimulus shoal in a 15-min trial.

trials; $Z = 1.503$, $n = 13$, $P = 0.13$; Fig. 3), but neither of these results were statistically significant. However, these two results were statistically significant when the data were combined with a binomial test (21 out of 30 trials: $P = 0.021$), which suggests that guppies from this population can distinguish between individuals differing in relatedness of 0.25.

There was no correlation between the age difference of the two stimulus groups and the percentage of time spent associating with the more related group (mean of age difference = 2.9 days; range = 0–8 days; $r = 0.03$, $n = 89$, $P = 0.79$). Thus, the preferences by focal fish for kin or familiar individuals could not be explained by matching for age (or body size inasmuch as size and age are correlated; Grether *et al.* 2001). We also tested for an effect of sex bias in the stimulus groups on association time in both full-sibling vs. unrelated and familiar vs. unfamiliar trials. Neither males nor females showed discrimination between groups on the basis of how many more males there were in one stimulus group than in the other group ($P > 0.2$ for all). There was no correlation between the age of the focal fish and the time spent associating with groups that had more males in either sex ($P > 0.2$ for all).

Adult relatedness in shoals

Across the two pools, we caught 59 adults from 16 natural shoals (mean shoal size = 3.7; range = 1–9). There was no difference in the mean relatedness of fish from the two pools ($t = 0.919$, d.f. = 764, $P = 0.36$). The overall relatedness for the population, calculated as the mean of all pairwise

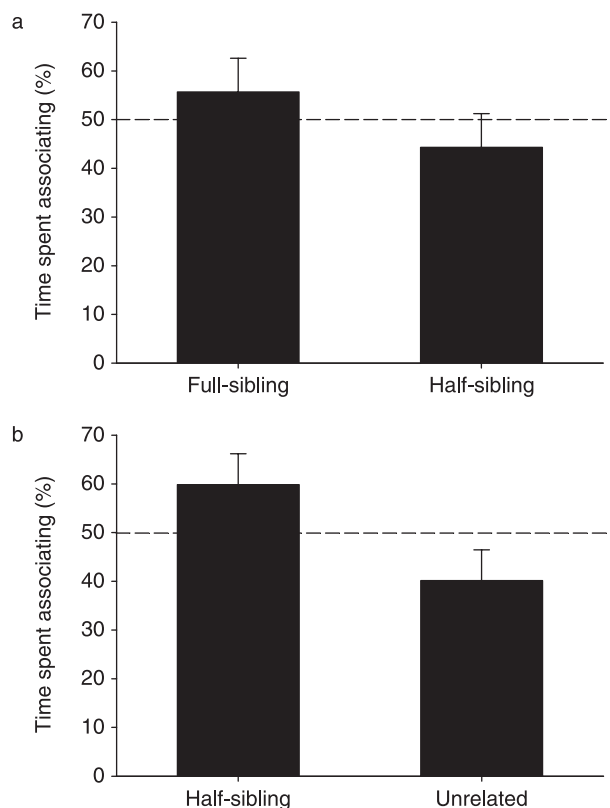


Fig. 3 Relative association times \pm SE of juvenile guppies from a tributary in the Paria River in Trinidad for (a) full-siblings over half-siblings ($n = 17$), or (b) half-siblings over unrelated individuals ($n = 13$). The dashed line represents the expectation of 50% association time with either stimulus group. Association time was calculated as time spent associating with one stimulus shoal as a percentage of the total time spent associating with either stimulus shoal in a 15-min trial.

relatedness values, was 0.099 (see Table S1, Supplementary material). Sixteen per cent of all pairs in the population were more closely related than half-siblings ($R = 0.25$). There was no significant difference between the mean within-shoal relatedness ($R = 0.090$) and the mean outside-shoal relatedness ($R = 0.093$) ($t = 0.10$, d.f. = 10, $P = 0.93$).

Discussion

We used microsatellite markers to find that 95% of broods collected from a tributary in the Paria River (Trinidad) were sired by more than one male and 50% of broods were sired by more than two males. Within the family Poeciliidae, this level of multiple paternity is high (Soucy & Travis 2003; Luo *et al.* 2005). Previous studies of poeciliids have found that the percentage of multiply mated females ranges from 23% in *Poeciliopsis monacha* (Leslie & Vrijenhoek 1977) to 90% in *Gambusia holbrooki* (Zane *et al.* 1999). Furthermore, an average of 3 sires per brood is also

the highest yet reported among poeciliids (reviewed in Soucy & Travis 2003).

Our paternity data indicate that multiple mating among guppies within tributaries of the Paria River is much higher than has been previously reported. Kelly *et al.* (1999) reported that only approximately 20% of Paria broods had more than one sire. This discrepancy may be explained in three ways. First, Kelly and colleagues used a conservative approach of counting unique paternal alleles to detect multiple mating. For example, two sires were detected only if there were either three or four paternal alleles in a brood. We used a more sophisticated and powerful program called COLONY that reconstructs putative sires based on a maximum-likelihood method (Wang 2004). This program could infer a multiply sired brood when only two unique paternal alleles were observed in a brood when, for example, the two alleles deviated significantly from the expected Mendelian inheritance ratio of 1:1. The program was not available to Kelly and colleagues. Second, the broods examined by Kelly and colleagues were smaller than the broods examined here (their mean brood size was 7 whereas our mean brood size was 12.9). We found a significant positive correlation between brood size and the number of sires detected. Thus, assays of small broods may have missed additional sires that would be detected in large broods. Third, Kelly and colleagues used a single microsatellite locus with relatively low variation (four alleles) to detect multiple paternity. Here we used three loci with greater variation (10–13 alleles per locus), which increases the likelihood of detecting multiple sires. Indeed, using the paternal allele counting method of Kelly and colleagues, the probability of detecting a multiply mated brood in their analysis was estimated to be 0.363 and in our analysis it was 0.987 (see Neff & Pitcher 2002).

Our study was able to detail the recognition mechanisms used by guppies from a tributary of the Paria River. Based on our paternity data of natural broods, we determined that the average relatedness within a brood was $R = 0.36$. If broods continue to associate post-parturition, this level of relatedness may be sufficiently high for familiarity to be a reliable method of distinguishing kin from nonkin. Indeed, we have shown that juvenile Paria guppies choose shoaling partners based in part on familiarity. However, because guppies in tributaries of the Paria River typically are found in small pools, predation rates are low, adult sex ratio is female-biased (1.7:1; authors' unpublished data; also see Rodd & Reznick 1997) and each female mates with an average of 3 males (this study), it is likely that individuals will come into contact with unfamiliar paternal half-siblings as well as unfamiliar half- or full-siblings from their mothers' previous or subsequent broods. Thus, phenotype matching is expected to evolve as a kin recognition mechanism (Holmes & Sherman 1982; Sherman *et al.* 1997; Hauber & Sherman 2001). Consistent with this hypothesis,

we also found that juvenile Paria guppies preferred to associate with related over unrelated individuals, independent of their level of familiarity. Thus, our data show that these guppies are able to use both familiarity and phenotype matching recognition mechanisms.

The use of both familiarity and phenotype matching as kin recognition mechanisms in Paria guppies is perhaps surprising. Individuals clearly require phenotype matching to distinguish between unfamiliar siblings and unrelated individuals or between familiar full- and half-siblings. It is less clear why they would also use familiarity. Familiarity may be used because it is more reliable (i.e. less error prone) or because it is cognitively 'cheaper' to utilize than phenotype matching. To our knowledge, there are no data available on the sophistication of the neurology needed to perform either mechanism. Furthermore, shoaling with familiar individuals may have added benefits outside of kin selection such as reciprocal altruism (Wilkinson 1984; Trivers 1985). Guppies have been shown to have stable social networks (Croft *et al.* 2004), which facilitates the development of reciprocal altruism (Milinski 1987). Reciprocal altruism may be important in the context of foraging and predator inspection (Croft *et al.* 2006).

It is unlikely that Paria guppies were using familiarity developed *in utero* and not phenotype matching in some of our trials. First, the preference of guppies for paternal half-siblings over unrelated individuals could not be explained by familiarity because all individuals were unfamiliar (i.e. gestated separately). Second, Griffiths & Magurran (1997) have previously shown in another population of guppies that familiarity with shoal-mates develops only after 12 days of association post-parturition. The guppies used in our experiment were separated within 24 h of birth. Furthermore, a kin template formed based on the phenotypes of brood-mates is likely to provide a heterogeneous signal (e.g. mean relatedness ranged from 0.29 to 0.5 across broods; see Table 2) and is unlikely to allow discrimination between individuals of differing relatedness. Thus, phenotype matching, and specifically self-referencing, is the most likely mechanism to explain some of the patterns of discrimination observed in our study.

Our study was not designed to test for a particular cue used in kin recognition by phenotype matching. However, the relationship between odour phenotype and genotype at loci associated with the major histocompatibility complex (MHC) makes MHC a strong candidate for providing cues of kinship (Penn 2002; Milinski *et al.* 2005). For example, juvenile Arctic charr, *Salvelinus alpinus*, discriminate between shoal-mates based on differences in MHC (Olsén *et al.* 1998). Furthermore, MHC has been implicated as a cue of kin recognition in several other taxa, including mice (Manning *et al.* 1992) and rats (Brown *et al.* 1987). Thus, MHC may be a recognition cue involved in phenotype matching in guppies, but this remains to be explored.

Little is known about the specific benefits gained by juvenile guppies from shoaling preferentially with kin. However, it is known that in two species of salmon, *Salmo salar* and *Oncorhynchus mykiss*, juveniles in shoals of related individuals grow faster and have fewer antagonistic behaviours than do shoals of unrelated individuals (Brown & Brown 1996). Guppies may similarly benefit by shoaling with kin. However, the behavioural interactions of relatives in shoals, and the specific benefit to shoaling with kin, such as increased growth rate, has yet to be investigated in the guppy.

Although the Paria guppies we studied can recognize kin, we found no evidence of kin structure in adult shoals. This is perhaps surprising given that we found that a large number of close relatives are present within the population; for example, 16% of pairs were more related than half-siblings (see Table S2, Supplementary material). The absence of kin structure in adult shoals may in part be explained by reduced shoaling behaviour by adults. Shoaling is a common defence against fish predation (Pitcher & Parrish 1993) and adults in the Paria River population are subjected to low predation. Our results are consistent with other studies from the Quare River and Lower Tacarigua River populations (Russell *et al.* 2004). It is possible that adult guppies do not shoal with kin because they are actively seeking mates.

Finally, the contrasting results from our study and that of Griffiths & Magurran (1999) provide support for the kin recognition hypothesis put forward by Holmes & Sherman (1982). The Paria River tributary and Lower Tacarigua River differ in critical aspects of their ecology and mating system, which should lead to the evolution of different recognition mechanisms. Guppies from the Lower Tacarigua River are characterized by broods that are sired predominantly by a single male, with one male typically siring about 96% of the brood (Evans & Magurran 2001). This population also experiences high predation (Reznick *et al.* 1996). As such, unfamiliar full-siblings are unlikely to be encountered and familiarity with brood- and shoal-mates provides a reliable indicator of full-sibling relatedness. Thus, kin recognition by familiarity is expected. Conversely, the tributaries of the Paria River are characterized by a high degree of multiple mating and low predation (this study, Reznick *et al.* 1996). Thus, consistent with our results, phenotype matching is expected to evolve. Together these studies provide the first within-species support for the kin recognition hypothesis that local ecology and mating system are associated with the evolution of kin recognition mechanisms.

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References

- Becher SA, Russell ST, Magurran AE (2002) Isolation and characterization of polymorphic microsatellites in the Trinidadian guppy (*Poecilia reticulata*). *Molecular Ecology Notes*, **2**, 456–458.
- Breden F, Stoner G (1987) Male predation risk determines female preference in the Trinidad guppy. *Nature*, **329**, 831.
- Brown GE, Brown JA (1996) Does kin-biased territorial behavior increase kin-biased foraging in juvenile salmonids? *Behavioral Ecology*, **7**, 24–29.
- Brown RE, Singh PB, Roser R (1987) The major histocompatibility complex and the chemosensory recognition of individuality in rats. *Physiology and Behavior*, **40**, 65–74.
- Burke T, Davies NB, Bruford MW, Hatchwell BJ (1989) Parental care and mating behaviour of polyandrous dunnocks *Prunella modularis* related to paternity by DNA fingerprinting. *Nature*, **338**, 249–251.
- Crispo E, Bentzen P, Reznick DN, Kinnison MT, Hendry AP (2005) The relative influence of natural selection and geography on gene flow in guppies. *Molecular Ecology*, **15**, 49–62.
- Croft DP, Albanese B, Arrowsmith BJ, Botham M, Webster M, Krause J (2003) Sex-biased movement in the guppy (*Poecilia reticulata*). *Oecologia*, **137**, 62–68.
- Croft DP, Krause J, James R (2004) Social networks in the guppy (*Poecilia reticulata*). *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **271** (Supplement), 516–519.
- Croft DP, James R, Thomas POR *et al.* (2006) Social structure and co-operative interactions in a wild population of guppies (*Poecilia reticulata*). *Behavioral Ecology and Sociobiology*, **59**, 644–650.
- Evans JP, Magurran AE (2001) Patterns of sperm precedence and predictors of paternity in the Trinidadian guppy. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **268**, 719–724.
- Evans JP, Pitcher TE, Magurran AE (2002) The ontogeny of courtship, colour and sperm number in guppies. *Journal of Fish Biology*, **60**, 495–498.
- Grether GF, Millie DF, Bryant MJ, Reznick DN, Mayea W (2001) Rain forest canopy cover, resource availability, and life history evolution in guppies. *Ecology*, **82**, 1546–1559.
- Griffiths SW, Magurran AE (1997) Familiarity in schooling fish: how long does it take to acquire? *Animal Behaviour*, **53**, 945–949.
- Griffiths SW, Magurran AE (1999) Schooling decisions in guppies (*Poecilia reticulata*) are based on familiarity rather than kin recognition by phenotype matching. *Behavioral Ecology and Sociobiology*, **45**, 437–445.
- Hamilton WD (1964) The genetical evolution of social behaviour, I & II. *Journal of Theoretical Biology*, **7**, 1–52.
- Hauber ME, Sherman PW (2001) Self-referent phenotype matching: theoretical considerations and empirical evidence. *Trends in Neuroscience*, **24**, 609–616.
- Holmes WG (1986) Identification of paternal half-siblings by captive Belding's ground squirrels *Spermophilus beldingi*. *Animal Behaviour*, **34**, 321–327.
- Holmes WG, Sherman PW (1982) The ontogeny of kin recognition in two species of ground squirrels. *American Zoologist*, **22**, 491–517.

- Houde AE (1997) *Sex, Color, and Mate Choice in Guppies*. Princeton University Press, Princeton, New Jersey.
- Jennions MD, Petrie M (2000) Why do females mate multiply? A review of the genetic benefits. *Biological Reviews*, **75**, 21–64.
- Kalinowski ST, Wagner AP, Taper ML (2006) ML-RELATE: a computer program for maximum likelihood estimation of relatedness and relationship. *Molecular Ecology Notes*, **6**, 576–579.
- Kelly CD, Godin J-GJ, Wright JM (1999) Geographical variation in multiple paternity within natural populations of the guppy (*Poecilia reticulata*). *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **266**, 2403–2408.
- Leslie JF, Vrijenhoek RC (1977) Genetic analysis of natural populations of *Poeciliopsis monacha*. *Journal of Heredity*, **68**, 301–306.
- Luo J, Sanetra M, Schartl M, Meyer A (2005) Strong reproductive skew among males in the multiply mated swordtail *Xiphophorus multilineatus*. *Journal of Heredity*, **96**, 346–355.
- Magurran AE (2005) *Evolutionary Ecology: The Trinidadian Guppy*. Oxford University Press, London, UK.
- Manning CJ, Wakeland EK, Potts WK (1992) Communal nesting patterns in mice implicate MHC genes in kin recognition. *Nature*, **360**, 581–583.
- Milinski M (1987) TIT FOR TAT in sticklebacks and the evolution of co-operation. *Nature*, **325**, 433–435.
- Milinski M, Griffiths S, Wegner KM, Reusch TBH, Haas-Assenbaum A, Boehm T (2005) Mate choice decisions of stickleback females predictably modified by MHC peptide ligands. *Proceedings of the National Academy of Sciences, USA*, **102**, 4414–4418.
- Neff BD, Pitcher TE (2002) Assessing the statistical power of genetic analyses to detect multiple mating in fishes. *Journal of Fish Biology*, **61**, 739–750.
- Neff BD, Fu P, Gross MR (2000) Microsatellite multiplexing in fish. *Transactions of the American Fisheries Society*, **129**, 584–593.
- Olsén KH, Grahn M, Lohm J, Langefors Å (1998) MHC and kin discrimination in juvenile Arctic charr, *Salvelinus alpinus* (L.). *Animal Behaviour*, **56**, 319–327.
- Paterson IG, Crispo E, Kinnison MT, Hendry AP, Bentzen B (2005) Characterization of tetranucleotide microsatellite markers in guppy (*Poecilia reticulata*). *Molecular Ecology Notes*, **5**, 269–271.
- Penn DJ (2002) The scent of genetic compatibility: sexual selection and the major histocompatibility complex. *Ethology*, **108**, 1–21.
- Pitcher TE, Neff BD, Rodd FH, Rowe L (2003) Multiple mating and sequential mate choice in guppies: females trade up. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **270**, 1623–1629.
- Pitcher TJ, Parrish JK (1993) Functions of shoaling behaviour in teleosts. In: *Behaviour of Teleost Fishes* (ed. Pitcher TJ), pp. 363–440. Chapman & Hall, London, UK.
- Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.
- Reznick DN, Endler JA (1982) The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). *Evolution*, **36**, 160–177.
- Reznick DN, Butler MJ, Rodd FH, Ross P (1996) Life history evolution in guppies (*Poecilia reticulata*) 6. Differential mortality as a mechanism for natural selection. *Evolution*, **50**, 1651–1660.
- Rodd FH, Reznick DN (1997) Variation in the demography of guppy populations: the importance of predation and life histories. *Ecology*, **78**, 405–418.
- Russell ST, Kelley JL, Graves JA, Magurran AE (2004) Kin structure and shoal composition dynamics in the guppy, *Poecilia reticulata*. *Oikos*, **106**, 520–526.
- Sherman PW, Reeve HK, Pfennig DW (1997) Recognition systems. In: *Behavioural Ecology: an Evolutionary Approach* (eds Krebs JR, Davies NB), pp. 69–96. Oxford University Press, Oxford, UK.
- Soucy S, Travis J (2003) Multiple paternity and population genetic structure in natural populations of the poeciliid fish, *Heterandria formosa*. *Journal of Evolutionary Biology*, **16**, 1328–1336.
- Trivers RL (1985) *Social Evolution*. Benjamin/Cummings Publishing, Menlo Park, California.
- Wang J (2004) Sibship reconstruction from genetic data with typing errors. *Genetics*, **166**, 1963–1979.
- Wilkinson GS (1984) Reciprocal food sharing in the vampire bat *Desmodus rotundus*. *Nature*, **308**, 181–184.
- Wilson EO (1975) *Sociobiology: The New Synthesis*. Harvard University Press, Cambridge, Massachusetts.
- Zane L, Nelson WS, Jones AG, Avise JC (1999) Microsatellite assessment of multiple paternity in natural populations of a live-bearing fish, *Gambusia holbrooki*. *Journal of Evolutionary Biology*, **12**, 61–69.

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Supplementary material

The following supplementary material is available for this article:

Table S1 Pairwise relatedness values for 54 guppies from a tributary of the Paria River in Trinidad

Table S2 The number of individuals and mean pairwise relatedness for 11 guppy shoals from a tributary of the Paria River in Trinidad

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6 **Table 2.** The number of individuals in 11 guppy shoals from a tributary of the Paria River in
 7 Trinidad with their within-shoal (along diagonal, in bold) and between-shoal mean relatedness.

8

Shoal ID	Number of individuals	A	B	C	D	E	F	G	H	I	J	K
A	7	0.11										
B	5	0.10	0.06									
C	2	0.10	0.10	0.00								
D	4	0.04	0.05	0.10	0.02							
E	7	0.08	0.08	0.06	0.13	0.17						
F	2	0.19	0.17	0.07	0.04	0.07	0.00					
G	6	0.14	0.12	0.16	0.09	0.14	0.09	0.15				
H	2	0.04	0.04	0.04	0.08	0.08	0.00	0.08	0.00			
I	8	0.13	0.07	0.13	0.07	0.08	0.07	0.10	0.11	0.14		
J	9	0.12	0.09	0.03	0.05	0.12	0.17	0.10	0.04	0.11	0.08	
K	2	0.03	0.05	0.00	0.13	0.02	0.09	0.00	0.00	0.00	0.05	0.24

9

Group ID	Number of A	B	C	D	E	F	G	H	I	J	K	
A	7	0.11										
B	5	0.10	0.06									
C	2	0.11	0.10	0.00								
D	4	0.04	0.05	0.10	0.02							
E	7	0.08	0.08	0.06	0.13	0.17						
F	2	0.19	0.17	0.07	0.04	0.07	0.00					
G	6	0.14	0.12	0.16	0.09	0.14	0.09	0.15				
H	2	0.04	0.04	0.04	0.08	0.08	0.00	0.08	0.00			
I	8	0.13	0.07	0.13	0.07	0.08	0.07	0.10	0.11	0.14		
J	9	0.12	0.09	0.03	0.05	0.12	0.17	0.10	0.04	0.11	0.08	
K	2	0.03	0.05	0.00	0.13	0.02	0.09	0.00	0.00	0.00	0.05	0.24