Geographic variation in sperm production by Trinidadian guppies

Jonathan P. Evans* and Anne E. Magurran

Institute of Environmental and Evolutionary Biology, University of St Andrews, St Andrews, Fife KY16 9TS, UK

Population comparisons yield important insights into adaptive differentiation. However, despite the current interest in sperm competition and spermatogenesis, geographic variation in these traits has received little attention. We tested the hypothesis that sperm production covaries with risk by comparing five natural populations of Trinidadian guppies (*Poecilia reticulata*): two that inhabited dangerous *Crenicichla* localities, two from low-risk *Rivulus* sites and a 'transplant' population comprised of the descendants of guppies moved from one of the high-risk sites to a low-predation environment. As predicted, males from the three low-risk sites performed significantly more courtship displays and had larger sperm reserves than their high-risk counterparts. This result implies higher rates of sperm competition in the low-risk sites.

Keywords: *Poecilia reticulata*; guppy; sneaky mating

1. INTRODUCTION

Sperm competition has been likened to a raffle (Parker 1990a) in which the probability of a male fathering offspring rises in proportion to the number of tickets (sperm) he enters (Parker 1970). It follows that males should invest more heavily in sperm production as the risk of sperm competition increases (Parker 1990b). Marked inter- and intraspecific variation in investment in spermatogenesis, as predicted by sperm competition theory, has now been documented. For example, a comparative study of 89 fish species from 26 families revealed a significant positive association between the intensity of sperm competition and sperm number per stripped ejaculate (Stockley *et al.* 1997). Males also adjust their sperm production in line with the social environment in which they develop or the mating strategy that they adopt. Gage (1995), for instance, found that when male moth larvae (*Plodia interpunctella*) develop at higher population densities, they grow bigger testes and produce ejaculates containing more sperm in anticipation of higher levels of sperm competition. Gage *et al.* (1995) also showed that precocious male salmon parr (*Salmo salar*), which mate by sneaking, have greater numbers of sperm in relation to body size than their anadromous male counterparts.

Although there is considerable interest in the relationship between male mating strategy, sperm competition and spermatogenesis, geographic variation in sperm production amongst populations of a single species has been largely ignored. Yet, as Foster & Endler (1999) pointed out, population comparisons yield important insights into adaptive differentiation. Natural populations often exhibit cascades of effects that stem from the correlation of behavioural, morphological and geographical

traits. One of the best examples of this multiple-trait coevolution is provided by guppy (*Poecilia reticulata*) populations in Trinidad (Endler 1995). Ever since Haskins *et al.* (1961) proposed that geographic variation in male colour patterns results from the interplay of (natural) selection by predators against conspicuousness and the (sexual) selection of colourful males by females (Endler 1978, 1980, 1983) it has been recognized that predators play a key role in generating the remarkable diversity of guppy populations in Trinidad (for reviews, see Endler 1995; Magurran *et al.* 1995; Houde 1997). Indeed, virtually every morphological, life-history or behavioural trait that has been examined is now known to covary with risk, which in turn covaries with biotic and abiotic factors such as forest cover and stream size (Endler 1995). Many of these traits exhibit complex interactions. For example, sexual selection is reduced in high-predation sites due to the combined effects of elevated predator avoidance behaviour, greater sexual harassment, more sneaky copulation, increased food availability, higher temperature and other factors (Endler 1995).

Experimental manipulations in the wild have confirmed that a shift in predation regime results in evolution in guppy populations. For example, Endler (1980) conducted a transplant experiment which demonstrated heritable change in male colour patterns following an amelioration of predation risk. In 1976, 200 guppies were moved from a section of the Lower Aripo River in Trinidad to a previously guppy-free tributary further upstream. Guppies in the Lower Aripo River co-occur with dangerous cichlid and characoid predators including *Crenicichla alta* and *Hoplias malabaricus* (Reznick *et al.* 1990; Magurran & Seghers 1994) whereas the tributary to which the transplanted fishes were moved was originally home to just one species of fish (*Rivulus hartii*) (Reznick *et al.* 1990). *Rivulus* is a member of the Cyprinodontidae and is, at most, a minor predator of guppies (Liley & Seghers

*Author for correspondence (jpe1@st-and.ac.uk).

1975; Magurran *et al.* 1995). Within two years the male descendants of the transplanted guppies had become more conspicuous due to an increase in the size and number of their colour spots. Reznick & Endler (1982) and Reznick *et al.* (1990) demonstrated further evolutionary change in the transplanted population. In most respects, guppies introduced to the transplant site had evolved life-history (Reznick *et al.* 1990) and sexually selected (Endler 1980) traits characteristic of low-predation populations. Artificial introductions in other Trinidadian rivers have provided additional evidence of predator-mediated evolution in behaviour (Magurran 1998) and life history (Reznick *et al.* 1997).

Given the cascades of effects in guppy populations, as highlighted by Endler (1995), it is possible that any geographic variation in sperm production will be complex and unpredictable. Nonetheless, since the strength of female preferences is reduced in high-risk populations (Houde 1997) and female receptivity is further depressed in the presence of predators (Gong 1997), it seems likely that male investment in spermatogenesis will be lower in these populations too. Our aim in this paper is therefore to test the hypothesis that sperm reserves are greater in low-predation populations. To do this, we contrast the sexual behaviour and sperm production of male guppies in Endler's (1980) transplanted population in the Aripo River, Trinidad, with males in two high-predation (*Crenicichla*) populations (including the ancestral one) and two low-risk (*Rivulus*) populations. The inclusion of Endler's (1980) transplant population allows us to control for historical effects and exclude the possibility that observed variation has arisen independently of predation regime. An increased level of sperm production in the transplant and *Rivulus* sites, relative to the *Crenicichla* ones, would provide evidence that sperm competition, like sexual selection, is more intense in low-risk localities.

2. METHODS

(a) *The study populations and their maintenance*

We obtained male and female guppies from five populations: two high-predation populations (Lower Aripo River, grid reference PS 938 786 and Middle Tacarigua River, grid reference PS 787 804), two low-predation populations in the same river system (Naranjo River (Aripo), grid reference PS 936 809 and Tunapuna River (Tacarigua), grid reference PS 759 797) and Endler's (1980) Aripo River transplant population (grid reference PS 932 799). Fishes were returned to the laboratory and maintained in 'stock' aquaria with roughly 1:1 sex ratios and tested within one month of collection. Observations of male mating behaviour were made in a 45 cm × 23 cm × 20 cm deep aquarium containing an airstone, filter and natural gravel, and maintained at 26 °C. The fishes were fed to satiation with commercially prepared flake food (TetraminTM) at least 1 h before mating behaviour was recorded.

(b) *Mating behaviour*

Males from each population were observed in groups of five in the presence of five non-virgin females. The fish were allowed to settle for 1 h before observations commenced. The number of sigmoid displays and gonopodial thrusts performed by each male was recorded over a 10 min period. A sigmoid display is characterized by the male orientating itself in front of a female

and vibrating its body in an S-shaped posture (Liley 1966). Sigmoids were only recorded if they were directed at a particular female. Females are receptive to males either when virgins or in the few days following parturition and are very unlikely to respond to male displays outside this period (Liley 1966). A gonopodial thrust is an alternative mating tactic and does not require female cooperation. During a thrust attempt a male approaches a female from behind, swings his gonopodium (intromittent organ) forward and attempts to insert it in the female's genital opening (Liley 1966). We judged a male to have successfully performed a thrust if his gonopodium made contact with a female's genital region. Differences in male coloration allowed us to recognize each male individually.

(c) *Measuring sperm number*

Following behavioural observations, males were isolated and humanely killed in a water bath containing a lethal dose of 0.4 g l⁻¹ Benzocaine (ethyl *p*-amino benzoate). After removing excess water from each male, the total body length (snout to the tip of the caudal fin) was measured and sperm were extracted. To collect sperm each male was placed on a Petri dish under a low-power dissection microscope. The gonopodium was swung forward and gentle pressure was applied to the side of the abdomen, just anterior to the base of the gonopodium. This action released sperm in the form of a number of spermatozeugmata (sperm bundles). We repeated this procedure in order to ensure all sperm bundles were removed. Following removal, the sperm bundles were drawn up a GilsonTM pipette and added to 100 µl of water. To break down the sperm bundles and distribute sperm cells evenly, samples were repeatedly drawn up and expelled from the pipette. The sperm count was estimated by counting sperm cells on an 'improved Neubauer chamber' haemocytometer under ×400 magnification. The distribution of sperm cells across the haemocytometer was checked visually for evenness before counts commenced. The sperm number was determined by multiplying the mean sperm count (from five counts) by the sample's dilution factor and initial volume. Sperm counts were expressed as the total number of spermatozoa per stripped ejaculate. The sample sizes (number of male fish tested per population) were as follows: high-predation sites, Aripo $n = 24$ and Tacarigua $n = 20$; low-predation sites, Aripo $n = 24$ and Tunapuna $n = 20$; and the Aripo transplant site, $n = 21$.

3. RESULTS

Males from the high-predation sites performed significantly fewer sigmoid displays than those from the low-predation and transplant sites (figure 1a; ANOVA, $F_{4,102} = 10.82$ and $p < 0.001$). This was confirmed using a post-hoc test for multiple comparisons (Tukey test, all values tested at 95% confidence level). Thus, the courtship behaviour of the transplanted males resembled that of males from the low-predation sites more than it did the males from the ancestral (high-predation) site. The rate at which males performed gonopodial thrusts also differed significantly between populations (figure 1b; $F_{4,102} = 8.34$ and $p < 0.001$).

To test the hypothesis that males from low-risk sites have higher sperm reserves than their high-risk counterparts, we compared the sperm counts of males from the five populations. Although the sperm counts appeared to differ between predation regimes (figure 1c), we also found differences in male body size among the populations

Figure 1. Sexual behaviour (a,b), sperm number (c) and body size (d) in male guppies from five localities in Trinidad. Figures are represented by the means $\pm$ s.e. Sample sizes: high predation $n = 24$ and 20, low predation $n = 24$ and 20, and transplant $n = 21$. Stippled bars represent the Tunapuna (low predation) and Middle Tacarigua (high predation) Rivers and open bars represent the Lower Aripo (high predation), Naranjo (low predation) and Endler's (1980) Aripo transplant (low predation) Rivers.

(figure 1d). Males from the high-risk sites were significantly smaller than those from the low-risk sites (ANOVA, $F_{4,102} = 42.9$ and $p < 0.001$). We controlled for body size as a possible confounding factor by performing an ANCOVA with body length as a covariate. The difference in sperm number between the populations remained significant when controlling for body size; males from the three low-predation sites (the two low-predation and transplant sites) had higher reserves of sperm than those from the high-predation sites (figure 1c; $F_{4,101} = 7.34$ and $p < 0.0001$). This was confirmed with a post-hoc test for multiple comparisons (Tukey pairwise test, $p < 0.05$).

4. DISCUSSION

Møller & Ninni (1998) argued that sperm competition is an important component of sexual selection because it increases the variance in male mating success. Our finding, that sperm reserves are higher in the guppies from the low-predation sites, supports the idea that sperm competition, like sexual selection, is more intense when predation pressure is relaxed.

One simple explanation for the observed increase in sperm reserves is that, for reasons to do with water temperature (Liley & Seghers 1975) and selection on life-history traits (Reznick *et al.* 1997), males, which have determinate growth, are larger in *Rivulus* populations. Nevertheless, as our analysis showed, their investment in sperm production cannot be accounted for by body size alone. Males in low-predation populations could also have greater sperm reserves because they have fewer opportunities to mate. We cannot discount this possibility entirely, but note that, when male guppies are housed in standard conditions in the laboratory, with ample access to females, it is the individuals with most sperm that exhibit consistently high courtship rates (Matthews *et al.* 1997). Alternatively, fishes inhabiting high-risk sites may divert resources away from reproduction in favour of those aiding predator avoidance (Havel & Dodson 1987). However, there is no evidence for this in wild guppy populations and, when the problem was investigated for captive descendants of wild guppies, no difference was found between the gonadosomatic index of high- and low-predation males (Matthews *et al.* 1997).

An intriguing possibility is that conventional courtship displays, followed by solicited copulations (as opposed to sneaky matings), require high numbers of sperm to be successful. Males inhabiting low-risk sites tend to rely more heavily on 'conventional' courtship displays to attract females than those in high-risk ones. This was confirmed by our study and our results parallel those of others (Luyten & Liley 1985; Magurran & Seghers 1990; but see Farr 1975). Of course, males may also need larger sperm reserves in low-risk populations if the incidence of multiple mating is higher. As we noted in §1, females in these *Rivulus* sites are more choosy during their receptive phases (Endler & Houde 1995; Houde 1997) and are unlikely to have their receptivity curtailed by the presence of predators. There is an urgent need for data on the relative incidence of multiple paternity in guppy populations in Trinidad.

Although sperm competition may be reduced in high-predation populations it is unlikely to be absent. Guppy populations located in high-risk (*Crenicichla*) localities are less female biased than those in low-risk (*Rivulus*) ones (Seghers 1973; Rodd & Reznick 1997). This means that males in *Crenicichla* sites may have proportionally more competitors for each mating than their counterparts in *Rivulus* ones. The rate at which male guppies encounter one another could be increased further in risky localities as these fishes school more (Seghers 1974; Magurran *et al.* 1992); predation by piscivorous fishes selects for a greater schooling tendency in guppies (Seghers 1974; Magurran *et al.* 1992). Furthermore, for reasons including risk-sensitive courtship behaviour (Magurran & Seghers 1990), males in *Crenicichla* sites generally have a higher incidence of sneaky mating attempts than those in *Rivulus* sites (Magurran & Seghers 1994). Although the high-predation males in our study did not consistently exhibit this strategy, sneaky copulations may demand high reserves of sperm if they are to undermine female choice and compete with previously stored sperm.

A number of factors may account for the differences in male sexual behaviour among populations. These include short-term behavioural responses to predators (Endler 1987), differences in social environment (Rodd & Sokolowski 1995) and genetic variation (Luyten & Liley 1985). It has been shown that the presence of predators influences the degree to which males perform sigmoid displays and gonopodial thrusts (Endler 1987), although this can depend on the predation regime from which the males originate (Magurran & Seghers 1990). Generally, males reduce their sigmoid display rate in favour of sneaky mating as predation risk increases (Endler 1987; Magurran & Seghers 1990; Magurran & Nowak 1991). Since our experimental design was such that males from all localities were observed under standardized conditions, short-term behavioural responses to predators cannot account for the observed difference in behaviour between populations. However, we are not able to rule out the possibility that past experience, rather than inherent genetic variation, shaped the behaviour (and sperm production characteristics) of the males in our study populations. The degree to which males perform sigmoid displays and sneaky mating attempts is closely linked to the population demography they experienced as juveniles, as well as to heredity (Rodd & Sokolowski 1995). Indeed, the intensity of courtship by males in the

Lower Aripo River population has varied over time (Luyten & Liley 1985; Magurran & Seghers 1990, 1994; this study). Although our finding that male display behaviour and sperm number covaried in the transplant population, along with the fact that both traits resembled those seen in the two low-risk sites, is consistent with the evolution of the transplant population, fish from these localities will have to be bred and reared under controlled conditions before any heritable changes can be claimed.

We express our sincere gratitude to Dr Indar Ramnarine and staff at the University of the West Indies, Trinidad, for travel assistance and the loan of equipment essential to our research. Thanks are also due to the Pax Guesthouse, Trinidad, for use of laboratory space and equipment. Leon R. Hockham and anonymous referees provided useful comments on the manuscript. Jeff A. Graves gave statistical advice. Financial support from the Natural Environment Research Council is also gratefully acknowledged.

REFERENCES

- Endler, J. A. 1978 A predator's view of animal color patterns. *Evol. Biol.* **11**, 319–364.
- Endler, J. A. 1980 Natural selection on color patterns in *Poecilia reticulata*. *Evolution* **34**, 76–91.
- Endler, J. A. 1983 Natural and sexual selection on color patterns in poeciliid fishes. *Environ. Biol. Fish.* **9**, 173–190.
- Endler, J. A. 1987 Predation, light intensity and courtship behaviour in *Poecilia reticulata* (Pisces: Poeciliidae). *Anim. Behav.* **35**, 1376–1385.
- Endler, J. A. 1995 Multiple-trait coevolution and environmental gradients in guppies. *Trends Ecol. Evol.* **10**, 22–29.
- Endler, J. A. & Houde, A. E. 1995 Geographical variation in female preferences for male traits in *Poecilia reticulata*. *Evolution* **49**, 456–468.
- Farr, J. A. 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.
- Foster, S. A. & Endler, J. A. 1999 *Geographic variation in behavior*. Oxford University Press.
- Gage, M. J. G. 1995 Continuous variation in reproductive strategy as an adaptive response to population density in the moth *Plodia interpunctella*. *Proc. R. Soc. Lond.* **B 261**, 25–30.
- Gage, M. J. G., Stockley, P. & Parker, G. A. 1995 Effects of alternative male mating strategies on characteristics of sperm production in the Atlantic salmon (*Salmo salar*): theoretical and empirical investigations. *Phil. Trans. R. Soc. Lond.* **B 350**, 391–399.
- Gong, A. 1997 The effects of predator exposure on the female choice of guppies (*Poecilia reticulata*) from a high predation population. *Behaviour* **134**, 373–389.
- Haskins, C. P., Haskins, E. F., McLaughlin, J. J. A. & Hewett, R. E. 1961 Polymorphism and population structure in *Lebistes reticulatus*, an ecological study. In *Vertebrate speciation* (ed. W. F. Blair), pp. 320–395. Austin, TX: University of Texas Press.
- Havel, J. E. & Dodson, S. I. 1987 Reproductive costs of *Chaoborus*-induced polymorphism in *Daphnia pulex*. *Hydrobiologia* **150**, 273–281.
- Houde, A. E. 1997 *Sex, color, and mate choice in guppies*. Princeton University Press.
- Liley, N. R. 1966 Ethological isolating mechanisms in four sympatric species of poeciliid fishes. *Behaviour* **13**(Suppl.), 1–197.
- Liley, N. R. & Seghers, B. H. 1975 Factors affecting the morphology and behaviour of guppies in Trinidad. In *Function and evolution in behaviour* (ed. G. Baerends, C. Beer & A. Manning), pp. 92–118. Oxford, UK: Clarendon Press.

- Luyten, P. H. & Liley, N. R. 1985 Geographical variation in the sexual behaviour of the guppy, *Poecilia reticulata* (Peters). *Behaviour* **95**, 164–179.
- Magurran, A. E. 1998 Population differentiation without speciation. *Phil. Trans. R. Soc. Lond. B* **353**, 275–286.
- Magurran, A. E. & Nowak, M. A. 1991 Another battle of the sexes: the consequences of sexual asymmetry in mating costs and predation risk in the guppy, *Poecilia reticulata*. *Proc. R. Soc. Lond. B* **246**, 31–38.
- Magurran, A. E. & Seghers, B. H. 1990 Risk sensitive courtship in the guppy (*Poecilia reticulata*). *Behaviour* **112**, 194–201.
- Magurran, A. E. & Seghers, B. H. 1994 Sexual conflict as a consequence of ecology: evidence from guppy, *Poecilia reticulata*, populations in Trinidad. *Proc. R. Soc. Lond. B* **255**, 31–36.
- Magurran, A. E., Seghers, B. H., Carvalho, G. R. & Shaw, P. W. 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. *Proc. R. Soc. Lond. B* **248**, 117–122.
- Magurran, A. E., Seghers, B. H., Shaw, P. W. & Carvalho, G. R. 1995 The behavioural diversity and evolution of guppy, *Poecilia reticulata*, populations Trinidad. *Adv. Study Behav.* **24**, 155–202.
- Matthews, I. M., Evans, J. P. & Magurran, A. E. 1997 Male display rate reveals ejaculate characteristics in the Trinidadian guppy *Poecilia reticulata*. *Proc. R. Soc. Lond. B* **264**, 695–700.
- Møller, A. P. & Ninni, P. 1998 Sperm competition and sexual selection: a meta-analysis of paternity studies of birds. *Behav. Ecol. Sociobiol.* **43**, 345–385.
- Parker, G. A. 1970 Sperm competition and its evolutionary consequences in the insects. *Biol. Rev.* **45**, 525–567.
- Parker, G. A. 1990a Sperm competition games: raffles and roles. *Proc. R. Soc. Lond. B* **242**, 120–126.
- Parker, G. A. 1990b Sperm competition games: sneaks and extra-pair copulations. *Proc. R. Soc. Lond. B* **242**, 127–133.
- Reznick, D. N. & Endler, J. A. 1982 The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). *Evolution* **36**, 125–148.
- Reznick, D. N., Bryga, H. & Endler, J. A. 1990 Experimentally induced life-history evolution in a natural population. *Nature* **346**, 357–359.
- Reznick, D. N., Shaw, F. H., Rodd, F. H. & Shaw, R. G. 1997 Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). *Science* **275**, 1934–1937.
- Rodd, F. H. & Reznick, D. N. 1997 Variation in the demographics of guppy populations: the importance of predation and life histories. *Ecology* **78**, 405–418.
- Rodd, F. H. & Sokolowski, M. B. 1995 Complex origins of variation in the sexual behaviour of male Trinidadian guppies, *Poecilia reticulata*: interactions between social environment, heredity, body size and age. *Anim. Behav.* **49**, 1139–1159.
- Seghers, B. H. 1973 An analysis of geographic variation in the antipredator adaptations of the guppy, *Poecilia reticulata*. PhD thesis, University of British Columbia.
- Seghers, B. H. 1974 Schooling behavior in the guppy (*Poecilia reticulata*): an evolutionary response to predation. *Evolution* **28**, 486–489.
- Stockley, P., Gage, M. J. G., Parker, G. A. & Møller, A. P. 1997 Sperm competition in fishes: the evolution of testis size and ejaculate characteristics. *Am. Nat.* **149**, 933–954.