Geographical variation in multiple paternity within natural populations of the guppy (*Poecilia reticulata*)

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Mating can increase an individual's risk of mortality by predation. In response to predation hazards, males in some species court females less often, but alternatively engage in coerced copulations more frequently and females become less selective. Such predator-mediated shifts in mating tactics may result in higher levels of multiple inseminations in females and, thus, in greater frequencies of females with broods of mixed paternity. We tested this hypothesis using two polymorphic microsatellite loci to estimate conservatively multiple paternity in broods of female guppies (*Poecilia reticulata*) originating from ten natural populations that have evolved under different fish predation regimes in Trinidad. The frequency of broods that were multiply sired was significantly greater on average in populations experiencing high predation pressure compared to populations experiencing a relatively low predation risk. These results suggest that the intensity of male sperm competition covaries geographically with predation pressure in this species and that the local risk of predation mediates the opportunity for sexual selection within populations.

Keywords: multiple paternity; predation risk; population; geographical variation; guppy; *Poecilia reticulata*

1. INTRODUCTION

Sexual selection favours the evolution and maintenance of elaborate secondary sexual traits in males, such as courtship displays and bright coloration, and mating preferences for such traits in females (Andersson 1994). However, conspicuous traits in males and mate choice in females are costly. The potential costs include increased time and energy expenditures, lost foraging opportunities and increased risks of harassment, injury, disease and predation (Lima & Dill 1990; Magnhagen 1991; Andersson 1994; Sih 1994). Although much research has been directed at understanding the fitness benefits of conspicuous traits in males and mating preferences in females (Andersson 1994), relatively little attention has been paid to their associated fitness costs and the consequences for the process of sexual selection (Lima & Dill 1990; Magnhagen 1991; Andersson 1994; Sih 1994).

Both theory (e.g. Pomiankowski 1987; Pomiankowski *et al.* 1991) and experimental studies (e.g. Magurran & Seghers 1990; Forsgren 1992; Berglund 1993; Hedrick & Dill 1993; Godin 1995; Godin & Briggs 1996) have shown that, as the costs of mating (including the risk of predation) increase, females become less selective in their choice of mates and males decrease their courtship efforts and/or increase their use of apparently less costly mating tactics, such as sneak copulations (coerced copulation

which circumvents female mate choice), in a manner that maximizes fitness. Such predator-mediated shifts in mating tactics may result in proportionally more males achieving successful matings and in more females being multiply inseminated by different males within a population, potentially resulting in more broods with mixed paternity. In turn, this may reduce the variance in male mating success and, thus, weaken the intensity of sexual selection (Wade 1979; Wade & Arnold 1980) within the population. Because predation pressure can vary geographically among populations of the same species (Endler 1995; Foster 1999; Magurran 1999), the relative frequency of females that are inseminated by different males may be higher in populations experiencing strong predation pressure than in populations experiencing weaker predation pressure. We tested this hypothesis using two polymorphic microsatellite loci to estimate conservatively multiple paternity in broods of female guppies (*Poecilia reticulata*) originating from ten natural populations that have evolved under different fish predation intensities in Trinidad.

The guppy is a small, live-bearing fish species with internal fertilization which exhibits a promiscuous, non-resource-based mating system and occurs in mixed-sex shoals in nature (Houde 1997). Males have two alternative mating tactics. Individuals may either court a receptive female prior to attempting to copulate with her or they may attempt to sneak copulate quickly with a female without first displaying to her or without a prior receptive response from her. Although males may be more likely to achieve matings by courting females than by sneak

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Table 1. *Summary statistics for the guppy populations sampled* (*identity number in parentheses*)

(The numbers of broods and embryos genotyped for paternity determination, mean ($\pm$ s.e.) number of embryos per brood (range in parentheses), total number (n) of pregnant and non-pregnant females assayed at each of the loci, number of alleles per loci and expected heterozygosity (H_E) for each locus separately and combined (in bold type) are shown for each population.)

predation regime	population (river name)	Trinidad map grid reference	number of broods assayed	number of embryos assayed	number of embryos per brood	locus	n	number of alleles	H_E
low	Paria (1)	PS 9088	21	147	7.00 ± 0.63 (3–12)	TTA	34	4	**0.586**
low	Upper Aripo (2)	PS 9482	29	139	4.79 ± 0.43 (3–14)	TTA	44	7	**0.445**
low	Marianne (3)	PS 8590	27	229	8.48 ± 0.97 (3–20)	TTA	40	14	0.865
						CA	30	2	0.264
									0.565
low	Yarra (4)	PS 8387	22	130	5.91 ± 0.51 (3–12)	TTA	22	5	0.645
						CA	22	2	0.173
									0.409
low	Tunapuna (5)	PS 7680	25	129	5.16 ± 0.42 (3–11)	TTA	25	5	0.791
						CA	25	4	0.654
									0.723
high	Oropuche (6)	QS 0479	30	259	8.63 ± 1.00 (3–28)	TTA	39	17	**0.888**
high	Quaré (7)	PS 9780	25	161	6.44 ± 0.48 (3–12)	TTA	35	13	**0.884**
high	Arima (8)	PS 8780	25	202	8.08 ± 0.88 (3–22)	TTA	37	10	0.879
						CA	24	5	0.743
									0.811
high	Guanapo (9)	PS 9082	26	230	8.85 ± 0.71 (3–18)	TTA	28	5	0.667
						CA	28	3	0.416
									0.542
high	Tacarigua (10)	PS 8084	23	186	8.09 ± 0.83 (3–16)	TTA	36	12	0.802
						CA	25	4	0.643
									0.723

copulation, they probably incur greater costs in terms of energy expenditure and predation risk by using the former tactic over the latter. Laboratory studies have revealed that females can store sperm and individual broods can potentially be multiply sired (Houde 1997).

Fish predation pressure on guppies varies geographically in Trinidad (Liley & Seghers 1975; Endler 1978, 1995; Houde 1997; Magurran 1999). Guppy populations which coexist with piscivorous characin and cichlid fishes, particulary the cichlid *Crenicichla alta*, are generally categorized as experiencing a high-predation risk. Populations that do not coexist with these predators, but only with the small, gape-limited cyprinodontid *Rivulus hartii*, are categorized as being at low-predation risk. As an evolved anti-predator adaptation, guppies in high-predation populations spend more time shoaling and live in larger shoals than those in low-predation populations (Magurran & Seghers 1991). Moreover, male guppies in populations exposed to high fish predation intensity court females less often and attempt sneak copulations more often than males in populations with a lower risk of predation (Luyten & Liley 1985; Magurran & Seghers 1994). Within populations, males reduce their courtship display rate and, conversely, increase their rate of sneak copulation (Endler 1987; Magurran & Seghers 1990; Magurran & Nowak 1991; Godin 1995) and females become less selective when choosing mates (Godin & Briggs 1996; Gong & Gibson 1996) in response to increased local risk of fish predation.

This is as expected if courtship and active mate selection are associated with an elevated risk of predation (Lima & Dill 1990; Magnhagen 1991; Sih 1994). These interpopulation differences in guppy behaviour which covary with predation risk led us to hypothesize that the opportunity for multiple insemination of females by males and the resulting potential increase in concurrent multiple paternity per brood should be greater in populations experiencing strong predation pressure than in those experiencing weaker predation pressure.

2. MATERIAL AND METHODS

(a) *Sampling and populations*

We collected gravid females under permit from each of five high-predation and five low-predation populations (table 1) in the Northern Range Mountains of Trinidad during the dry season in April–May 1997 and 1998. Each population was categorized as either experiencing high-predation pressure from fishes or low-predation pressure based on previous studies (Liley & Seghers 1975; Endler 1978, 1995; Houde 1997; Magurran 1999) and our own sampling of fish predators during on-site visits. Guppies were collected using a beach seine or a dip-net (rarely and only where seining was not possible). All rivers were sampled haphazardly at various locations to ensure genetic variability within the samples. The fishes were immediately preserved in absolute ethanol upon collection for later genetic analysis.

(b) *DNA extraction*

We dissected each female in the laboratory and removed all embryos present. Approximately 0.01 g of muscle was excised from the caudal peduncle of each female and embryo for DNA extraction and analysis. The samples were placed separately in 100 mM Tris and 40 mM EDTA, pH 8.0, for 15 min to remove all residual ethanol. Each tissue sample was then suspended in 30 μl of extraction buffer (10 mM Tris, pH 8.3, 50 mM KCl and 0.8% Tween) and 3 μl of proteinase K (20 mg ml^{-1} stock solution) and incubated for 2.5 h at 65 °C with periodic mixing.

(c) *Genetic analysis*

To estimate conservatively the number of putative sires contributing to a particular brood, we genotyped mothers and offspring using two highly polymorphic microsatellite DNA markers (namely loci TTA and CA) which are specific to poeciliid fishes, including the guppy. The TTA locus was isolated and characterized by J. S. Taylor (unpublished data; GenBank accession number AF164205; see also Taylor *et al.* 1999). It has a core (TTA)$_n$ repeat, with primer sequences 5′-GTGACCGAACGAAAG-GATA-3′ and 5′-CCCCAAAGGAACACTGTAT-3′. The CA locus was isolated and characterized by M. Schartl (unpublished data; GenBank accession number AF170707). It has a core (CAA(CA)8)$_n$ repeat, with primer sequences 5′-GAGCTC-CACTTGAATGACA-3′ and 5′-AACCACTCACGAGCCACT-3′. Both loci display Mendelian inheritance, low mutation rates, a low incidence of null alleles and no sex differences (J. S. Taylor and F. Breden, unpublished data). To our knowledge, these two loci are the only markers available which amplify consistently and are sufficiently polymorphic for investigating multiple paternity in the guppy. We attempted to use a third locus (Pooc-G49) which is specific to the Gila topminnow, *Poeciliopsis occidentalis occidentalis* (Parker *et al.* 1998); unfortunately, this locus failed to amplify consistently.

Following established procedures (Sambrook *et al.* 1989), we labelled each primer radioactively with [γ-^{33}P] ATP, amplified the DNA samples using the polymerase chain reaction (PCR) and ran the PCR products on denaturing polyacrylamide gels. Fish genotypes were scored from the autoradiographs using an M13 DNA standard and internal standards (samples of known genotype) run on each gel.

(d) *Estimation of multiple paternity and genetic diversity*

We determined the frequency of multiple paternity by genotyping only those broods comprising three or more embryos. Broods from all ten populations were surveyed at the TTA locus for a total of 253 gravid females and 1812 embryos and six of these populations (representing 58.5% of the above females and 61.0% of the embryos; table 1) were additionally surveyed at the CA locus. For each brood, we estimated multiple paternity conservatively by direct observation of the offspring genotypes. Maternal genotypes were determined directly and observed in the offspring genotypes, whereas paternal genotypes were inferred from the offspring genotypes. Any brood possessing three or more paternal alleles was considered multiply sired (cf. Travis *et al.* 1990; Kellogg *et al.* 1995; Trexler *et al.* 1997). This method is conservative because each paternal allele that was contributed to a brood could have been donated by a different male if each male was homozygous for a different allele or, alternatively, possessed the same di-locus genotype. As a result, the true number of sires per brood may be greater than direct-observation estimates based on the assumption of all sires being

heterozygous. There is no *a priori* reason to suspect that the magnitude of any underestimation of multiple paternity would differ on average between the predation regimes in our study. Moreover, estimates of multiple paternity obtained separately by direct observation of genotypes and by a maximum-likelihood statistical method have been shown to be closely correlated across populations in at least one fish species (Trexler *et al.* 1997).

To ascertain the frequency of concurrent multiple paternity in populations within each of the two predation regimes, we calculated the proportion of broods that were multiply sired in each population and then averaged these proportions for each predation regime (low predation and high predation) separately. Because the average female brood size varied between populations (table 1) and could potentially affect the level of multiple paternity that we could detect (Travis *et al.* 1990; Trexler *et al.* 1997), we treated brood size as a covariate and held it constant statistically using ANCOVA when testing for an effect of predation regime on the population level of multiple paternity.

To estimate the genetic diversity within populations, we genotyped 340 and 154 adult females at the TTA and CA loci, respectively. For each locus at each population, we noted the number of alleles present and calculated the expected heterozygosity (Nei & Roychoudhury 1974).

3. RESULTS

Both loci were variable, with the number of alleles detected at each locus varying between two and 17 depending on the population (table 1). Most (56%) alleles were present at a frequency of < 10%. Low-predation populations generally had fewer alleles and exhibited lower heterozygosity at both loci than high-predation populations (table 1). We observed only one case each of an apparent null allele and a mutation. A comparison of the observed and expected frequencies of homozygous genotypes within populations using the χ^2-test (Paetkau *et al.* 1995) did not reveal the presence of null alleles (i.e. an excess of homozygotes) at either locus in any of our study populations ($p > 0.05$ for all 16 tests). However, direct observation of brood genotypes revealed two broods (out of 253) which did not fit the expected Mendelian class types. Regardless of whether these two cases represent null alleles, mutations or scoring errors, they did not prove troublesome to our paternity analysis (cf. O'Reilly *et al.* 1998). We did not detect any mutation at the CA locus. The single mutation detected at the TTA locus represents a mutation rate of 5.5×10^{-4}, which is not atypical of known mutation rates for microsatellite DNA in general (reviewed in Jarne & Lagoda 1996).

We detected multiple paternity in all ten populations (figure 1). Broods originating from high-predation populations on average ($\pm$s.e.) had a significantly higher frequency ($64.2 \pm 8.0\%$) of concurrent multiple paternity than broods from low-predation populations ($24.6 \pm 6.1\%$), with the population-specific mean brood size held constant statistically as a covariate (one-way ANCOVA $F_{1,7} = 8.31$ and $p = 0.024$).

4. DISCUSSION

These findings support our hypothesis that the broods of female guppies originating from high-predation
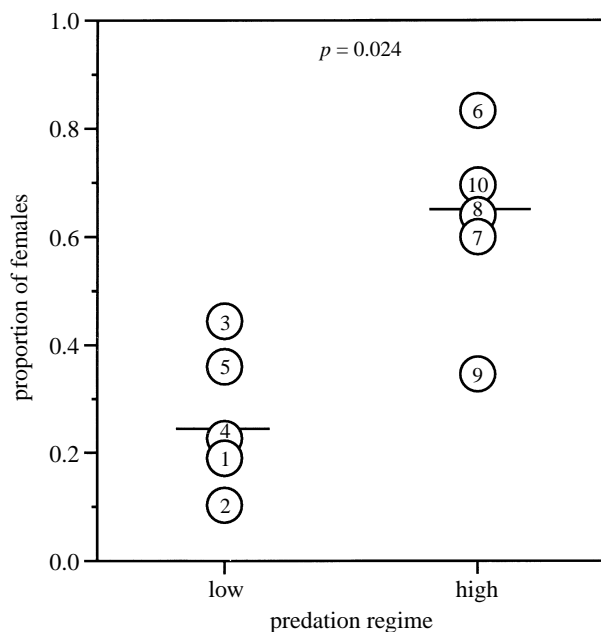
Figure 1. Proportion of females with multiply sired broods observed in each of the ten guppy populations which were categorized as experiencing either low or high fish predation pressure. Numbers inside the data points denote individual population identities (see table 1). The mean proportions of each of the two predation regimes are indicated by the horizontal lines. The difference between regimes was tested using ANCOVA and its statistical significance is noted by the *p*-value.

localities in Trinidad should be more frequently sired by two or more males than the broods of females originating from low-predation sites. This pattern of interpopulation variation in the level of concurrent multiple paternity may have resulted from differences in the predation regimes between guppy populations (Liley & Seghers 1975; Endler 1978, 1995; Houde 1997; Magurran 1999) and, more specifically, from predator-mediated shifts from courtship to sneak copulations in males (Endler 1987; Magurran & Seghers 1990; Magurran & Nowak 1991; Godin 1995) and weakening of female mating preferences (Godin & Briggs 1996; Gong & Gibson 1996) in those populations experiencing a greater predation hazard (table 1). As a consequence, male sperm competition is probably more intense in high-predation populations compared with low-predation populations.

Sperm competition tends to favour the evolution of a high sperm density and ejaculate size in males in general (Birkhead & Møller 1998; Møller 1998). Similar to our prediction of multiple paternity being correlated with predator-mediated shifts in mating behaviour, Matthews *et al.* (1997) independently predicted multiple paternity to be positively correlated with sperm count in the Trinidadian guppy. They found that males from the high-predation Tacarigua River population exhibited proportionately more sneaky matings and possessed higher sperm counts than males from the low-predation Tunapuna River population. Based on their results, we would expect a higher incidence of multiple paternity in females in the former population than in the latter. In the current study, we indeed found proportionately more broods that were

multiply sired in the Tacarigua River (69.6%) than in the Tunapuna River (36.0%).

We attribute the observed pattern of concurrent multiple paternity in our study to predator-mediated shifts in guppy mating and shoaling behaviour. At present, we favour this explanation as the most plausible one for our findings, given the known important effects of fish predators on guppy evolution and the geographical variation in predation pressure among guppy populations in Trinidad (Liley & Seghers 1975; Endler 1978, 1995; Houde 1997; Magurran 1999). However, there are alternative explanations.

First, the adult sex ratio in natural guppy populations is typically female biased, but on average tends to be less so (i.e. proportionately more males) in high-predation populations than in low-predation populations (Seghers 1973; Rodd & Reznick 1997). However, because the differences in the sex ratio between high- and low-predation populations are not consistent and not statistically significant (Seghers 1973; Rodd & Reznick 1997), they do not explain our observed pattern of multiple paternity among the populations studied.

Second, females should tolerate male sneak copulation attempts when the cost of rejecting such unwanted matings is greater than the cost of accepting them (Rowe 1992). If this is the case when the local predation risk is high, then higher frequencies of insemination via sneak copulations and resulting higher frequencies of multiply sired broods might be expected in guppy populations experiencing strong fish predation pressure. To our knowledge, this causal mechanism has not been studied in the guppy.

Third, multiple mating by female guppies in high-predation populations may be under direct selection. By mating multiply, females may gain greater fitness benefits than otherwise, for example through enhanced offspring quality, fertility assurance and/or genetic complementarity (Birkhead & Møller 1998; Møller 1998). This remains to be investigated in the guppy.

Fourth, other environmental factors are strongly correlated with predation regimes in Trinidadian rivers (Endler 1995) and, alternatively, could explain our results. For example, high-predation rivers are typically larger, are more exposed to higher ambient light intensities, have higher water temperatures and have greater benthic flora and microfauna abundances than low-predation rivers (Endler 1995). These factors generally translate into increased food availability for guppies in high-predation localities, which in turn may reduce the required daily foraging time and, thus, allow more time for anti-predator (e.g. shoaling) and mating behaviour (Endler 1995). Notwithstanding the potentially greater amount of time available for mating in high-predation habitats, male guppies nonetheless court less frequently and sneak copulate more often in high-risk than in low-risk localities (Luyten & Liley 1985; Magurran & Seghers 1994). A likely outcome of this is a greater incidence of concurrent multiple paternity in high-predation habitats.

Sneak mating probably undermines female mate choice and may result in multiple mating in females (Farr 1980; Petersen & Warner 1998), thereby potentially reducing the variance in male mating success within a population, particularly in high-predation habitats. Because the intensity of sexual selection is directly related to the variance

in male mating success (Wade 1979; Wade & Arnold 1980), the potential for sexual selection and, thus, the evolution of sexually selected traits in males will be constrained by any predator-mediated increase in the frequency of sneak copulations and weakening of female preferences for certain male traits, such as bright male coloration in the guppy (Houde 1997). Our finding of higher levels of concurrent multiple paternity in populations under strong selection pressure from predators is noteworthy and suggests that the potential for sexual selection in Trinidadian guppy populations covaries inversely with the intensity of local predation. This in turn may increase the intrapopulation gene flow, maintain phenotypic polymorphisms and constrain population differentiation and speciation in the guppy (Endler 1995; Houde 1997; Magurran 1998), as well as in other species (Foster 1999; Foster & Endler 1999).

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