## NOTES

# Geographical variation in the male intromittent organ of the Trinidadian guppy (*Poecilia reticulata*)

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**Abstract**: Sexual selection may favour the evolution of elaborated genital traits in males, particularly when phenotypic variation in such traits results in corresponding variation in reproductive success among males in the population. Compared with insects, very little is known about the natural variation in any male genital trait, and its causes, in vertebrates. Here we report on variation in a male intromittent organ both within and between natural populations of a vertebrate, the Trinidadian guppy (*Poecilia reticulata*). Male guppies inseminate females using an intromittent organ called the gonopodium. We demonstrate that males from populations that have evolved under high fish-predation intensity have, on average, a relatively longer gonopodium than males originating from populations under low fish-predation intensity. Compared with body coloration, the gonopodium exhibited relatively low phenotypic variation, but nonetheless was within the range of known variation for sexually selected traits. The male gonopodium was positively allometric in general. To our knowledge, this is the first report of within-species variation in an intromittent organ and of a positive allometric relationship between male genitalia and body size in a vertebrate species. Our results suggest that the length of the male intromittent organ in the guppy is under selection, which varies geographically.

**Résumé** : La sélection sexuelle peut favoriser l'apparition de caractères génitaux élaborés chez les mâles, particulièrement lorsque la variation phénotypique de tels caractères résulte en une variation correspondante du succès de la reproduction chez les mâles de la population. Comparativement à ce que nous savons des insectes, nous connaissons peu de choses de la variation naturelle des caractères génitaux mâles, ni sur leurs causes, chez les vertébrés. On trouvera ici les résultats d'une étude sur la variation au sein d'une population et d'une population à l'autre de l'organe d'intromission du mâle chez le guppy de Trinidad (*Poecilia reticulata*). Les guppys mâles fécondent les femelles au moyen d'un organe d'intromission, le gonopodium. Nous démontrons que les mâles de la population qui ont évolué avec une forte pression de prédation par les poissons ont généralement un gonopodium relativement plus long que les mâles de populations qui ont moins fait l'objet de prédation. Comparativement à la coloration du corps, le gonopodium a subi une variation phénotypique relativement faible, mais en-deçà des limites de la variation qui affecte ordinairement les caractères apparus par sélection sexuelle. Le gonopodium des mâles a une allométrie positive dans l'ensemble. Nous croyons que cet article est le premier compte-rendu de la variation intraspécifique d'un organe d'intromission et de la relation allométrique positive entre les genitalia mâles et la taille du corps chez un vertébré. Nos résultats indiquent que la longueur de l'organe d'intromission du mâle chez le guppy est soumise à la sélection, laquelle varie géographiquement.

[Traduit par la Rédaction]

## Introduction

In animals with internal fertilization, male intromittent organs show remarkable and rapid morphological diversification (Eberhard 1985; Arnqvist 1997). Eberhard (1985, 1993, 1996) suggested that variation in post-insemination paternity

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success among males is responsible for divergent evolution of genital traits. Although this hypothesis has recently been supported by both comparative (Arnqvist 1998) and experimental (Arnqvist and Danielsson 1999) studies, the evolutionary mechanisms underlying the relationship between variation in genitalia and paternity are poorly understood and require further investigation (Arnqvist 1997; Birkhead 1998). Variation in male genitalia and any associated variation in paternity success among males are thought to be derived from three different, but not mutually exclusive, sexual selection mechanisms, namely, sperm competition (Birkhead and Møller 1998), cryptic female choice (Eberhard 1985, 1996), and conflict between the sexes over the control of fertilization (Arnqvist and Rowe 1995; Alexander et al. 1997).

Evolutionary theory predicts that sexually selected traits, including intromittent genitalia (Arnqvist 1997), should show

**Table 1.** Slope and standard error (SE) of slope of the allometric relationships between the logarithms of body length (BL) and gonopodium length (GL), and between body length (BL) and area of black colour (CA) in male guppies originating from eight Trinidadian populations differing in predation risk.

| Predation regime | Population[a] | Trinidad map grid reference | N | Log(BL) vs. log(GL) | | | Log(BL) vs. log(CA) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Slope | SE | P | Slope | SE | P |
| Low | Marianne (1) | PS 8590 | 50 | 1.403 | 0.192 | 0.021 | 4.813 | 1.394 | 0.004 |
| Low | Tunapuna (2) | PS 7680 | 50 | 1.002 | 0.166 | ns | 5.753 | 1.367 | <0.001 |
| Low | Upper Aripo (3) | PS 9482 | 34 | 0.988 | 0.166 | ns | 6.365 | 1.723 | 0.002 |
| Low | Yarra (4) | PS 8387 | 50 | 1.314 | 0.197 | ns | 4.799 | 1.283 | 0.002 |
| High | Arima (5) | PS 8780 | 30 | 2.092 | 0.568 | 0.033 | 6.029 | 1.459 | <0.001 |
| High | Guanapo (6) | PS 9082 | 50 | 2.002 | 0.468 | 0.019 | 6.338 | 1.630 | 0.001 |
| High | Oropuche (7) | QS 0479 | 31 | 1.164 | 0.270 | ns | 3.950 | 1.078 | 0.005 |
| High | Tacarigua (8) | PS 8084 | 50 | 1.390 | 0.271 | ns | 4.427 | 1.082 | 0.001 |

**Note:** N denotes the number of fish sampled. Allometric values (slopes) were obtained using reduced major axis regression analysis.
[a]The population name corresponds to river name and the numbers in parentheses are the identity numbers.

relatively high phenotypic variation (Price et al. 1993; Rowe and Houle 1996). Moreover, Eberhard et al. (1998) have suggested that sexually selected male intromittent organs should show positive allometric relationships (slopes > 1.0) with body size, similar to those observed in other sexually selected traits (Petrie 1988, 1992; Møller 1991; Green 1992). They predict high allometric values if females use genitalia to evaluate overall male size, which is commonly correlated with competitive ability and survival (Andersson 1994), or if male genitalia function as weapons in intersexual conflicts over control of copulation events (Alexander et al. 1997), much like the weapons used by males in intrasexual competition for mates (Andersson 1994).

To date, studies examining the evolution of elaborated male genital traits by sexual selection have predominantly focused on insects. Comparatively very little is known about the natural variation in any male genital trait, and its causes, in vertebrates. Here we report on variation in a male intromittent organ both within and among natural populations of a vertebrate, the Trinidadian guppy (*Poecilia reticulata*; Poeciliidae).

The guppy has internal fertilization and internal gestation (Houde 1997). The male possesses an intromittent organ, the gonopodium, which is a modified anal fin used to transfer sperm into the female reproductive tract (Houde 1997). Sperm competition (Luyten and Liley 1991; Matthews et al. 1996) and conflict between the sexes over mating decisions (Magurran and Nowak 1991; Magurran 1996, 1998) occur in this species. Female guppies exert mate choice and base their mating preferences on a number of morphological and behavioural traits in males, and on social cues (Houde 1997). Male guppies have two alternative mating tactics. Individuals may either court a receptive female prior to attempting to copulate with her or they may attempt a coerced (sneak) copulation. Sneak copulation entails a male approaching a female from behind and thrusting his gonopodium at the female's urogenital pore without first displaying to her or without a prior receptive response from her. Females typically attempt to avoid sneaking males by rapidly swimming away from them (Houde 1997). Although sneaking is a successful sperm-transfer (insemination) tactic, it is a less efficient one than the courtship tactic in the guppy (Pilastro and Bisazza 1999).

Fish predation on guppies varies geographically in Trinidad (Liley and Seghers 1975; Endler 1978, 1995; Magurran 1999) and is known to affect their morphology, life history, and behaviour (Houde 1997). Males are more inclined to utilize sneak matings when females are preoccupied by the threat of predation (Houde 1997) and males in high-predation localities sneak-copulate more often than males in populations experiencing low predation risk (Magurran and Seghers 1994). This may result in higher frequencies of multiple paternity in females in high-predation populations compared with low-predation populations (Kelly et al. 1999).

Assuming that a longer gonopodium facilitates sneak copulation we predict that the male gonopodium will be, on average, relatively longer in populations that have evolved under high fish predation, and apparently more intense sexual conflict (Magurran and Nowak 1991), than in populations evolving under comparatively low predation. Moreover, we predict that the gonopodium will exhibit a coefficient of phenotypic variation and an allometric value (slope > 1.0) typical of known sexually selected traits. We use standard body length (generally a non-sexually selected, non-genital character) and area of black (melanin) coloration (sexually selected, non-genital character) as morphometric variables for comparison with variation in the gonopodium. We tested these predictions using samples of male guppies collected from each of eight natural populations that have evolved under different fish predation intensity in Trinidad.

## Methods

### Fish collections

We collected a minimum of 30 male guppies from each of four low-predation and four high-predation populations (Table 1) in the Northern Range mountains of Trinidad during the dry season in April–May 1997 and 1998, for a total of 345 fish. These fish were caught using either a beach seine or a dip net (rarely and only when seining was not possible) and immediately preserved in absolute ethanol for analysis several months later.

All rivers were sampled haphazardly at various locations to ensure genetic variability within samples. Each population was categorized as either "low-predation" or "high-predation," based on previous work (Endler 1978, 1995; Houde 1997; Magurran 1999) and our qualitative assessment of predator assemblages during

**Fig. 1.** Scanning electron micrograph (25×) of a typical male gonopodium. "A" denotes the hook and "B" denotes the hood (palp). The longest white horizontal line indicates the measured length of the gonopodium (from its base to the tip of the hook).
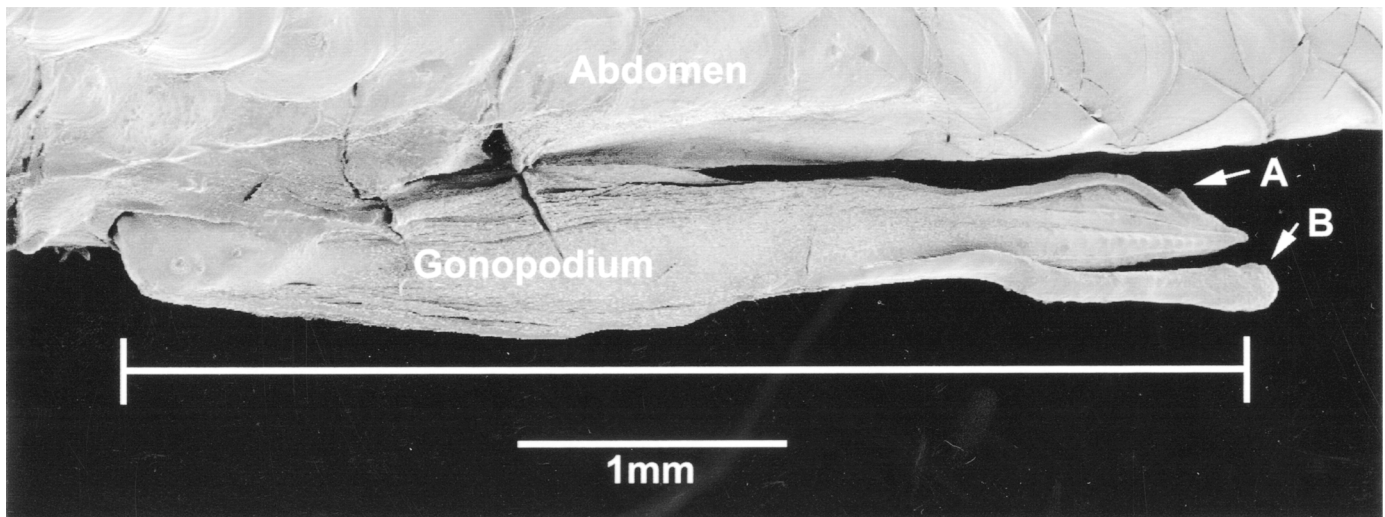


on-site visits. Guppy populations that coexist with piscivorous characin and cichlid fishes, particularly the cichlid *Crenicichla alta*, are generally categorized as experiencing high-predation risk. Those that do not coexist with these predators, but only with the small gape-limited cyprinodontid *Rivulus hartii*, are categorized as low-predation risk.

## Morphometric variables

In the laboratory, morphometric variables of the sampled fish were measured by only one of us (G.A.) to avoid interobserver differences. Measurements were taken blindly of the fish's population of origin. Each male was placed on its right side directly onto a UMAX® Astra 1220S scanner and its image scanned into an Apple Macintosh™ computer. The fish's image was magnified 73× on a 17-in. colour monitor. Using National Institutes of Health public domain image-analysis software (NIH Image, version 1.62, available at <http://rsb.info.nih.gov/nih-image/>), the standard body length, gonopodium length, standard body area (excluding all fins), and the area of each black (melanin) spot on the standard body area were measured from the fish's scanned image. The gonopodium (Fig. 1) was measured from its base on the fish's abdomen to the distal tip of the hook. Measurements of orange (carotenoid) spots were not taken because the carotenoid pigment did not preserve well in absolute ethanol. In the guppy, there is evidence for sexual selection through female choice on melanin pigmentation in males (Endler and Houde 1995; Brooks 1996) and on male body length, but only for a limited number of populations (Reynolds and Gross 1992; Endler and Houde 1995). Following Yezerinac et al. (1992), two measurements were repeatedly taken of each of the traits described above and the mean value for each trait was used in further analysis. Following Lessells and Boag (1987), Yezerinac et al. (1992), and Sokal and Rohlf (1995), we used a model II ANOVA to estimate the repeatability of our measurements (*r*, coefficient of intraclass correlation) and percent measurement error for gonopodium length, body length, body area, and black colour area, separately.

## Statistical analyses

Linear measurements (body length and gonopodium length) were log-transformed to meet the assumption of normality for the ANOVA and ANCOVA (Sokal and Rohlf 1995). The coefficient of variation (standard deviation / mean) for each of body length, gonopodium length, and percent colour area (percentage of male body covered

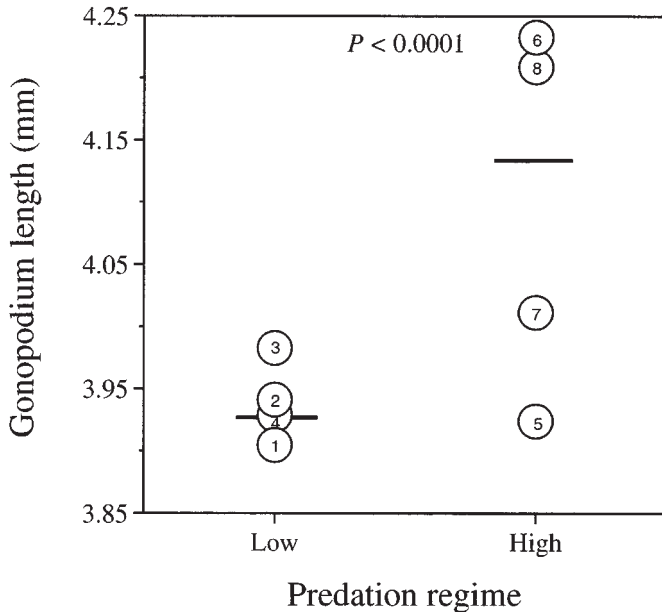on the right side with black pigment) was calculated separately using untransformed data.

Gonopodium length and standard body length were positively correlated in all populations (see Table 1), and standard body length differed significantly between the two predation regimes (two-way ANOVA, $F_{[1,337]} = 104.28$, $P < 0.0001$) and among populations within regimes ($F_{[3,337]} = 37.39$, $P < 0.0001$). Within each predation regime, the population-specific slopes of the relationship between body length and gonopodium length were homogeneous (homogeneity of slopes test, $P > 0.10$ for both regimes). Similarly, between predation regimes, the slopes of the relationship between body length and gonopodium length did not differ significantly (nested ANCOVA, interaction term, $F_{[1,341]} = 3.05$, $P > 0.05$). Consequently, we used the nested ANCOVA (Sokal and Rohlf 1995), with population nested within predation regime, to statistically control for variation in standard body length in testing for differences in mean gonopodium length between predation regimes and among populations. For both the ANOVA and nested ANCOVA, the factors "predation regime" and "populations within regime" were fixed (cf. Bennington and Thayne 1994) and, because sample sizes were unequal among populations, the sums of squares were Type III (cf. Wilkinson 1990).

To test for and compare allometric relationships between body length and gonopodium length and between body length and body area covered by black colour for each population separately, we used reduced major axis regression analysis. This type of analysis is most appropriate because it overcomes the scale dependence of the major axis and because both the dependent and independent variables above are subject to measurement error (Harvey and Pagel 1991; Sokal and Rohlf 1995). If the relationship between gonopodium length and body length were a simple linear (proportional) one, one would expect an isometric relationship with the slope of the regression of log gonopodium length versus log body length equal to 1.0. Similarly, if the relationship between the total area of black (melanin pigment) colour and body length were isometric, then the slope of the regression of log area of black colour versus log body length should also be unity.

To determine whether phenotypic variation in standard body length, gonopodium length and black colour area each differed between predation regimes, we calculated their respective mean coefficient of variation separately for each of the four populations within each predation regime, averaged them, and then compared the average values for the two predation regimes.

All analyses were performed using SYSTAT (Wilkinson 1990) and Microsoft Excel®.

**Fig. 2.** Mean gonopodium length of males (adjusted for individual body standard length) originating from each of eight guppy populations that were categorized as experiencing either low or high risk of fish predation. Numbers inside the data points denote individual population identities (see Table 1). The adjusted mean gonopodium length for each of the two predation regimes is indicated by the horizontal line. The difference between regimes was tested using the nested ANCOVA and its statistical significance is noted by the *P* value.



**Fig. 3.** Coefficient of variation for standard body length ($\bigcirc$), gonopodium length ($\square$), and body area covered by black colour ($\triangle$) in guppies originating from each of eight guppy populations that were categorized as experiencing either low or high risk of fish predation. The mean coefficients of variation for each trait separately, within each of the two predation regimes, are indicated by the horizontal lines. The difference in the mean coefficients for the three traits, within each of the two predation regimes, was tested using Tukey's HSD test and its statistical significance is noted by the *P* values. Population identity numbers inside the data points were omitted for clarity.



## Results

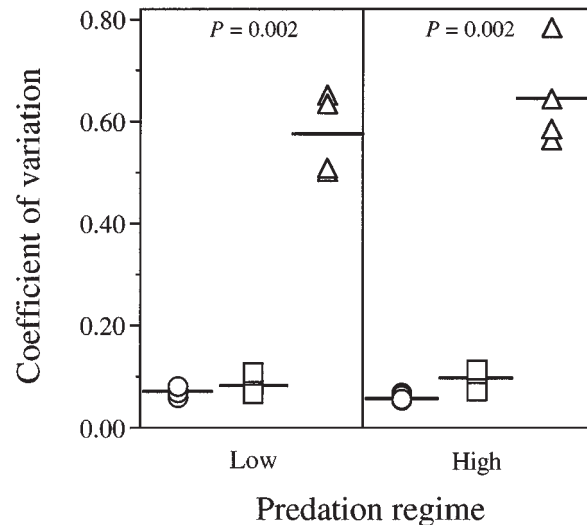### Measurement repeatability and error

All linear and area measurements were highly repeatable ($r$ = 0.979, 0.989, 0.992 and 0.997 for gonopodium length, body length, body area, and black colour area, respectively) and measurement errors were very small (% ME = 2.07, 1.04, 0.79 and 0.27 for gonopodium length, body length, body area, and black colour area, respectively).

### Variation in gonopodium length

As we predicted a priori, the male gonopodium (adjusted for body length) was significantly longer on average (nested ANCOVA, $F_{[1,336]}$ = 14.95, $P$ < 0.0001; Fig. 2) for males originating from high-predation populations ($\bar{x} \pm$ SE = 4.13 ± 0.03 mm, $n$ = 161) compared with males from low-predation populations (3.93 ± 0.03 mm, $n$ = 184). This represents a relative difference of 5.09% in mean gonopodium length between these two predation regimes.

Notwithstanding the aforementioned difference between predation regime, considerable variability in gonopodium length occurred among populations (nested ANCOVA, $F_{[6,336]}$ = 4.11, $P$ < 0.001; Fig. 2). Gonopodium length varied significantly between the populations that experienced high-predation intensity (one-way ANCOVA, $F_{[3,156]}$ = 5.84, $P$ = 0.0008), but not between those experiencing low-predation intensity (one-way ANCOVA, $F_{[3,179]}$ = 0.72, $P$ = 0.54).

### Allometric relationships

Gonopodium length and body length were positively correlated in all populations, but the slope of this relationship was significantly greater than 1.0 for only three of the eight populations (Table 1). In comparison, all populations showed significant positive allometric relationships between area of black coloration and body length. Overall, allometric values for gonopodium length were, on average, significantly lower than allometric values for black colour area (paired *t* test, $t$ = 12.42, df = 7, $P$ < 0.0001).

### Coefficient of variation

The mean coefficients of variation for body length, gonopodium length, and black area differed significantly within each predation regime (two-way ANOVA, $F_{[1,18]}$ = 272.16, $P$ < 0.0001; Fig. 3). The coefficient of variation for black area was significantly greater than the coefficients of variation for body length and gonopodium length in both the high- and low-predation regimes (Tukey's HSD test, $P$ = 0.002 for both predation regimes).

## Discussion

We demonstrated that the length of the gonopodium in male guppies varies both within and among populations in Trinidad, even when differences in body length are statistically controlled. As predicted a priori, males from populations experiencing high local fish predation intensity have, on average, a significantly longer gonopodium than males from low-predation risk populations. The relative difference

in the gonopodium length of males between the two predation regimes was 5.09%, on average, which cannot be attributed solely to measurement error.

Although we do not fully understand the functional significance of these observed differences in gonopodium length, we hypothesize that males possessing a relatively longer gonopodium will be more successful at achieving sneak inseminations, thereby circumventing female mate choice, than counterparts with a shorter gonopodium. This could generate variation in insemination success, and in turn variation in fertilization success, among males within a population. This hypothesis seems reasonable given that, in insects, small differences (<10%) in the size of certain genital traits among males can result in differences in their mating (Preziosi and Fairbairn 1996) and fertilization success (Arnqvist and Danielsson 1999). A test of this hypothesis was beyond the scope of the current study.

In response to increased predation hazard, male guppies increase their rate of sneak mating attempts and reduce courtship display rate, as sneaking behaviour is much less conspicuous to potential predators than courtship (Magurran and Seghers 1990; Magurran and Nowak 1991; Godin 1995). In the wild, male guppies in high-predation populations devote a greater proportion of their time to pursuing females and attempt sneak copulations more frequently than males in low-predation populations (Magurran and Seghers 1994). This finding parallels an intrapopulation pattern found in guppies originating from the high-predation Quaré River by Reynolds et al. (1993). They observed that male guppies possessing a relatively longer gonopodium attempted to sneak copulate more often than those males with a relatively shorter gonopodium. Furthermore, Brooks and Caithness (1995) reported evidence that males with a longer gonopodium are preferred as mates by females in a feral South African guppy population, but they did not statistically control for variation in male body size. Their finding nonetheless suggests that gonopodium length is a sexually selected trait. Our results are consistent with the hypothesis that sexual conflict, which is apparently more intense in high-predation populations (Magurran and Seghers 1994), selects for a longer male gonopodium to circumvent female mate choice, but other sexual selection mechanisms (i.e., female behavioural mating preference, cryptic female choice, and sperm competition) are not excluded (Arnqvist 1997). Another possibility is that predation risk indirectly selects for longer gonopodium by increasing the frequency of use of the sneak-mating tactic in high-predation populations.

Evolutionary theory predicts that sexually selected traits should show relatively high phenotypic variance (Price et al. 1993; Rowe and Houle 1996); this should also be true for intromittent organs that function to increase fertilization success (Arnqvist 1997). The mean coefficients of phenotypic variation ($CV_P$) for the guppy gonopodium in our study were 8.1% and 9.6% in the low- and high-predation regimes, respectively. These values are below the average $CV_P$ (22.3%), but well within the range (3.3–90.5%), for traits experiencing directional sexual selection in general (reviewed in Pomiankowski and Møller 1995).

A recent comparative study of closely related insect species (Arnqvist 1998) revealed that genitalia are more divergent in groups in which females mate several times than in groups in which females mate only once per reproductive episode. Among poeciliid fishes, species that specialize in gonopodial thrusting have relatively longer gonopodia than those specializing in elaborate precopulatory courtship display and female mate choice (Rosen and Tucker 1961). In addition, we have recently shown using genetic paternity analysis that female guppies in high-predation populations are more often multiply inseminated than females in low-predation populations (Kelly et al. 1999), presumably mainly as a result of the higher rates of gonopodium thrusting by males in high-predation populations (Magurran and Seghers 1994). These studies support the proposition that inter- and intra-specific divergence in genital evolution is related partly to differences in mating system and in the intensity of sexual conflict, both of which can be mediated by environmental factors such as predation risk.

To our knowledge, the current study represents the first report of within-species variation in an intromittent organ and of a positive allometric relationship between male genitalia and body size in a vertebrate species. Unlike genital traits in numerous insect species (Eberhard et al. 1998), gonopodium allometric values were all near or above unity (three significantly so), which suggests that gonopodium length is a sexually selected trait (cf. Møller 1991; Petrie 1988, 1992; Green 1992) in the guppy. The allometric values for the guppy gonopodium were lower on average than those for black (melanin) coloration, a non-genital secondary sexual character, perhaps because the intensity of sexual selection on the gonopodium is generally weaker or the intensity of opposing natural selection (e.g., energetic costs; Constantz 1989) stronger, or both, than on melanin colour in the guppy. This remains to be investigated.

The guppy gonopodium displays levels of phenotypic variation and allometries typical of known sexually selected traits, and the intensity of such selection may be indirectly mediated by local predation risk, which varies geographically in Trinidad. At present, we cautiously favour sexual selection as an explanation for the observed population divergence in the guppy gonopodium. Other plausible mechanisms proposed for the evolution of animal genitalia include the lock-and-key hypothesis and the pleiotropy hypothesis (reviewed in Arnqvist 1997). The lock-and-key hypothesis, which posits that genitalia evolve by pre-insemination hybridization avoidance, does not explain the observed differences in gonopodium length between guppy populations in Trinidad because these populations have been allopatric for at least 100 000 years (Fajen and Breden 1992; Magurran et al. 1995), and thus opportunities for interpopulation gene exchange are extremely limited. Our data do not allow for the testing of the pleiotropy hypothesis.

Further investigation into the evolutionary causes and consequences of morphological divergence of the guppy gonopodium is warranted. Following Arnqvist and Danielsson (1999), future studies of genital morphology in the guppy and related poeciliid species should examine the relationship between gonopodium shape–length and fertilization success in individual males. This would allow us to ascertain whether longer or more elaborate genitalia evolve through cryptic female choice and (or) sperm competition in these fish species.

## Acknowledgements

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