

Inter-population variation in multiple paternity and reproductive skew in the guppy

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Abstract

We use microsatellite loci to detail the multiple paternity patterns in broods from 10 wild populations of the guppy (*Poecilia reticulata*) found in Northern Trinidad. The populations span two major drainages comprising the Caroni and the Oropouche, and include sites that are characterized by either high or low predation. Across the populations the frequency of multiple paternity is high with 95% (range: 70%–100%) of broods having multiple sires. Broods have an average of 3.5 sires (range: 1–9) and a mixed-model analysis suggests that broods from high predation sites have marginally more sires than do those from low predation sites, but this is true only in the Oropouche drainage. There is no difference in sire number between predation sites in the Caroni drainage. Brood size, but not female body length, is correlated with the number of sires and the correlation cannot be attributed solely to the stochastic process associated with sperm competition and a ‘fair raffle’. Within broods there is significant skew in reproductive success among males, which may reflect variation in sperm competitiveness or female choice. There is, however, no difference in the skew among populations from different predation regimes or drainages. Finally, high predation populations were characterized by increased genetic variability at the microsatellite loci, suggesting a larger effective population size. We discuss explanations for the high degree of multiple paternity but the general lack of any major differences among broods from ecologically different populations.

Keywords: guppy, landscape genetics, microsatellite, multiple paternity, reproductive skew, sperm competition

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Introduction

Evolutionary and behavioural ecologists have long been interested in mate choice and multiple mating (Darwin 1871; Bateson 1983; Andersson 1994; Kokko *et al.* 2003). In nonresource based mating systems — those in which males provide only sperm to females — females may multiply mate to increase the genetic quality of their offspring, or to ensure they obtain sufficient sperm to fertilize all their eggs (Trivers 1972; Jennions & Petrie 2000; Neff & Pitcher 2005). Alternatively, in some mating systems males have evolved mating behaviours to circumvent female choice and thus females

may have to endure some level of multiple mating even if it is detrimental to them (Arnqvist & Rowe 2005).

In the present study we investigate the patterns of multiple paternity among broods in wild populations of the guppy (*Poecilia reticulata*). 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 sites. In particular, waterfalls exclude larger guppy predators from upstream sites resulting in lower predation in these populations. The level of predation risk is an important ecological variable that explains variation in many guppy characteristics, including life history (Reznick & Endler 1982), mate choice (Breden & Stoner 1987) and shoaling

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behaviour (Magurran & Seghers 1991). For example, females from low predation sites typically give birth to broods containing fewer but larger offspring than females from high predation sites. This variation in brood and offspring size has been shown to be an adaptation to predation, as high predation leads to selection for larger broods and consequently smaller offspring (Reznick & Endler 1982). Low predation populations also tend to be smaller and with unidirectional downstream gene flow, they typically have low genetic variation (Shaw *et al.* 1994; Crispo *et al.* 2005).

Female guppies are selective among males when mating and generally prefer to mate with males that have more orange colouration on their integument or sometimes males that are larger (Houde 1989; Reynolds & Gross 1992; Endler & Houde 1995). Females have also been shown to 'trade-up' and mate with a second male that is more colourful, and presumably of higher genetic quality, than a previous mate (Pitcher *et al.* 2003). Mating with multiple males may be adaptive as multiply mated females have shorter gestation times and produce larger broods with offspring that have better developed schooling behaviour and predatory escape response than do singly mated females (Evans & Magurran 2000; Ojanguren *et al.* 2005). Conversely, males can use forced copulation to circumvent female choice and the tactic appears to be effective in many guppy populations; one study on eight wild populations found that 45% of females contained sperm from unsolicited copulations (Evans *et al.* 2003a; also see Matthews & Magurran 2000). Males are more likely to use force copulation in high predation sites (Magurran & Seghers 1994) and, consistent with this observation, females from high predation populations tend to have greater numbers of sperm from unsolicited copulations than females from low predation populations (Evans *et al.* 2003a). However, there appears to be no difference in the proportion of females with sperm from unsolicited copulations across the predation regimes (Evans *et al.* 2003a).

Given the well characterized differences in ecology between upstream and downstream sites, we postulated that there would be differences in the patterns of multiple mating and subsequent paternity. We anticipated that multiple paternity would be more prevalent in downstream sites (high predation populations) because of increased genetic variation and hence potential benefits to multiple mating (e.g. Petrie *et al.* 1998), large brood sizes and perhaps sperm limitation (e.g. Kamimura 2003; Pai *et al.* 2005; Uller & Olsson 2005), increased frequency of forced copulations, or reduced precopulatory cues of male genetic quality due to reduced courtship frequency (e.g. Magurran & Seghers 1994). Consistent with this expectation, one study has already suggested that multiple paternity is more common in high predation populations (Kelly *et al.* 1999). Kelly *et al.* (1999) found that 64% of broods were multiply sired in high predation sites, whereas only 25% of broods were multiply

sired in low predation sites. However, their study may have been confounded by lower statistical power to detect multiple paternity in low predation sites. The authors used one or two microsatellite loci and the variability of the loci was lower in the low vs. high predation populations (see Neff & Pitcher 2002; Neff *et al.* 2002). Indeed, a study by Hain & Neff (2007) on kin recognition, which used three highly variable microsatellite loci and a more sophisticated statistical method for detecting multiple paternity, found that the proportion of multiply sired broods in one population was nearly fivefold higher than the proportion reported by Kelly *et al.* (1999) for the same population. Kelley *et al.* (1999) also did not examine the number of sires or the reproductive skew among sires within broods. Thus, a broader analysis of interpopulation variation in multiple paternity in guppies is warranted.

Here, we use three highly variable microsatellite loci to detail the patterns of multiple paternity, number of sires, and reproductive skew among males in broods from 10 populations of the guppy. The populations span two major drainages comprising the Caroni and the Oropouche, and include sites that are characterized by high or low predation. We discuss our results in terms of the known behavioural and ecological differences among populations and the potential selective pressures on multiple mating.

Materials and methods

Guppy collection

In May 2005, pregnant female guppies were collected from 10 populations in Northern Trinidad. The populations came from upper and lower regions of five rivers occurring in two drainages (Table 1). All upper regions are characterized by low predation with the exception of the upper Oropouche, which is characterized by high predation (Endler & Houde 1995). The regions encompassed three stream types comprising (i) open rivers; (ii) pools connected by riffles; and (iii) pools. The pregnant females were isolated into individual tanks until they gave birth. Within 24 h of parturition, newborn guppies were euthanized, enumerated and preserved in 95% ethanol. Females were then euthanized, measured for standard length, and a fin clip was preserved in 95% ethanol.

Microsatellite analysis

We used a Wizard Genomic DNA purification kit (Promega) to extract genomic DNA from the fin (dams) or caudal peduncle (offspring) of each fish. Briefly, each tissue sample was soaked in water for 2 h, and incubated in 100 µL nuclei lysis solution at 55 °C for 3 h. RNA was removed by 37 °C incubation with RNase A solution (final concentration of 4 µg/mL) for 30 min. All proteins were precipitated by

Table 1 List of the 10 guppy (*Poecilia reticulata*) populations from Northern Trinidad. Data comprise population, collection location, predation regime and stream type, total number of individuals analysed, locus, number of alleles, observed heterozygosity, expected heterozygosity and *PrDM*

Population	Location	Predation, stream type*	N†	Locus	Alleles	H_O	H_E	<i>PrDM</i> ‡
<i>Caroni Drainage</i>								
Upper Aripo	N 10°41.743' W 061°12.406'	Low, Open river	83	<i>Pre8</i>	8	0.740	0.748	0.801
				<i>Pre9</i>	7	0.794	0.710	
				<i>Pr39</i>	3	0.246	0.312	
Lower Aripo	N 10°39.036' W 06°13.380'	High, Open river	173	<i>Pre8</i>	16	0.858	0.839	1.000
				<i>Pre9</i>	19	0.974	0.908	
				<i>Pr39</i>	8	0.801	0.806	
Tunapuna	PS 759 797§	Low, Pools	157	<i>Pre8</i>	7	0.421	0.572	0.963
				<i>Pre9</i>	5	0.641	0.705	
				<i>Pr39</i>	7	0.619	0.734	
Lower Tacarigua	N 10°40.736' W 061°19.168	High, Open river	172	<i>Pre8</i>	15	0.435	0.903	1.000
				<i>Pre9</i>	16	0.849	0.878	
				<i>Pr39</i>	8	0.613	0.702	
<i>Oropouche Drainage</i>								
Quare Tributary	N 10°40.553' W 061°11.792'	Low, Pools/riffles	83	<i>Pre8</i>	8	0.586	0.692	0.868
				<i>Pre9</i>	9	0.770	0.784	
				<i>Pr39</i>	5	0.689	0.601	
Lower Quare	N 10°40.418' W 06°11.833'	High, Open river	165	<i>Pre8</i>	21	0.808	0.909	1.000
				<i>Pre9</i>	14	0.877	0.876	
				<i>Pr39</i>	7	0.664	0.667	
Upper Oropouche	N 10°43.060' W 061°08.800'	High, Open river	109	<i>Pre8</i>	16	0.807	0.891	1.000
				<i>Pre9</i>	16	0.939	0.861	
				<i>Pr39</i>	10	0.773	0.703	
Lower Oropouche	N 10°39.570' W 061°07.868'	High, Open river	153	<i>Pre8</i>	28	0.684	0.902	1.000
				<i>Pre9</i>	15	0.919	0.895	
				<i>Pr39</i>	12	0.709	0.801	
Upper Turure	N 10°40.775' W 061°10.002'	Low, Pools	107	<i>Pre8</i>	11	0.810	0.826	0.980
				<i>Pre9</i>	10	0.737	0.771	
				<i>Pr39</i>	5	0.670	0.701	
Lower Turure	N 10°39.394' W 061°10.059'	High, Open river	145	<i>Pre8</i>	17	0.741	0.882	1.000
				<i>Pre9</i>	15	0.928	0.855	
				<i>Pr39</i>	8	0.594	0.780	

*Predation pressure from Endler & Houde (1995) and field observations by the authors.

†Includes mothers and offspring.

‡*PrDM* is the probability of detecting a multiple mating based on all three loci and is calculated according to Neff & Pitcher (2002) using the observed brood size and reproductive skew data.

§National grid reference as GPS coordinates could not be obtained due to poor signal reception.

adding 35 µL of the protein precipitation solution. The DNA was then precipitated from the supernatant with 130 µL isopropanol, pelleted using centrifugation and washed with 70% ethanol.

For genotyping, we used a dinucleotide microsatellite locus (*Pr39*; primers published in Becher *et al.* 2002) and two tetranucleotide microsatellite loci (*Pre8* and *Pre9*; primers published in Paterson *et al.* 2005). A T1 Thermocycler (Whatman-Biometra) or PTC-200 (MJ Research) was used to amplify the microsatellites with the following program: 94 °C for 10 min, 35 cycles of 30 s at 94 °C, 30 s at 58 °C (for

Pre9 and *Pr39*) or 61 °C (for *Pre8*), 30 s at 72 °C and final elongation at 72 °C for 10 min. Each 10 µL polymerase chain reaction (PCR) contained ~75 ng of total DNA, 3 mM MgCl₂, 1 × PCR buffer (Invitrogen Life Technologies), 0.25 mM of each deoxynucleotide (Sigma-Aldrich), 0.25 units Taq DNA polymerase (Invitrogen Life Technologies) and 0.25 µM of each forward and reverse primer. The forward primers were fluorescently labelled (Sigma-Proligo) and the PCR product was analysed following the standard protocol for the CEQ 8000 Genetic Analysis System (Beckman Coulter).

Paternity and statistical analysis

Offspring were assigned to parents using COLONY version 1.2, a parentage assignment program that reconstructs putative sires based on a maximum-likelihood (ML) method (Wang 2004). For this analysis, we set the typing error rate at the suggested 0.025 (Wang 2004). Thus, for each female we were able to determine the number of sires contributing to her brood as well as the reproductive skew among males. Skew was summarized by first calculating the effective number of sires from $1/\sum(rs_i/\text{brood size})^2$; where rs_i is the number of offspring assigned to sire i , and the summation is over all sires contributing to a brood. Skew was then expressed as $1 - (\text{effective number of sires}/\text{actual number of sires})$. Thus, a value of zero implies no skew in which case all sires contribute equally to the brood, and a value approaching 1 implies maximal skew in which case nearly all offspring are assigned to a single sire.

We first used an analysis of variance (ANOVA) to compare variability among the microsatellites while controlling for differences among populations. We also used an ANOVA to examine microsatellite variability among populations, and between drainages and predation regimes. Next, we used a restricted ML mixed-model nested analysis of covariance (ANCOVA) to examine variation in the number of sires or reproductive skew among sites and the ecological parameters. For this model, the number of sires or reproductive skew was entered as the dependent variable, drainage, river, and predation regime were entered as independent variables, and female body length and brood size were entered as covariates. River was coded as a random factor (because we did not sample all of the rivers within a drainage) and was nested within drainage.

Based on a fair raffle process in sperm competition (Parker 1990), we anticipated that there would be a positive correlation between brood size and number of sires. That is, larger broods are more likely to contain offspring from each of the males a female mates with simply because of the chance probability that each male's sperm contributes to at least one of the offspring. To assess whether such a correlation was a consequence of a raffle process or represented differences in the actual degree of multiple mating by females producing larger broods, we conducted a Monte Carlo simulation using the C++ programming language. The simulation first disassociated the observed brood size and number of sires data by randomization. Next, it generated 101 pseudo-broods (the number we actually analysed) with offspring being probabilistically assigned to males within broods based on the observed reproductive skew data. Thus, it was possible that not all males that a female 'mated' with would end-up contributing to a brood because of the random chance that a male was not sampled. A male is more likely to not be sampled when a female mates with several males but produces a small brood. We

then determined the number of males contributing to each pseudo-brood and used these data to calculate the correlation between brood size and number of sires. Simultaneously, we generated a second set of 101 pseudo-broods but this time we did not disassociate the brood size and number of sires data (i.e. we used the actual data). Offspring within broods were again assigned probabilistically to males based on the reproductive skew data and the correlation between brood size and number of sires was determined. The entire analysis was repeated 1000 times and we compared the pairs of correlation coefficients to determine the probability that the observed data was a result of a stochastic process associated with sperm competition, i.e. if the correlations from the nonrandomized data were not consistently higher than the correlations from the randomized data.

All statistical analyses were performed in JMP (version 4.0.4, SAS Institute Incorporated). Means are reported with range or plus or minus one standard deviation unless otherwise noted.

Results

In total we analysed 1256 offspring from 101 broods at three microsatellite loci. Across the populations, the loci were variable with between three alleles (*Pr39* in Upper Aripo) and 28 alleles (*Pre8* in Lower Oropouche; Table 1). *Pr39* had the fewest alleles (7.3 ± 2.6 SD), *Pre9* was intermediate (12.6 ± 4.6), and *Pre8* was the most variable (14.7 ± 6.6 ; ANOVA controlling for population: $F_{2,18} = 15.5$, $P < 0.001$). These loci also differed significantly at the observed heterozygosity, although *Pre9* and not *Pre8* had the highest level of heterozygosity (*Pr39*: 0.64 ± 0.15 ; *Pre9*: 0.84 ± 0.10 ; *Pre8*: 0.69 ± 0.16 ; ANOVA controlling for population: $F_{2,18} = 8.50$, $P = 0.002$); the allele frequencies at *Pre8* were more skewed than those at *Pre9* (data not shown). There was a significant difference in allele number and observed heterozygosity among the populations (ANOVAs controlling for locus: allele number $F_{9,18} = 5.50$, $P = 0.001$; heterozygosity $F_{9,18} = 2.43$, $P = 0.052$; Table 1). Furthermore, although there was no difference in microsatellite variability between the two drainages, populations from high predation sites were more variable at the three loci than populations from low predation sites (ANOVAs controlling for locus, allele number: drainage, $F_{1,25} = 1.19$, $P = 0.28$; predation regime, $F_{1,25} = 44.4$, $P < 0.001$; heterozygosity: drainage, $F_{1,25} = 0.78$, $P = 0.38$; predation regime, $F_{1,25} = 8.40$, $P = 0.008$; Table 1). Nevertheless, the probability of detecting a multiple mating, as measured by *PrDM* (see Neff & Pitcher 2002), was high in both types of populations (high predation *PrDM* = 1.0 ± 0.0 ; low predation *PrDM* = 0.92 ± 0.08).

Among populations, there was significant variation in the number of mates ($r^2 = 0.21$, $F_{9,91} = 2.72$, $P = 0.008$), but not the reproductive skew among sires within broods ($r^2 = 0.14$, $F_{9,91} = 1.72$, $P = 0.10$) (Table 2). The mixed-model

Table 2 Multiple paternity and reproductive skew for 10 guppy (*Poecilia reticulata*) populations from Northern Trinidad. Data comprise population, number of broods analysed, female body length, offspring per brood, proportion of broods multiply sired, number of sires and reproductive skew

Population	Broods	Female body length*		Offspring per brood		Multiply sired	Number of sires		Reproductive skew	
		Mean	Range	Mean	Range		Mean	Range	Mean	Range
<i>Caroni Drainage</i>										
Upper Aripo	10	25.0	21.2–28.0	7.3	4–11	1.00	2.8	2–4	0.20	0.00–0.36
Lower Aripo	11	24.8	18.5–28.8	14.7	5–32	1.00	3.6	2–5	0.22	0.04–0.49
Tunapuna	10	27.7	23.1–31.0	14.7	4–27	1.00	3.6	2–6	0.14	0.00–0.30
Lower Tacarigua	10	25.9	21.8–29.8	16.2	6–36	0.70	2.9	1–7	0.09	0.00–0.27
<i>Oropouche Drainage</i>										
Quare Tributary	10	24.2	22.0–29.5	7.3	5–10	0.90	2.6	1–4	0.14	0.00–0.24
Lower Quare	10	28.9	23.3–35.0	15.5	9–32	1.00	4.6	3–9	0.23	0.08–0.50
Upper Oropouche	11	23.6	19.9–28.1	8.9	4–21	0.91	3.2	1–5	0.16	0.00–0.46
Lower Oropouche	10	24.4	21.0–29.3	14.3	8–21	1.00	4.4	3–6	0.24	0.04–0.35
Upper Turure	10	23.6	21.1–25.9	9.7	6–15	1.00	3.3	2–5	0.16	0.00–0.42
Lower Turure	9	24.2	21.1–25.2	16.2	8–28	1.00	3.8	2–6	0.27	0.00–0.61

*Standard length measured in mm.

Table 3 Mixed-model ANCOVA results examining multiple paternity and reproductive skew for 10 guppy (*Poecilia reticulata*) populations from Northern Trinidad. Data comprise the independent variables, degrees of freedom (d.f.), *F* statistic, and *P*-value

Independent variable	d.f.	<i>F</i>	<i>P</i>
<i>Number of sires</i>			
Drainage	1, 3	3.99	0.14
River [Drainage]*	3, 92	0.72	0.54
Predation	1, 92	0.23	0.63
Drainage × Predation	1, 92	3.02	0.085
Female body length	1, 92	0.48	0.49
Brood size	1, 92	53.9	< 0.001
<i>Reproductive skew</i>			
Drainage	1, 3	0.45	0.55
River [Drainage]*	3, 92	1.98	0.12
Predation	1, 92	0.13	0.72
Drainage × Predation	1, 92	2.38	0.13
Female body length	1, 92	0.05	0.82
Brood size	1, 92	2.65	0.11

*River was nested-within-drainage and coded as a random factor.

nested ANCOVA captured 55% of the variance in the number of mates ($r^2 = 0.55$, $F_{11,89} = 10.1$, $P < 0.001$), but brood size was the only significant effect (Table 3). Females that produced larger broods tended to have more sires contributing to their broods (Fig. 1). Female body length was correlated with brood size ($r = 0.49$, $n = 101$, $P < 0.001$), but it did not appear to relate to the number of sires independent of brood size (Table 3; partial correlation analysis: female body length, $r = 0.06$, $n = 101$, $P = 0.55$; brood size, $r = 0.59$,

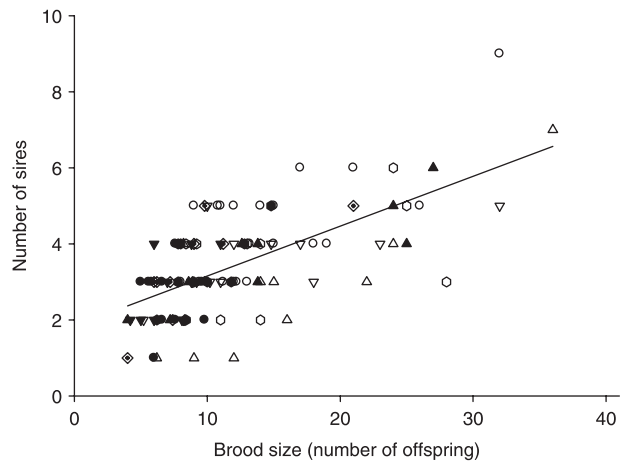


Fig. 1 The relationship between brood size and the number of sires contributing to broods in the guppy (*Poecilia reticulata*). The line is from a simple linear regression, although statistical analysis involved a mixed-model nested ANCOVA (see text). Filled symbols denote low predation sites and open symbols denote high predation sites from 10 populations: inverted triangle for upper and lower Aripo, circle for Quare tributary and Quare, diamond for upper (with inset +) and lower Oropouche, triangle for Tunapuna and lower Tacarigua, and hexagon for upper and lower Turure. Overlapping data are offset slightly in the x-axis to increase clarity.

$n = 101$, $P < 0.001$). The Monte Carlo analysis revealed that the observed correlation between brood size and number of sires was significantly greater than that expected by chance ($P < 0.001$). Thus, the observed difference in sire number could not be explained simply by differences in

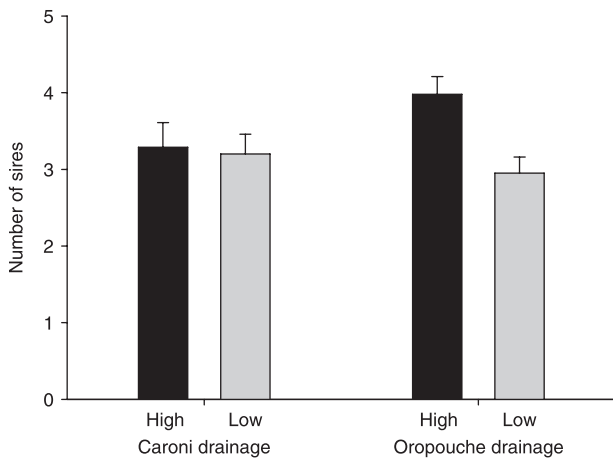


Fig. 2 Variation in the number of sires contributing to broods from the guppy (*Poecilia reticulata*). The number of sires did not differ between high and low predation sites in the Caroni drainage. Conversely, there were more sires contributing to broods from high predation sites than low predation sites in the Oropouche drainage. Across all broods ($n = 101$), an average of 3.5 ± 1.4 (SD) males sired offspring within each brood. Error bars denote one standard error.

brood sizes. There was a marginally nonsignificant interaction between drainage and predation and this effect was significant when the nonsignificant covariate (female body length) was removed from the model ($F_{1,93} = 3.95$, $P = 0.049$). There was no difference in the number of sires between high and low predation sites in the Caroni drainage (high: 3.3 ± 1.4 ; low: 3.2 ± 1.2), but there was a difference in the Oropouche drainage (high: 4.0 ± 1.5 ; low: 2.9 ± 0.9 ; Fig. 2).

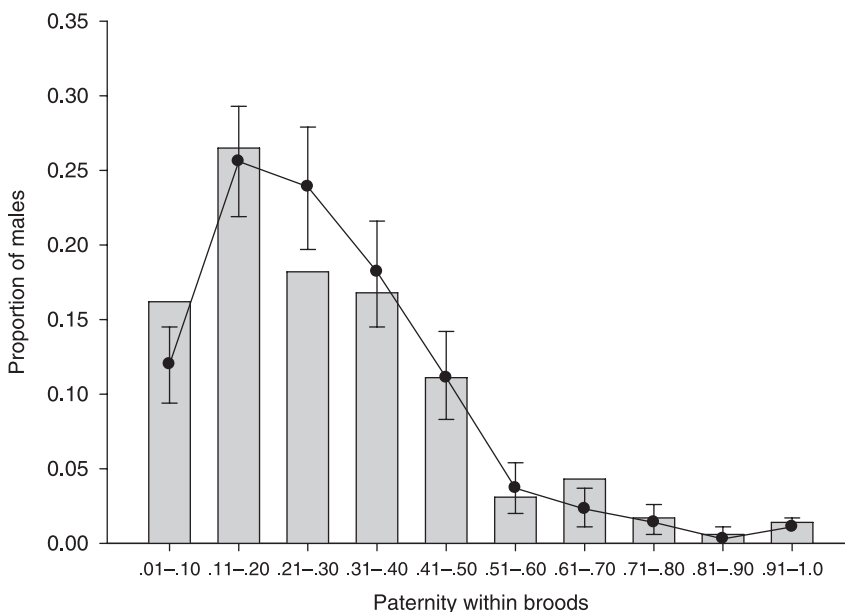


Fig. 3 Variation in paternity within broods of the guppy (*Poecilia reticulata*). Bars denote the proportion of all males with a given paternity within broods. Filled circles and error bars denote expected proportions and associated 95% confidence interval assuming a fair raffle in sperm competition. The expected proportions were generated using a sampling routine based on the observed number of sires contributing to each brood and assuming that each sire had an equal chance of fertilizing each offspring within the brood. There were more males with paternity of 0.01–0.10 and 0.61–0.70 and fewer males with paternity of 0.21–0.30 than expected given a fair raffle.

Across all broods the mean reproductive skew was 0.19 ± 0.14 and this was significantly different than 0 (i.e. no skew; one sample t -test, $t_{100} = 13.8$, $P < 0.001$). However, there was no apparent relationship between skew and any of the variables in the ANCOVA (Table 3). Examining all broods, males' paternity within broods ranged from 0.03 to 1.0 and the distribution of paternities differed from the expectation given a fair raffle in sperm competition (Fig. 3). Finally, there was no difference in the proportion of broods that were multiply sired between high and low predation sites (high: 0.94 ± 0.12 ; low: 0.98 ± 0.05 ; $t_8 = 0.62$, $P = 0.55$), or between drainages (Caroni: 0.94 ± 0.15 ; Oropouche: 0.97 ± 0.06 ; $t_8 = 0.52$, $P = 0.52$).

Discussion

We used three highly polymorphic microsatellite loci to genetically dissect patterns of multiple paternity across 10 wild populations of the guppy. There was significant variation among populations in the number of sires per brood and, although there was significant reproductive skew within broods, there was no difference in the mean skew across populations. It is possible that it was more difficult to detect a difference in skew among populations because many of our brood sizes were not overly large. For example, with four sires and a brood size of 12 (the mean in our sample), the maximum possible skew is 0.57 (the theoretical maximum is 1). We found that some of the variation in number of sires could be attributed to differences in predation regime, at least in the Caroni drainage. Perhaps surprisingly, across all the populations, nearly all broods were multiply sired (95%) with an average of 3.5 sires per brood, and although females from high predation populations tended to have

more sires than those from low predation populations, the difference was not remarkable (high predation: 3.8 ± 0.7 ; low predation: 3.1 ± 0.4). Furthermore, we found that brood size, but not female size, was correlated with the number of sires, and this correlation was not simply a function of an increased likelihood of detecting more sires in larger broods. Instead the correlation likely reflects a greater degree of multiple mating by females that produce larger broods.

The results from our study differ from those of a previous study of multiple paternity in guppies. Kelly *et al.* (1999) found that 64% of broods were multiply sired in high predation populations, whereas this proportion was only 25% in broods from low predation populations. We found no statistical difference in the proportion of broods multiply sired from high and low predation populations, and our overall rate was much higher at 94% and 98% for high and low predation populations, respectively. It is unlikely that the difference between the two studies reflects variation in the actual populations studied because six of the 10 populations studied by Kelly and colleagues were also studied here, and in all six of these populations, our estimate of the frequency of multiple paternity was higher than that of Kelly and colleagues. Instead, it is possible that the discrepancy between the two studies reflects a difference in the statistical power to detect multiple paternity. For example, Kelly and colleagues used one or two microsatellite loci and the probability of detecting a multiple mating ranged from 0.31 to 0.80 (see Neff & Pitcher 2002). Our values ranged from 0.80 to 1.00. We also used a more sophisticated and powerful program called COLONY that reconstructs putative sires based on an ML method (Wang 2004). This program can infer a multiply sired brood when only two unique paternal alleles are 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 and they instead used a conservative approach of counting unique paternal alleles to detect multiple mating (i.e. two sires were detected only when there were at least three paternal alleles detected in a brood).

The frequency of multiple paternity in guppies that we uncovered is one of the highest reported of any vertebrate. We found that 95% of all broods were multiply sired (range across populations = 70%–100%). Within the Poeciliidae family, reports of the proportion of multiply sired broods range from 23% in *Poeciliopsis monacha* (Leslie & Vrijenhoek 1977) to 90% in *Gambusia holbrooki* (Zane *et al.* 1999). Compared to other vertebrates, a review of 20 polygamous mammals found that an average of 29% of broods were multiply sired with a high of 80% in the red fox, *Vulpes vulpes* (Isvaran & Clutton-Brock 2007). In birds, a review of over a hundred species found that, on average, 18% of broods were multiply sired with a high of 95% in the superb fairy wren, *Malurus cyaneus* (Griffith *et al.* 2002). Interestingly, both reviews implicate female-biased operational sex ratios —

as may result in species with shorter breeding seasons or when females otherwise breed more synchronous — as resulting in higher rates of multiply sired broods. Guppy populations tend to be characterized by highly female-biased sex ratios (up to about 6 : 1; Rodd & Reznick 1997) and this may contribute to the high rate of multiple paternity in guppies. On the other hand, the relationships between sex ratio and multiple mating in mammals and birds presumably relates to males' ability to mate guard and prevent females from engaging in extra-pair matings, but there is no evidence that male guppies attempt to mate guard females. Thus, it is unclear as to whether or not female-biased sex ratios have similar effects on the frequency of multiple paternity across these different taxa.

Given the striking differences in ecology and life history between high and low predation populations of guppies, it was surprising that we did not find greater differences in the frequency of multiply sired broods or the number of sires contributing to broods across the predation regimes. High predation populations are characterized by high genetic variability (Shaw *et al.* 1994; Crispo *et al.* 2005), large broods (Reznick & Endler 1982), and a high frequency of forced copulation attempts (Magurran & Seghers 1994), all of which may lead to a higher degree of multiple paternity in high vs. low predation populations. However, we found no difference in the proportion of broods that were multiply sired between high and low predation populations, and only a small difference in the number of sires, which was only significant in one drainage (see Fig. 2). There is nothing obviously different about the two drainages that would suggest why the trend was found in one drainage and not the other. Furthermore, the lowest frequency of multiply sired broods was in the Lower Tacarigua, a high predation population, and this same population had one of the lowest mean number of sires contributing to broods. We consider below several possible explanations for the lack of a large difference in the degree of multiple paternity in high vs. low predation populations.

First, it is possible that female control of precopulatory mate choice is circumvented by male forced copulations in all populations. Males frequently switch from courtship displays to forced copulation attempts via gonopodial thrusting (Magurran & Seghers 1994). Forced copulations have been shown to be widely successful at circumventing female choice in several populations (Evans *et al.* 2003a). Furthermore, although females from high predation populations tend to have more sperm from unsolicited copulations in their gonoducts than females from low predation populations, there is no difference in the proportion of females containing sperm from unsolicited copulations (Evans *et al.* 2003a). Thus, it is possible that the patterns of multiple paternity are a direct function of the frequency of force copulation attempts by males. To test this hypothesis, we compared our data with the data from Evans *et al.* (2003a)

for the eight populations that were common to both studies. We found that the number of sires contributing to broods was positively correlated with both the proportion of females containing sperm from unsolicited copulations ($r = 0.64$, $n = 8$, $P = 0.085$) and the number of sperm in females' gonoducts from unsolicited copulations ($r = 0.30$, $n = 8$, $P = 0.47$). Although neither correlation is significant, the data suggest that the frequency of forced copulations could contribute to the patterns of multiple paternity across populations. Further study is warranted, particularly behavioural estimates of the frequency that males use forced copulations across the different populations.

Second, it is possible that all females multiply mate for fertility assurance. Most guppy populations are female biased (Rodd & Reznick 1997) and there is substantial variation in the amount of sperm males possess (Evans & Magurran 1999; Pitcher & Evans 2001; Evans *et al.* 2002; Pitcher *et al.* 2007). Thus, it is possible that some males become sperm depleted and by mating with multiple males a female ensures that she obtains a sufficient sperm supply to fertilize all of her ova. Such a mechanism has been invoked to explain multiple mating patterns in several species (e.g. Kamimura 2003; Pai *et al.* 2005; Uller & Olsson 2005), and it is consistent with the correlation between brood size and number of sires that we uncovered in guppies (Fig. 1). However, there appears to be no relationship between the number of sperm artificially inseminated in females and the number of offspring produced, which suggests that females are not typically sperm limited (Pilastro *et al.* 2008). Thus, it is unclear as to whether or not female choice for fertility assurance explains the positive relationship between brood size and number of sires or the broader interpopulation patterns of multiple paternity in guppies.

Third, other aspects of population ecology outside of predation pressure may contribute to the patterns of multiple paternity. The populations reside in streams that differ considerably in size and flow, and range from small pools, to pools connected by riffles, to open rivers (see Table 1). In so much as small pools restrict gene flow and increase the probability of inbreeding, multiple mating may be more valuable in populations occupying small pools as opposed to pools connected by riffles or open rivers. Indeed, females from small pools or housed in aquaria tend to discriminate against familiar males, perhaps because they are more likely to be related to the female (Kelley *et al.* 1999). Furthermore, female guppies that multiply mate may avoid some of the costs of inbreeding (Pitcher *et al.* 2008; for examples in other animals see, Tregenza & Wedell 2002; Garant *et al.* 2005). Only two of the populations we studied were from small pools and both of these populations were ranked about in the middle of the distribution for number of sires (ranked five and tied for six out of 10 populations), and there was no difference in the mean number of sires between populations in pools vs. those in pools connected

by riffles or open rivers ($t_g = 0.98$, $P = 0.36$). Furthermore, populations from open rivers had some of the smallest and largest values for sire number. Thus, it does not appear that stream type and connectivity affects the patterns of multiple paternity in guppies. However, a more thorough analysis of genetic load among populations and post-copulatory mechanisms of sperm choice would help to understand the role of inbreeding depression on multiple mating in guppies.

Finally, it is possible that females obtain some other indirect benefit by mating with multiple males. Previous work has shown that multiply mated females from the Tacarigua river have shorter gestation times and produce larger broods with offspring that have better developed schooling behaviour and predatory escape response than do singly mated females (Evans & Magurran 2000; Ojanguren *et al.* 2005). These benefits may be obtained via a postcopulatory mechanism that differentiates among males' sperm based on genetic quality such as sperm competitiveness (Evans *et al.* 2003b). Given that the populations in our study showed significant skew in reproductive success among males contributing to broods, some males appear to be better sperm competitors than other males. Females may also benefit from bet hedging (Cohen 1966) given that stream environments can change considerably between the wet and dry seasons in Trinidad (Magurran 2005). Bet hedging has been invoked as a possible explanation for multiple mating in other species that experience variable environments (e.g. Mäkinen *et al.* 2007). Presumably a female that mates with multiple males produces a brood with greater genetic diversity and increases the chance that she has some offspring with genes that are well suited for the offspring's environment.

In summary, we found that across 10 populations of Trinidadian guppies most broods were multiply sired (96 of 101 broods). There was no difference in the proportion of multiply sired broods between high and low predation populations, but there was a difference in the number of sires contributing to the broods in one of two drainages. The difference in sire number, however, was smaller than anticipated given the striking differences in population ecology and life history between the two predation regimes. It is conceivable that all females benefit from multiple mating, or that males largely circumvent female choice by using unsolicited, forced copulations. Additional research, particularly behavioural studies of male and female mating interactions in the wild, and studies of the genetic benefits from multiple mating, is required to further elicit the mechanisms underlying this intriguing pattern.

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- The authors study fish mating systems and enjoy an ongoing collaboration working on the Trinidadian guppy.
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