

# Sperm transfer through forced matings and its evolutionary implications in natural guppy (*Poecilia reticulata*) populations

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In species in which individuals alternate between mating strategies, males may respond to elevated predation risk by switching from conspicuous courtship displays to less risky or more profitable sneaky mating attempts. As a consequence, in such species female choice is likely to be undermined more frequently in relatively dangerous localities. We tested this prediction using the guppy, a species of fish in which individual males alternate between courtship (solicited) and forced (unsolicited) copulations according to prevailing levels of predation. We collected females at late stages of gestation from four high- and four low-predation populations in Trinidad and examined them for the presence of sperm in their gonoducts. Due to the patterns of sperm storage in guppies, sperm found in the gonoducts of such late-cycle females can only arise from unsolicited copulations. We anticipated that because female guppies are subject to greater sexual harassment in the form of forced mating attempts in high-risk localities, a higher proportion of females in these populations would contain sperm in their gonoducts arising from recent unsolicited copulations. Contrary to this prediction, only one of the four paired comparisons (from the Quaré River) revealed a significant difference in the proportion of females recently inseminated through forced copulations. The paired comparisons for the remaining three rivers revealed no significant differences in the proportion of females with recoverable sperm in their gonoducts. However, overall, we found that 44.5% ( $\pm 4.3$  SE) of females had sperm in their gonoduct arising from sneaky mating, a figure three times higher than previously reported for this species. We discuss these findings in relation to recent predictions concerning the strength of sexual selection in natural populations. © 2003 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2003, 78, 605–612.

**ADDITIONAL KEYWORDS:** alternative mating tactics – forced copulation – guppies – sexual conflict – sexual selection – sperm competition.

## INTRODUCTION

Predation has been implicated as a major selective force in the evolution of animal reproductive strategies (Lima & Dill, 1990). In particular, conspicuous activities associated with courtship (e.g. mate searching, displaying and mate guarding) frequently place males at increased risk of predation, and the strategies employed by individuals at a given location often reflect the need to balance survival with future mating

success (Lima & Dill, 1990; Magnhagen, 1991). Numerous studies have shown that conspicuous behaviours associated with courtship are suppressed in the presence of predators (e.g. Sih, 1994; Candolin, 1997; Cooper, 1999). Female reproductive behaviour may also be influenced by prevailing levels of predation and a number of studies have demonstrated that under increased risk females become sexually unresponsive (Jennions & Petrie, 1997) or reduce their preference for showy males (Crowley *et al.*, 1991; Godin & Briggs, 1996; Gong & Gibson, 1996). As a consequence of predator-mediated changes in male and female reproductive behaviour, selection can often favour the use of alternative male mating tactics when

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the cost of engaging in conspicuous courtship is high (Andersson, 1994).

In species where males alternate between distinct mating strategies it is possible to study how individuals resolve trade-offs between mating success and the risk of mortality (Lima & Dill, 1990). One species that has been particularly well studied in this regard is the guppy (*Poecilia reticulata* Peters), a small poeciliid fish found in freshwater streams throughout Trinidad. Trinidadian guppies are of particular interest to evolutionary biologists because much of the variation that exists among geographically isolated populations has been attributed predominantly to the selective force of predation (reviewed by Endler, 1995). Importantly, predation imposes costs on the expression of male reproductive behaviour, which in guppies is characterized by two distinct mating strategies. Individual males can achieve copulation by courting females using conspicuous displays or attempting forced copulations in the form of 'gonopodial thrusts' (gonopodium = male intromittent organ) (Baerends, Brouwer & Waterbolk, 1955; Liley, 1966). Courtship by male guppies is characterized by an elaborate 'sigmoid' display used to attract prospective mates, while gonopodial thrusting is a cryptic strategy in which males attempt to forcibly inseminate females without prior display (Houde, 1997). Although individuals employ both strategies interchangeably, numerous studies have revealed that under elevated predation risk, males tend to switch from conspicuous displays to more covert forced copulation attempts (Endler, 1987; Magurran & Seghers, 1990; Magurran & Nowak, 1991; Godin, 1995). The increased reliance on forced copulations by males in high-risk situations is mirrored by a population effect in which individuals inhabiting rivers characterized by high levels of predation rely more heavily on gonopodial thrusting than their upstream (low-predation) counterparts (Endler, 1987; Farr, 1975; Magurran & Seghers, 1990; Magurran & Nowak, 1991).

The facultative use of alternative mating strategies in response to predators can have important implications for sexual selection. It has been argued, for example, that in guppies, female choice may be undermined more frequently in populations that are subject to high levels of predation (Magurran & Seghers, 1994), which in turn may weaken the strength of sexual selection (Endler, 1983; Magurran, 2001) and impede the process of genetic differentiation among divergent populations (Magurran, 1998). However, the frequently reported observation that males rely more heavily on gonopodial thrusting under elevated predation risk (Endler, 1987; Magurran & Seghers, 1990; Godin, 1995; Evans *et al.*, 2002) does not imply that precopulatory female choice is undermined more often in dangerous localities. Indeed, females in high-risk

environments are likely to be under strong selective pressure to avoid the high incidence of (costly) forced copulations that occur in these populations, for example through behavioural or physiological adaptations that reduce the success of the strategy.

In this paper, we report our findings from a recent field survey in which we compared the proportion of females containing sperm arising from recent forced copulations among eight natural populations of guppy in Trinidad. Four of the populations chosen for the study were characterized by high levels of predation and four by relatively low predation intensity. At each of the eight collection points we also estimated the population sex ratio since this parameter is known to influence patterns of male mating behaviour in guppies (Evans & Magurran, 1999; Jirotkul, 1999). We determined (1) whether a greater proportion of females inhabiting high-risk sites contained sperm in their gonoducts resulting from forced copulations, and (2) whether females inhabiting high-predation localities had, on average, higher numbers of sperm arising from the strategy. Our results suggest that insemination efficiency through sneaky mating is largely independent of predation regime, but also that the proportion of females inseminated through forced copulations is far higher than previously estimated. We discuss the implications of these findings for the operation of sexual selection in natural populations.

## METHODS

### POPULATIONS AND COLLECTION TECHNIQUES

The eight populations sampled in this study were located in four rivers encompassing two major river drainages (Caroni and Oropuche) in Trinidad's Northern Range (see Magurran *et al.*, 1995). The fish communities inhabiting these southern flowing rivers are typically South American in origin and include several cichlids, characids and cyprinodontids (Magurran *et al.*, 1995). The eight localities from which fish were collected for the study were characterized by clearly defined levels of predation. Four of the populations were subject to low levels of predation, exerted mainly by the killifish (*Rivulus hartii*); in one of the low-predation populations (Quaré tributary) a species of freshwater prawn (*Macrobrachium carcinus*) was also present (Table 1). Both *Rivulus* and *Macrobrachium* exert relatively minor predation pressure on guppies (Seghers, 1973; Liley & Seghers, 1975; Endler, 1983; Mattingley & Butler, 1994). The four high-predation localities were inhabited by several cichlid (e.g. *Crenicichla alta* and *Aequidens pulcher*), characid (*Astyanax bimaculatus*, *Hemibrycon taeniurus* and erythrinid *Hoplias malabaricus*) species (see Table 1 for predator assemblage information and river characteristics at the collection sites).

**Table 1.** Biological and physical characteristics of river sites

Population	River drainage	National grid reference	Sex ratio*	N	Mean no. sperm extracted (±SE)	River type	Predation intensity	Predator assemblage
Lower Aripo	Caroni	PS 938 786	0.71	50	52 568 (25 988)	OR	High	C, A, Ho, As, H <sup>1-3</sup> , S†
Upper Aripo	Caroni	PS 931 817	0.59	50	36 827 (15 920)	OR	Low	R <sup>1,3</sup>
Middle Tacarigua	Caroni	PS 787 804	0.38	50	37 000 (15 883)	OR	High	C, A, Ho, As, H <sup>1</sup>
Tunapuna (Tac trib)	Caroni	PS 759 797	0.63	50	15 944 (4 984)	P	Low	R <sup>1,4</sup>
Quaré River	Oropuche	PS 971 805	0.61	50	31 450 (14 080)	OR	High	C, A, As, Ci <sup>2,5,6</sup>
Quaré tributary	Oropuche	PS 970 806	0.71	50	8 455 (4 686)	P/R	Low	R <sup>6</sup> M†
Lower Turure	Oropuche	QS 002 784	0.65	50	46 000 (12 976)	OR	High	C, A, Ho, As, S, Ci <sup>4</sup>
Upper Turure	Oropuche	QS 003 809	0.59	26	24 429 (5 835)	P	Low	R†

\*Proportion of females among mature adults at each site (see text for details).

†Species present at site but not indicated in literature.

OR = open river; P = pool; R = riffle; C = *Crenicichla alta*; A = *Aequidens pulcher*; Ho = *Hoplias malabaricus*; As = *Astyanax bimaculatus*; H = *Hemibrycon taeniurus*; S = *Synbranchus marmoratus*; R = *Rivulus hartii*; Ci = *Cichlasoma taenia*; M = *Macrobrachium carcinus*.

References: **1** Magurran & Seghers (1994); **2** Magurran *et al.*, (1992); **3** Endler (1980); **4** Magurran & Seghers (1991); **5** Godin (1995); **6** Endler & Houde (1995).

#### ESTIMATING THE SEX RATIO

Although sex ratios are known to fluctuate considerably over time (Haskins *et al.*, 1961; Seghers, 1973; Rodd & Reznick, 1997), we nonetheless estimated this parameter at each collection point since it is known that the operational sex ratio influences the propensity of males to engage in coercive copulations (Evans & Magurran, 1999; Jirotkul, 1999). At the open river sites (five of eight), fish were collected using a one-person seine. To estimate the population sex ratio at each of these locations, 10 separate seines comprising entire schools were collected. We counted the number of males and females per seine, ignoring juvenile fish that could not be sexed by eye. Specifically, females were included for the sex ratio data if they had non-differentiated anal fins and clearly visible pigmentation around the anal pore (see Houde, 1997). Likewise, males were easily identifiable due to the presence of colour patterns or, in the case of males that were not yet fully mature, by the presence of clearly differentiated anal fins (i.e. emerging gonopodia) and an absence of pigmentation around the anal pore (Houde, 1997). Following each seining event, fish were counted and placed in buckets. After determining the sex ratio,

50 heavily pregnant females were selected (see below) and the remaining fish were returned to the river. At the three sites where fish were collected from pools or riffles (Table 1), dip nets (or where appropriate the seine net) were used to capture every guppy in the pools (and riffle in the case of the Quaré tributary). In all populations, the sex ratio was expressed as the proportion of females among the fish for which sex could be determined (Table 1).

#### SELECTION CRITERIA FOR LATE-STAGE PREGNANT FEMALES

To estimate the proportion of females containing sperm from forced matings, we utilized the ability to recognize females at late stages of pregnancy in which sperm present in the gonoduct can only arise from non-solicited copulations. Female guppies do not exhibit superfetation (carrying two or more clutches at different stages of development) and gestation ranges from 25 to 30 days (e.g. Constantz, 1989). Previous work has confirmed that females do not solicit copulations more than 5 days after parturition (Liley, 1966) and are usually sexually non-receptive within

2–3 days of producing a brood (see also Houde, 1997). Additionally, recent work has confirmed that sperm are present in the female's gonopore for no more than 7 days following copulation (Matthews & Magurran, 2000).

Matthews & Magurran (2000) dissected wild-caught females to determine the developmental stage of their embryos. However, it is a simple procedure to use non-invasive (i.e. non-lethal) techniques to identify females at late stages of pregnancy. Near-term gravid females are easily identifiable due to their heavily distended abdomens (authors' pers. observ.), and through changes in pigmentation of the anal spot, which is enlarged shortly before parturition (Constantz, 1989). At each collection point, 50 females were selected using these criteria and returned to the laboratory at the University of the West Indies for sperm assays (see below). The only exception to this was the Upper Turure population from which only 26 pregnant females were tested. Thus, a total of 376 females were sampled in this study ( $N = 50$  for seven populations +  $N = 26$  for U. Turure). At each river site, test females were selected by one of the authors (JPE) and an experienced research technician (who was not involved in the study) confirmed the choice. A subsequent examination of each female in the laboratory under low-power magnification confirmed our original choice of females. Preliminary observations made prior to the study confirmed that females selected using these criteria always produced offspring within 14 days of isolation (J.P. Evans & A. Pilastro, pers. observ.). Importantly, for the purposes of this study, any sperm found in the gonoducts of females chosen on this basis can only result from unsolicited copulations since more than 7 days would have elapsed since the end of the females' receptive phase (Matthews & Magurran, 2000; see also Constantz, 1984 and references therein).

#### SPERM EXTRACTION AND COUNTS

Females were checked for the presence of sperm within 18 h of collection. Each female was anaesthetized in a water bath containing a mild dose of Benzocaine ( $0.4 \text{ g L}^{-1}$  ethyl p-amino benzoate). When fully subdued, the female was placed in a polystyrene 'cradle' with her genital pore exposed. After measuring standard length, a machine-pulled plastic micropipette (Clark Electromedical Instruments) was used to flush each female's gonopore with  $10 \mu\text{L}$  physiological solution (0.9% saline). The micropipette was used to flush the female's gonopore three times before the resultant sample was placed in a 0.5-mL sample tube containing  $20 \mu\text{L}$  physiological solution. This action was repeated to ensure that all recoverable sperm were retrieved from the gonopore (see Pilastro &

Bisazza, 1999; Matthews & Magurran, 2000). The pipette was washed thoroughly with distilled water between successive sampling events. Preliminary checks on 'sperm-positive' samples were made to ensure that no sperm were present on or in the pipette's tip following this cleaning process. Thus the possibility of cross-contamination among samples was avoided. Each sample was mixed thoroughly using a Gilson (P20) pipette and checked for the presence of sperm under high-power ( $\times 400$ ) magnification.

We noted the presence or absence of sperm in each sample, and the number of spermatozoa (when applicable) per sample using an 'improved Neubauer chamber' haematocytometer. To check for the presence of sperm, we selected at random five separate chambers of the haematocytometer and noted whether sperm cells were present or absent. If sperm were present, we estimated the total number of sperm per sample by multiplying the concentration of spermatozoa in a single chamber by the initial volume of the sample (Matthews, Evans & Magurran, 1997). Thus, we were able to compare the proportion of females containing sperm in their gonoducts and the average number of sperm per female among populations. Following sampling, each female was revived in conditioned fresh water and subsequently placed in a holding aquarium. After the study, all fish were returned to their respective rivers and released at the point of capture.

#### DATA ANALYSES

The proportion of females inseminated through forced mating was compared between high- and low-predation sites for each river (i.e. for Aripo, Tacarigua, Quaré & Turure rivers; see Table 1) using the chi-square test. Sperm count data were not normally distributed and variances were non-homogeneous following log or rank transformation. Thus, to compare the number of sperm retrieved from females in high- and low-predation localities (within each river system) we used the Mann–Whitney *U*-test. All probabilities are two-tailed and corrected for multiple comparisons.

#### RESULTS

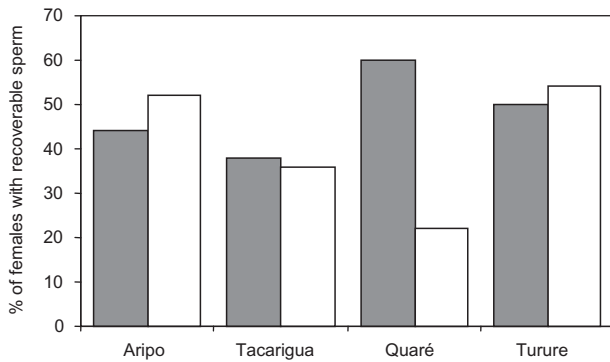
Our analysis revealed that in three of the four rivers (Aripo, Tacarigua & Turure) there were no significant differences between high- and low-predation sites in the proportion of females inseminated through forced mating (chi-square tests:  $\chi^2 = 0.61$ ,  $P = 0.42$ ;  $\chi^2 = 0.43$ ,  $P = 0.84$ ;  $\chi^2 = 0.101$ ,  $P = 0.75$ , respectively; Fig. 1). In contrast, the Quaré river comparison revealed that significantly more females were inseminated through forced copulation attempts in the high-risk group (chi square test:  $\chi^2 = 14.92$ ,  $P < 0.001$ ; Fig. 1). This differ-

ence remained significant after applying the Bonferroni correction for multiple comparisons ( $\alpha = 0.05/4$ ) (Sokal & Rohlf, 1995).

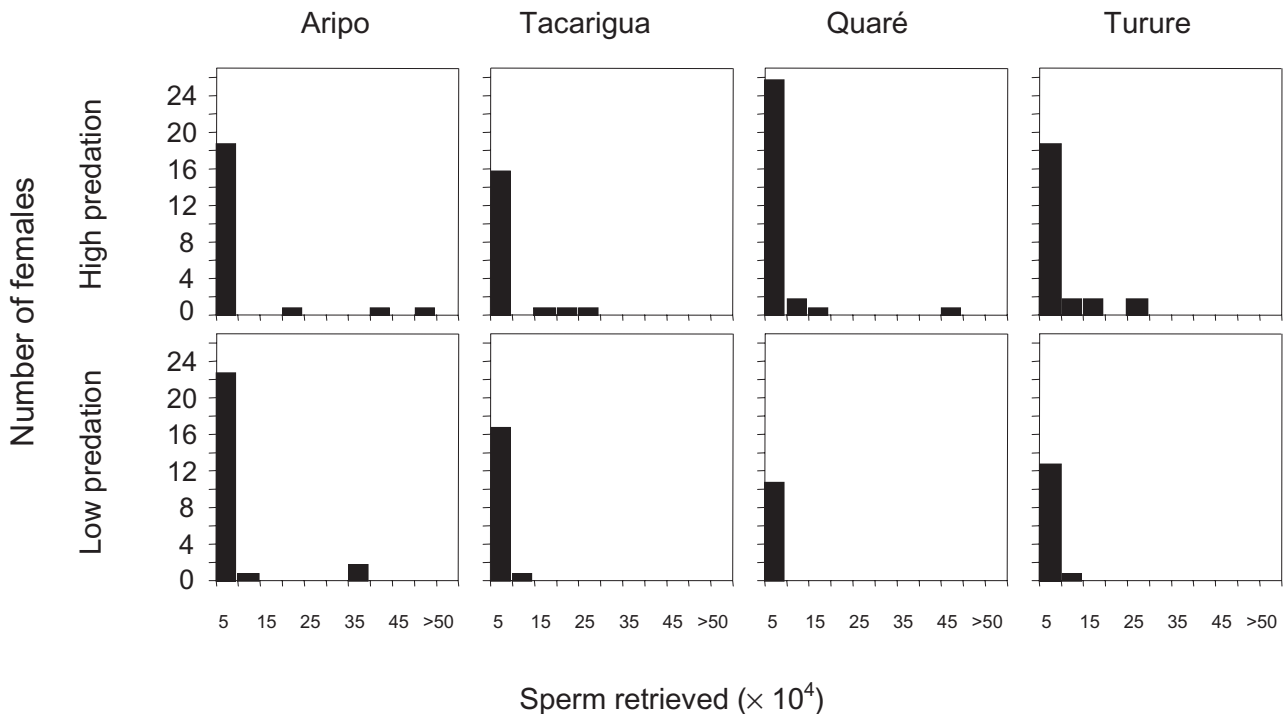
Consistent with the above analysis of the proportion of females inseminated through forced copulations, the Aripo, Tacarigua and Turure paired comparisons revealed no significant difference in the number of sperm retrieved from high- and low-predation fish (Mann–Whitney  $U$ -tests:  $Z_{22,26} = 0.89$ ,  $P = 0.38$ ;  $Z_{19,18} = 0.42$ ,  $P = 0.69$ ;  $Z_{24,14} = 0.24$ ,  $P = 0.83$ ,

respectively). In the Quaré populations, however, high-predation females had significantly more sperm in their gonoducts (Mann–Whitney  $U$ -test:  $Z_{30,11} = 2.36$ ,  $P = 0.017$ ). Interestingly, despite our observation that the mean number of sperm did not differ consistently in the predicted direction, in all four rivers the highest sperm counts were observed in the high-predation females. With the exception of the upper Aripo population, extractions yielding high numbers of sperm (>250 000 cells) were only recoverable from high-predation females (Fig. 2; see also Table 1).

When the data for the proportion of females with recoverable sperm were pooled to include all populations ( $N = 376$ ), an average of 44.5% ( $\pm 4.3$  SE; range 22–60%) of females had sperm in their gonoducts arising from forced matings, a figure three times higher than previously estimated for wild Trinidadian guppies (Matthews & Magurran, 2000). The sex ratio data collected at the eight sites did not vary consistently with the observed proportion of females with sperm, or with the number of sperm retrieved from females in the different populations (see Table 1). Finally, we found that low-predation females were significantly larger than their high-predation counterparts (ANOVA:  $F_{7,363} = 17.72$ ,  $P < 0.001$ ; Tukey HSD: all pairwise comparisons differ significantly at  $P < 0.05$ ), which is consistent with previous work documenting life-history patterns in



**Figure 1.** Percentage of females containing sperm arising from unsolicited copulations in eight populations of guppy in Trinidad. Shaded bars = high-predation sites; white bars = low-predation sites.



**Figure 2.** Frequency distribution of the number of sperm recovered from females from eight Trinidadian populations.

relation to predation regimes in guppies (Reznick & Endler, 1982).

## DISCUSSION

Our results indicate that the proportion of females containing sperm arising from unsolicited copulations is largely independent of predation regime, and thus do not support the widely held view that local levels of predation will mediate the success of the strategy in nature. Additionally, we found no evidence that the sex ratio, as estimated from the proportion of females among the adult fish captured at each site, is related to the proportion of females inseminated through forced copulations. We anticipated a general increase in the frequency of females containing sperm from unsolicited copulations in the high-risk populations, where forced mating attempts by males generally occur most frequently (e.g. Magurran & Seghers, 1994). In contrast, the survey revealed that in three of the four rivers, the proportion of females containing sperm, and the number of sperm recovered from females, do not differ significantly between predation regimes. Only one comparison (Quaré river) provided evidence that females are more likely to have sperm in their gonopores (and in higher numbers) in the high-risk site.

An intriguing possibility that may explain these findings is that females are under stronger selective pressure to avoid unsolicited mating attempts in populations where sexual coercion occurs most frequently. Any adaptation in females which reduces male copulation success would in turn favour counter-adaptations in males to undermine such defences, resulting in sexually antagonistic coevolution of male and female reproductive behaviour or physiology (Rice, 1996; Lessells, 1999). In support of this idea, recent evidence from guppies suggests that the male intromittent organ (the gonopodium) is longer – and presumably more effective – in high-predation populations (Kelly, Godin & Abdallah, 2000). It would be interesting to compare female genitalia and behaviour, and the relative success of sneaky mating in terms of sperm transfer efficiency, among populations to test these ideas further.

The evolutionary implications of sexual harassment in Trinidadian guppies have been discussed recently (Magurran & Seghers, 1994; Magurran, 1998, 2001). Particular attention has focused on the potential for coercive mating to undermine female choice, which is thought to play an important role in the behavioural and genetic differentiation of guppy populations in Trinidad (Endler, 1989; Luyten & Liley, 1991). In guppies, it is generally accepted that sexual selection (via precopulatory mate choice) operates most efficiently in the absence of predation, when the reduced cost of

mate choice facilitates directional selection on preferred male traits through female choice (Houde, 1997 p. 109). When sexual selection is unhindered in this way, geographically isolated populations may differentiate rapidly and ultimately undergo reproductive isolation (Butlin & Ritchie, 1993; Barraclough, Harvey & Nee, 1995). The lack of speciation in the guppy, despite high levels of genetic divergence among geographically isolated populations (Carvalho *et al.*, 1991; Fajen & Breden, 1992; Magurran *et al.*, 1995), has led Magurran (1998) to speculate that 'sneaky' (i.e. forced) mating may impede the process of population differentiation. However, until recently the importance of coercive mating as an effective mating strategy has been questioned (e.g. see Houde, 1997). Indeed, until the publication of two recent studies in which the insemination efficiency of coercive copulations was investigated under experimental conditions (Pilastro & Bisazza, 1999) and in natural populations (Matthews & Magurran, 2000), it was believed that the strategy carried a low rate of success. In the latter study, Matthews & Magurran (2000) reported that 15% of wild-caught females (from the Caroni drainage) contained sperm inseminated through forced mating, but did not specify the populations and predation regimes from which the fish were collected. Our finding that on average nearly 45% (range 22% – 60%) of wild females contained sperm arising from unsolicited copulations suggests that the strategy is likely to be far more important than previously thought.

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## REFERENCES

- Andersson M.** 1994. *Sexual selection*. Princeton: Princeton University Press.
- Baerends GP, Brouwer R, Waterbolk HT.** 1955. Ethological studies on *Lebistes reticulatus* (Peters), I. An analysis of the male courtship pattern. *Behaviour* **8**: 249–334.
- Barraclough TG, Harvey PH, Nee S.** 1995. Sexual selection and taxonomic diversity in passerine birds. *Proceedings of the Royal Society London B* **259**: 211–215.
- Butlin RK, Ritchie MG.** 1993. Behaviour and speciation. In: Slater PJB, Halliday TR, eds. *Behaviour and evolution*. Cambridge: Cambridge University Press, 43–79.

- Candolin U. 1997.** Predation risk affects courtship and attractiveness of competing threespine stickleback males. *Behavioural Ecology and Sociobiology* **41**: 81–87.
- Carvalho GR, Shaw PW, Magurran AE, Seghers BH. 1991.** Marked genetic divergence revealed by allozymes among populations of the guppy *Poecilia reticulata* (Poeciliidae), in Trinidad. *Biological Journal of the Linnean Society* **42**: 389–405.
- Constantz GD. 1984.** Sperm competition in poeciliid fishes. In: Smith RL, ed. *Sperm competition and the evolution of animal mating systems*. Orlando: Academic Press, 465–485.
- Constantz GD. 1989.** Reproductive biology of poeciliid fishes. In: Meffe GK, Snelson FF Jr., eds. *Ecology and evolution of livebearing fishes*. New Jersey: Prentice Hall, 33–50.
- Cooper WE. 1999.** Tradeoffs between courtship, fighting, and antipredatory behavior by a lizard, *Eumeces laticeps*. *Behavioural Ecology and Sociobiology* **47**: 54–59.
- Crowley PH, Travers SE, Linton MC, Cohn SL, Sih A, Sargent RC. 1991.** Mate density, predation risk, and the seasonal sequence of mate choices: a dynamic game. *American Naturalist* **137**: 567–596.
- Endler JA, ed. 1989.** *Conceptual and other problems in speciation*. Cambridge, Massachusetts: Sinauer.
- Endler JA. 1980.** Natural selection on color patterns in *Poecilia reticulata*. *Evolution* **34**: 76–91.
- Endler JA. 1983.** Natural and sexual selection on color patterns in poeciliid fishes. *Environmental Biology of Fishes* **9**: 173–190.
- Endler JA. 1987.** Predation, light intensity and courtship behaviour in *Poecilia reticulata* (Pisces: Poeciliidae). *Animal Behaviour* **35**: 1376–1385.
- Endler JA. 1995.** Multiple-trait coevolution and environmental gradients in guppies. *Trends in Ecology and Evolution* **10**: 22–29.
- Endler JA, Houde AE. 1995.** Geographical variation in female preferences for male traits in *Poecilia reticulata*. *Evolution* **49**: 456–468.
- Evans JP, Kelley JL, Ramnarine IW, Pilastro A. 2002.** Female behaviour mediates male courtship under predation risk in the guppy. *Behavioural Ecology and Sociobiology* **52**: 496–502.
- Evans JP, Magurran AE. 1999.** Male mating behaviour and sperm production characteristics under varying sperm competition risk in guppies. *Animal Behaviour* **58**: 1001–1006.
- Fajen A, Breden F. 1992.** Mitochondrial DNA sequence variation among natural populations of the Trinidad guppy, *Poecilia reticulata*. *Evolution* **46**: 1457–1465.
- Farr JA. 1975.** The role of predation in the evolution of social behaviour of natural populations of the guppy, *Poecilia reticulata* (Pisces: Poeciliidae). *Evolution* **29**: 151–158.
- Godin J-G. 1995.** Predation risk and alternative mating tactics in male Trinidadian guppies (*Poecilia reticulata*). *Oecologia* **103**: 224–229.
- Godin JGJ, Briggs SE. 1996.** Female mate choice under predation risk in the guppy. *Animal Behaviour* **51**: 117–130.
- Gong A, Gibson RM. 1996.** Reversal of a female preference after visual exposure to a predator in the guppy, *Poecilia reticulata*. *Animal Behaviour* **52**: 1007–1015.
- Haskins CP, Haskins EF, McLaughlin JJA, Hewett RE. 1961.** Polymorphism and population structure in *Lebistes reticulatus*, an ecological study. In: Blair WF, ed. *Vertebrate speciation*. Austin: University of Texas Press, 320–395.
- Houde AE. 1997.** *Sex, color, and mate choice in guppies*. Princeton: Princeton University Press.
- Jennions MD, Petrie M. 1997.** Variation in mate choice and mating preferences: a review of causes and consequences. *Biology Reviews* **72**: 283–327.
- Jirotkul M. 1999.** Operational sex ratio influences female preference and male-male competition in guppies. *Animal Behaviour* **58**: 287–294.
- Kelly CD, Godin J-G, Abdallah G. 2000.** Geographic variation in the male intromittent organ of the Trinidadian guppy (*Poecilia reticulata*). *Canadian Journal of Zoology* **78**: 1674–1680.
- Lessells CM. 1999.** Sexual conflict in animals. In: Keller L, ed. *Levels of selection in evolution*. Princeton: Princeton University Press, 77–99.
- Liley NR. 1966.** Ethological isolating mechanisms in four sympatric species of poeciliid fishes. *Behaviour* **13** (Suppl. XIII): 1–197.
- Liley NR, Seghers BH. 1975.** Factors affecting the morphology and behaviour of guppies in Trinidad. In: Baerends GP, Beer C, Manning A, eds. *Function and evolution in behaviour*. Oxford: Clarendon Press, 92–118.
- Lima SL, Dill LM. 1990.** Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology* **68**: 619–640.
- Luyten PH, Liley NR. 1991.** Sexual selection and competitive mating success of male guppies (*Poecilia reticulata*) from four Trinidad populations. *Behavioural Ecology and Sociobiology* **28**: 329–336.
- Magnhagen C. 1991.** Predation risk as a cost of reproduction. *Trends in Ecology and Evolution* **6**: 183–186.
- Magurran AE. 1998.** Population differentiation without speciation. *Philosophical Transactions of the Royal Society of London B* **353**: 275–286.
- Magurran AE. 2001.** Sexual conflict and evolution in Trinidadian guppies. *Genetica* **112–113**: 463–474.
- Magurran AE, Nowak MA. 1991.** Another battle of the sexes: the consequences of sexual asymmetry in mating costs and predation risk in the guppy, *Poecilia reticulata*. *Proceedings of the Royal Society London B* **246**: 31–38.
- Magurran AE, Seghers BH. 1990.** Risk sensitive courtship in the guppy (*Poecilia reticulata*). *Behaviour* **112**: 194–201.
- Magurran AE, Seghers BH. 1991.** Variation in schooling and aggression amongst guppy (*Poecilia reticulata*) populations in Trinidad. *Behaviour* **118**: 214–234.
- Magurran AE, Seghers BH. 1994.** Sexual conflict as a consequence of ecology: evidence from guppy, *Poecilia reticulata*, populations in Trinidad. *Proceedings of the Royal Society of London B* **255**: 31–36.
- Magurran AE, Seghers BH, Carvalho GR, Shaw PW.**

1992. Behavioural consequences of an artificial introduction of guppies, *Poecilia reticulata*, populations in N. Trinidad: evidence for the evolution of antipredator behaviour in the wild. *Proceedings of the Royal Society London B* **248**: 117–122.
- Magurran AE, Seghers BH, Shaw PW, Carvalho GR. 1995.** The behavioural diversity and evolution of guppy, *Poecilia reticulata*, populations in Trinidad. *Advances in the Study of Animal Behavior* **24**: 155–202.
- Matthews IM, Evans JP, Magurran AE. 1997.** Male display rate reveals ejaculate characteristics in the Trinidadian guppy *Poecilia reticulata*. *Proceedings of the Royal Society London B* **264**: 695–700.
- Matthews IM, Magurran AE. 2000.** Evidence for sperm transfer during sneaky mating in wild Trinidadian guppies. *Journal of Fish Biology* **56**: 1381–1386.
- Mattingley HT, Butler MJ. 1994.** Laboratory predation on the Trinidadian guppy: implications for the size-selective predation hypothesis and guppy life history evolution. *Oikos* **69**: 54–64.
- Pilastro A, Bisazza A. 1999.** Insemination efficiency of two alternative male mating tactics in the guppy (*Poecilia reticulata*). *Proceedings of the Royal Society of London B* **266**: 1887–1891.
- Reznick DN, Endler JA. 1982.** The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). *Evolution* **36**: 125–148.
- Rice WR. 1996.** Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. *Nature* **381**: 232–234.
- Rodd FH, Reznick DN. 1997.** Variation in the demographies of guppy populations: the importance of predation and life histories. *Ecology* **78**: 405–418.
- Seghers BH. 1973.** An analysis of geographic variation in the antipredator adaptations of the guppy, *Poecilia reticulata*. University of British Columbia. PhD Thesis.
- Sih A. 1994.** Predation risk and the evolutionary ecology of reproductive behaviour. *Journal of Fish Biology* **45**: 111–130.
- Sokal RR, Rohlf FJ. 1995.** *Biometry*. San Francisco: Freeman.