

Kit Magellan · Lars B. Pettersson · Anne E. Magurran

Quantifying male attractiveness and mating behaviour through phenotypic size manipulation in the Trinidadian guppy, *Poecilia reticulata*

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Abstract Although many studies have examined the effects of male size on attractiveness and mating behaviour, few have taken genetic background into consideration. Phenotypic manipulation permits the experimental adjustment of morphological traits while keeping genetic background constant. Here, male guppies, *Poecilia reticulata*, an ideal model for this type of manipulation, were raised at different temperatures to produce sibling pairs that differed in size. These were then used to investigate male mating behaviour and male attractiveness, assessed through female mate choice, in relation to this size dimorphism. Further, male–male competition, which is intrinsic to male mating behaviour, is also likely to be affected by their size. Through the use of repeated measures analyses we demonstrate that females significantly prefer larger males and male size and competition significantly affect several aspects of male mating behaviour. Larger siblings perform more sneaky mating attempts and spend more time chasing females. The frequencies of both these behaviours increase with competition. While display frequency is unaffected by male size and competition, display duration and the amount of time spent attending females are reduced in the presence of competitors. This study highlights the use of phenotypic manipulation as a valuable tool for investigating behavioural interactions and confirms that both male size and competition are significant factors in the guppy mating system.

Keywords Male size · Guppy · Mating behaviour · Life history decisions · Phenotypic manipulation

Introduction

Male body size plays a central role in many aspects of sexual selection. Larger males may have direct advantages when competing with other males for mating opportunities (Andersson 1994), but their body size may also be used by females as a cue to assess their overall quality and attractiveness (Jennions and Petrie 1997; Candolin 2003). There is today evidence that females actively choose larger males in groups as diverse as insects (Iyengar et al. 2002), lizards (Censky 1997; but see Olsson and Madsen 1998), mammals (Bro-Jørgensen 2002), and fish (Reynolds and Gross 1992; Quinn and Foote 1994). This female preference is often interpreted from a ‘good genes’ perspective (Møller and Alatalo 1999; Jennions et al. 2001), where size indicates general male vigour, but it can also evolve as a runaway process (Fisher 1958) or through sensory bias for exaggerated phenotypes (Ryan 1994). Males, on the other hand, also experience size effects. In particular, their relative body size commonly influences mating behaviour, e.g., through the expression of conditional mating tactics (Gross 1996; Moczek and Emlen 2000; Brockmann 2002; Aubin-Horth and Dodson 2004), or as context-dependent, flexible mating behaviour (Farr et al. 1986; Rodd and Sokolowski 1995; Shine et al. 2003). Given the attention these questions and sexual selection in general has received during the last decade (e.g., Andersson 1994; Jennions and Petrie 1997; Candolin 2003), it is somewhat surprising that a central feature of size-related effects remains largely unexplored. Traditionally, size-related effects have been investigated using males of unknown relatedness, matched for coloration or other secondary sexual characters (e.g., Andersson 1994; Houde 1997). Unfortunately, this also means that effects attributed to male size *per se* are confounded with differences in genetic background and related covarying factors. However, this problem can be circumvented through

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K. Magellan (✉) · A. E. Magurran
Gatty Marine Laboratory, School of Biology, University of St Andrews,
St Andrews, Fife,
KY16 8LB, Scotland, UK
e-mail: km51@st-andrews.ac.uk
Tel.: +44-7761-567-961
Fax: +44-1334-46-3443

L. B. Pettersson
Department of Ecology, Ecology Building, Lund University,
SE-223 62 Lund, Sweden

phenotypic manipulation (cf. Sinervo and Basolo 1996; Sinervo and Svensson 1998), a developmental approach recently applied to evolutionary biology. By experimentally generating relevant phenotypes rather than working with ones already available, phenotypic manipulation makes it possible to disentangle factors that would otherwise be beyond reach (Sinervo and Basolo 1996; Sinervo and Svensson 1998). Given that care is taken so that relevant treatments are used and unwanted correlated effects avoided (Rose et al. 1996; Sinervo and Svensson 1998), the approach can be successfully used to address life-history effects (Sinervo and Huey 1990), physiological performance (Pettersson and Brönmark 1999), growth strategies (Schmitt et al. 1999) and the expression of secondary sexual characters (Ketterson and Nolan 1999). To use phenotypic manipulation to address the effect of male body size on female choice and male mating behaviour while controlling for genetic background, a system is needed where males have phenotypically plastic growth, female choice is prominent, and male mating behaviour is flexible. The guppy, *Poecilia reticulata*, provides exactly this (Houde 1997; Magurran 2001).

The guppy is a small poeciliid native to Trinidad, where it is widely distributed and found in virtually every freshwater habitat (Magurran and Phillip 2001; Magurran 2001). Guppies have a promiscuous mating system (Houde 1997) with a central feature being female choice for colourful males (Houde and Endler 1990; Houde 1997). In contrast, males switch between two different mating strategies, either courting females using sigmoid display behaviour (Houde 1997) or engaging in forced mating attempts (sneaking), thereby potentially overriding female choice (Magurran 1998; 2001). The relative success of the two types of behaviour may be influenced by male size (Endler 1995). Growth patterns in guppies are phenotypically plastic and can be affected by factors such as water temperature (Liley and Seghers 1975), social environment (Rodd et al. 1997) and food abundance (Reznick et al. 2001). While female guppies have indeterminate growth, males cease growing after maturation (Houde 1997). The fact that male guppies have determinate growth while growth itself is a plastic trait provides a simple means of experimentally producing adult males that differ in size (cf. Sinervo and Basolo 1996). Water temperature during development is a suitable factor to manipulate (cf. Atkinson 1994), since guppy populations experience considerable variation in water temperature in nature (Liley and Seghers 1975; Magurran and Phillip 2001), and that temperature manipulation of ectotherm development generally leads to consistent effects on phenotypes, with low temperatures inducing larger body size and higher temperature leading to a smaller body size (Atkinson 1994; Johnston 2001).

The main aim of this study is to use phenotypic manipulation of male guppies to address effects of male body size on two aspects of sexual selection: male attractiveness, as judged by female choice, and male mating behaviour. The study uses sibling pairs raised at different temperatures in a split-brood design, generating full-sib pairs of small and large individuals. This design allows us to study the ef-

fect of male size separately while controlling for genetic differences. In line with Reynolds and Gross (1992), we expect females to prefer larger siblings. Further, we expect male size to influence the relative use of sigmoid display behaviour and forced mating attempts. In particular, we expect larger males to devote a larger proportion of time to display behaviour. Finally, we expect that the presence of male competitors will increase the overall time that males devote to mating behaviour and that there will be a switch from display to sneaking behaviour.

Methods

Experimental animals

The experimental guppies were descendants of wild-caught fish from the upper Aripo River in Trinidad's Northern Range. This population occurs in a locality where there are few predators, and is conventionally termed a low predation site (Endler 1995). Our goals were to quantify female preferences for male size (Experiment 1) and to examine size-dependent male mating behaviour in relation to male-male competition (Experiment 2). We took advantage of temperature-dependent phenotypic plasticity (Atkinson 1994) to produce eight size dimorphic full-sib pairs. In many ectotherms, including guppies, a lowered rearing temperature induces a larger size at maturity (Atkinson 1994) and can be used generate particular phenotypes while controlling for genetic background and other confounding effects (Sinervo and Basolo 1996; Sinervo and Svensson 1998). The same eight sib-pairs were used in both the experiments.

To standardize the effects of temperature on critical stages of early embryology (cf. Takahashi 1975; Stearns and Kawecki 1994; West-Eberhard 2003), parental males and females of the full-sib broods were raised and mated at intermediate temperature (26°C). Pregnant females were kept at this temperature until giving birth, thereby standardizing embryological development. Broods were then split and siblings transferred to either low (23°C) or high (29°C) water temperature, in which they were raised singly. The median water temperature of guppy sites in Trinidad is 25.8°C, with 90% of the populations within the range of 23–29°C (Liley and Seghers 1975; Magurran and Phillip 2001). The guppies were kept in transparent 5-l PVC jars and had visual contact with other guppies. They were fed live brine shrimp and commercial flake food *ad lib* daily, and apart from water temperature, rearing conditions were identical. Fish in the lower temperature treatment matured more slowly and achieved a larger size than those raised at a higher temperature (paired *t*-test, $t=8.59$, $df=10$, $P<0.0001$) with large males being, on average, 15% bigger than their smaller siblings (mean standard length \pm SE: larger males = 22.3 mm \pm 0.4 mm, smaller males = 19.4 mm \pm 0.6 mm). One large and one small male sibling from each brood were chosen to maximise size difference while matching for colour pattern. Their water temperature was adjusted to 25°C and the fish were then acclimatized to this temperature for a

minimum of 3 months to avoid any short term temperature dependent effects.

Twenty-four virgin females, from the same population as the males (Upper Aripo), were allowed to choose between large and small siblings (Experiment 1). Virgin females are receptive to male courtship, and exhibit clear preferences for particular male phenotypes (Liley 1966; Houde 1997). Wild females are rarely responsive to courtship and are often pursued by several males (Magurran and Seghers 1994). Experiment 2 therefore used non-receptive females and examined the effects of male–male competition when assaying the behaviour of large and small siblings. This set of trials involved a further 15 males (standard length: 15–18 mm) and 15 females from a mixed stock of laboratory-bred descendants from Trinidadian populations. These guppies were housed in separate tanks to ensure absence of familiarity between the sexes (Kelley et al. 1999). Throughout both experiments, fish were kept at 25°C with a 12-h light/dark regime and were fed live brine shrimp *ad lib* daily. Although males were no longer completely naïve of females at the start of Experiment 2, they had had no opportunity to mate and were matched in experience.

Experiment 1

The aim of the first experiment was to quantify female preferences for male size. To do this, two 61 × 30 cm tanks, filled to a depth of 25 cm were marked with vertical lines 22 cm from each side. The central (17 cm wide) area was designated the ‘neutral zone’. Two perforated plastic bottles (34 cm tall × 10 cm diameter) for males were placed against each side. A plastic, non-transparent 15 × 7 cm ‘start box’ with a 3 × 2 cm exit hole facing towards the back of the aquarium was suspended in the centre of the tank. The tank was illuminated from above using a standard 15-W, 45 cm fluorescent aquarium light. All other light sources were removed so that the observer, sitting 1 m away, was not likely to affect fish behaviour. Preliminary trials established that the fish did not react to the observer’s presence. Both tanks had two airstones each and the bottom was covered with gravel. Trials were initiated by placing a pair of male siblings in the tank, one in each bottle. A female was then placed in the start box and allowed to exit at will. If she had not left the start box after 15 min she was replaced with another female and re-tested at a later date. Each trial began when the female left the start box, and for 15 min the following measures were recorded: the time that the female spent in each male’s zone, and the time oriented towards each male whilst in its zone. During the trial, the female was able to see both males from anywhere in the tank and the males were able to see each other. At the end of the trial, all fish were removed. Each female was observed with one pair of males in two trials, with the males exchanging places for the second trial. Each male pair was placed with three different females (six trials), giving 48 trials in total. The order of males and females was randomised, as were the tanks and bottles used.

Experiment 2

The aim of the second experiment was to quantify size-dependent male mating behaviour in relation to male–male competition. All males were tested individually in two conditions, giving 16 trials each with and without competition. The same 61 × 30 cm tanks as in the previous experiment were used, but plastic bottles and start boxes were removed. Five non-virgin stock females, taken at random from the holding tank, were added to the experimental tank. They were fed, then left overnight to acclimate. The next day, the experimental male, either alone (no competition) or with five randomly selected stock males (competition), was added to the tank, fed, then left to acclimate for 2 h. During each 15 min trial the following variables were recorded for each focal male (identified by colour pattern): number of sneaky mating attempts; number of sigmoid displays; cumulative duration of sigmoids; total time spent chasing females; total time attending females. At the end of the observation, the males were removed. Females were left in the tank throughout one day’s observations. If any offspring were found in a tank, all the trials from that tank for that day were excluded from the analysis and the five females therein were not used in any trials for 3 days. This avoided changes in male behaviour due to female receptivity, which is high just after parturition (Liley 1966). The order of experimental males and of trial conditions was randomised.

Statistical procedures

Female preference in Experiment 1 was quantified as time spent with each male and as time spent observing each male whilst being close to it. These two measures were also combined into a choice index which evaluated the relative amount of time spent with each male as follows:

$$\text{Choice index} = \frac{\text{Time spent oriented towards male (s)}}{\text{Time spent in that male's area (s)}}$$

As the time spent with one male affects the time available to spend with the other, it is important to take this effect into account in the analysis (Houde 1997; Wagner 1998). One alternative has been to use the difference in time spent with each male and to standardize this by the total amount of time spent near the males (e.g., Houde 1997). However, this approach does not take the absolute values into account. As the negative correlation between the time spent with the two males is itself potentially dependent on the experimental setup (i.e., decreasing dependency with increasing size of the no-choice zone), we used an alternative method where we directly quantified the significance of the statistical dependency. We did this for all three measures of female preference. The three different measures were each evaluated with SAS Proc GLM (SAS Institute 1999) as a doubly repeated measures design, using family as a random factor (Littell et al. 1996; Hosn 1999; Stevens

2002). The double pairwise structure results from the fact that the first repeated factor (sibling size: large and small) is itself observed as a repeated factor (position in tank: left and right). As a consequence, the dependent measure, female preference, is a function of two repeated factors: size and position, each with two levels. By using a doubly repeated design, we are able to use the full dataset [i.e. three replicates (females) per sibling pair, each encountering the males in the two alternate positions] without either discarding information (Scheiner 2001) or inflating the degrees of freedom (Scheiner 2001; Stevens 2002). The female choice data also allowed us to investigate if females were responding to large and small guppies in a consistent way or if differences that were detected could be explained as correlated responses to males having been raised under cold and warm conditions (cf. Huey and Berrigan 1996; Rose et al. 1996). To quantify this effect, we analysed female preferences as functions of male size as separate (i.e. large and small siblings as two groups) and pooled regressions (Zar 1999). If separate regressions described the preference functions significantly better than the pooled regression, this would indicate that cold-raised and warm-raised males were not judged the same way. Conversely, if the pooled regression described the data well, this indicated that there was no significant correlated effect of the experimental treatment.

Male mating behaviour in Experiment 2 was evaluated using SAS Proc GLM (SAS Institute 1999) with respect to male size and presence or absence of male–male competition. The analysis was carried out in a two-step process. First, the full data set on all five measured male behavioural variables was analysed to assess overall effects. This main model was a mixed model repeated measures design without replication, with one random factor (family), two factorial variables (fixed effects: type of mating behaviour; male size), and one repeated factor (fixed effect: presence and absence of male competitors). Analogous to model formulation in randomised block designs, the main model was reduced by omitting the interaction between the random factor (family) and male size (c.f. Zar 1999). This formulation meant that both fixed factors, their interaction, and the random factor were quantifiable. Having identified significant overall effects, each behavioural variable was analysed separately. Tukey HSD post-hoc tests were used to identify significant differences between experimental groups (SAS Institute 1999; Stevens 2002). Model assumptions were tested using graphical methods according to standard practise (Lynch and Walsh 1998; Zar 1999). When necessary to meet model assumptions, data were $\log(X)$ or $\log(X+1)$ transformed to improve variance homogeneity and normality (Zar 1999).

Results

Experiment 1

Females did not spend more time with either of the two male phenotypes [small males: 308 ± 26 s (mean \pm SE); large

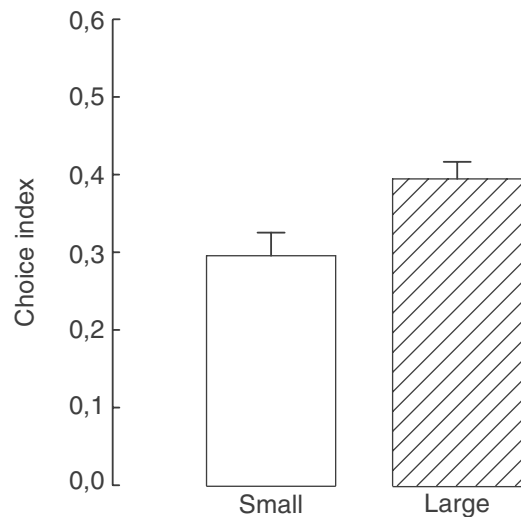


Fig. 1 Mean choice index (+SE), i.e. the ratio of time a guppy female spent oriented towards a male to the time she spent in that male's area. *Small* denotes smaller male sibling, *Large* denotes the larger male sibling

males: 379 ± 32 s; doubly repeated ANOVA, $F_{1,16}=3.40$, $P=0.084$]. However, they did spend more time observing the larger male [small males: 89 ± 11 s; large males: 152 ± 22 s; doubly repeated ANOVA, $F_{1,16}=12.31$, $P=0.003$]. Further, the choice index, i.e. the ratio of time a female spent oriented towards a male to the time she spent in that male's area, showed a strong female preference for larger males (Fig. 1; doubly repeated ANOVA, $F_{1,16}=19.11$, $P=0.0005$). Position effects and interaction effects were non-significant for all three measures (doubly repeated ANOVAs, $F_{1,16}=0.20-1.59$, $F_{7,16}=0.47-2.26$, $P>0.084$). Separate family effects and their corresponding interactions were also non-significant for all three measures (doubly repeated ANOVA, $F_{7,16}=0.72-1.90$, $P>0.14$). There was no evidence of significant negative correlations in the pairwise choice tests, i.e. that the time spent with one male reduced the time available to spend with the other (Pearson $r=0.113-0.257$, $P=0.077-0.446$, $N=48$). All three relations were weakly positive. Male behaviour was not formally quantified during the experiment, but no consistent behavioural differences between the phenotypes were observed (KM, pers. obs.).

The relation between male size and female preferences was similar for both groups of males. When each of the female choice criteria (time with male, time observing male, and the choice index) were regressed against male total length, females responded in the same way to males from the two different rearing conditions (separate vs. pooled regressions, $F_{2,13}=0.001-0.907$, $P=0.428-0.999$). The responses were stronger towards larger males, but regressions were not significantly different. Hence, as differences in male rearing temperature did not translate into significant influences on female preferences, the results suggest that the post-rearing acclimatisation period had been sufficiently long.

Table 1 Multivariate ANOVA of the combined set of five types of male mating behaviour (B, for details see Table 2) in relation to male size (S), and presence of male competitors (C). The model includes family effects (random) and full interactions, but these were all non-significant and are not shown here

Source	Numerator df	Denominator df	F	P
Behaviour (B)	4	4	258.73	<0.0001
Size (S)	1	7	0.03	0.869
Competition (C)	1	7	11.04	0.013
B × Size	4	4	6.64	0.047
B × Competition	4	4	41.18	0.002
S × Competition	1	7	1.41	0.274
B × S × C	4	4	1.29	0.406

Experiment 2

The analysis of the full set of all five measured male behavioural variables showed significant main effects and interactions (Table 1), while overall family effects were non-significant (results not shown). Subsequently, all five variables were analysed separately and four out of five proved to be significantly influenced by the experimental treatments (Fig. 2, Table 2), while family effects remained

non-significant (results not shown). Number of sneaky mating attempts was influenced by both male size and by the presence of competitors (Fig. 2a, Table 2). Larger siblings performed more sneaky mating attempts (mixed model repeated ANOVA, size: $F_{1,7}=5.73$, $P=0.048$; Table 2), and individual sneaking frequency more than doubled when competitors were present (Fig. 2a; mixed model repeated ANOVA, comp: $F_{1,7}=35.47$, $P=0.0006$; Table 2). The number of sigmoid displays were not affected by either male size or the presence of competitors (Fig. 2b; mixed model repeated ANOVA, both main treatments: not significant, see Table 2), while cumulative amount of time spent performing such displays was significantly reduced when competitors were present (Fig. 2c; mixed model repeated ANOVA, comp: $F_{1,7}=42.43$, $P=0.0003$; Table 2). There was no effect of male size on the cumulative amount of time spent performing sigmoids (Fig. 2c; mixed model repeated ANOVA, size: not significant, see Table 2). The fourth variable, the total time spent attending females, showed no difference between large and small siblings (Fig. 2d; mixed model repeated ANOVA, size: not significant, see Table 2), but that males significantly reduced the time attending females when competitors were present (Fig. 2d; mixed model ANOVA, comp: $F_{1,7}=8.46$, $P=0.023$; Table 2). Finally, the total time spent chasing females was

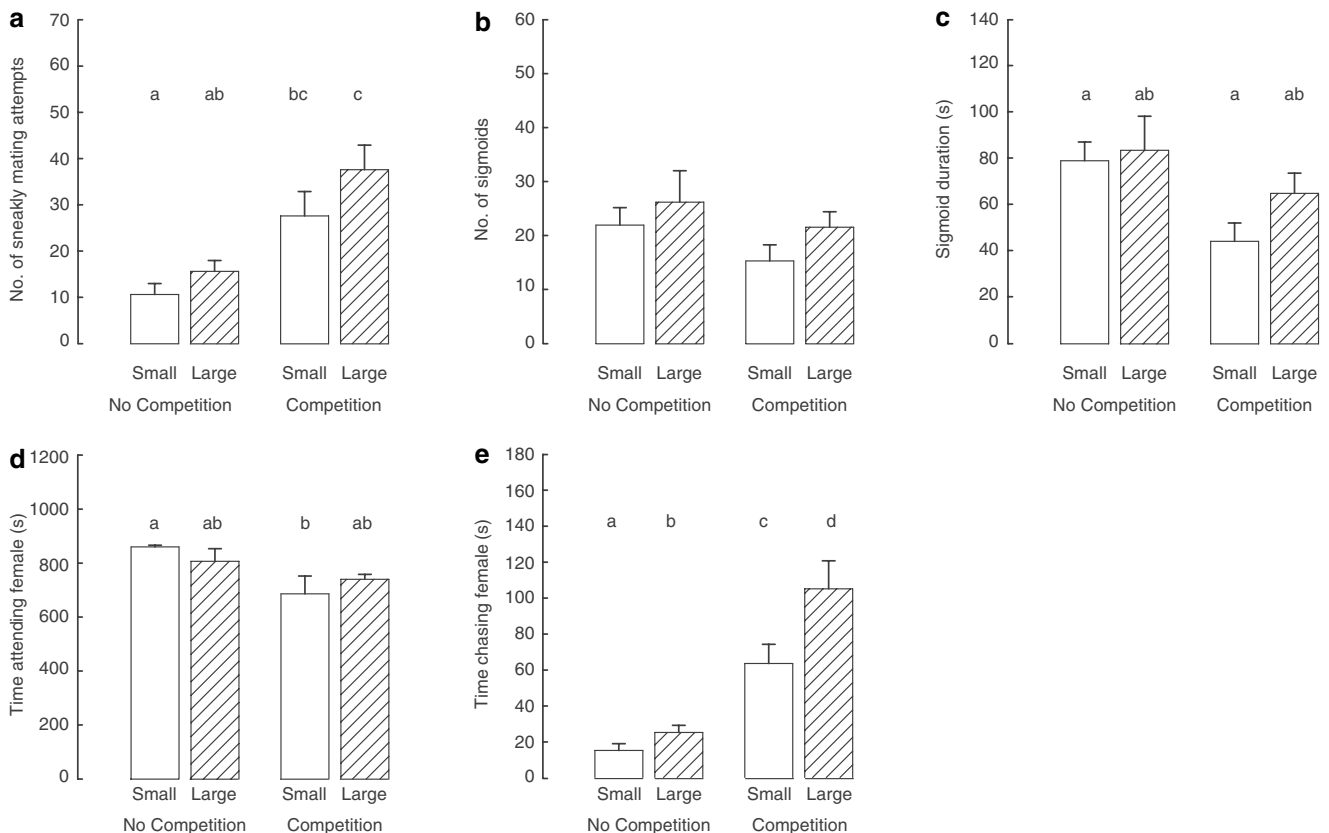


Fig. 2 Behavioural variables during male guppy mating behaviour (mean±SE): **a** the number of sneaky mating attempts; **b** the number of sigmoid displays; **c** the total (cumulative) time spent performing sigmoid displays; **d** the total time spent attending females; **e** the total time spent chasing females. In the *No Competition* treatment,

the experimental male encountered a group of five females, whereas in the *Competition* treatment, the experimental male encountered a group of five males and five females. *Small* denotes smaller male sibling, *Large* denotes the larger male sibling. Letters denote significant differences at $\alpha=0.05$ (Tukey HSD)

Table 2 Separate ANOVAs of five types of male mating behaviour in relation to size (*Size*) and presence of male competitors (*Comp*). *Sneaking* denotes the number of sneaky mating attempts, *sigmoid number* the number of sigmoid displays, *sigmoid duration* the total (cumulative) time spent performing sigmoid displays, *attending* the total time spent attending females, and *chase* the total time spent chasing females. Sneaking was log (X) transformed, and sigmoid duration and chase were log ($X+1$) transformed to meet model assumptions. The models include family effects (random) and interactions between family and *Size*, but these were all non-significant and are not shown here

Behaviour	Source	Numerator df	Denominator df	<i>F</i>	<i>P</i>
Sneaking	Size	1	7	5.73	0.048
	Comp	1	7	35.47	0.0006
	Size	1	7	0.06	0.813
	×Comp				
Sigmoid number	Size	1	7	1.33	0.287
	Comp	1	7	3.70	0.096
	Size	1	7	0.12	0.742
	×Comp				
Sigmoid duration	Size	1	7	0.43	0.531
	Comp	1	7	42.43	0.0003
	Size	1	7	0.33	0.586
	×Comp				
Attending	Size	1	7	<0.01	1.000
	Comp	1	7	8.46	0.023
	Size	1	7	1.68	0.236
	×Comp				
Chase	Size	1	7	9.54	0.018
	Comp	1	7	84.56	<0.0001
	Size	1	7	0.01	0.931
	×Comp				

strongly influenced by both male size and by the presence of competitors (Fig. 2e, Table 2). Larger siblings spent more time chasing females than small siblings did (Fig. 2e; mixed model ANOVA, size: $F_{1,7}=9.54$, $P=0.018$; Table 2), and the presence of competitors increased the chasing of females even further (Fig. 2e; mixed model ANOVA, comp: $F_{1,7}=84.56$, $P<0.0001$; Table 2). There was no interaction between male size and the presence of competitors for any of the behavioural variables (Table 2).

Discussion

Our results demonstrate that experimentally generated male size variation has marked effects on male attractiveness as well as on male mating behaviour. By controlling for genetic background, we quantified the relative importance of a 15% difference in male body size and found that it resulted in a significant female preference for the larger siblings in terms of time spent observing males, as well as in terms of the choice index. Further, the size difference had strong effects on the relative use and intensity of alternative mating behaviours. Larger siblings performed more sneaky mating attempts and spent significantly more time chasing females than smaller siblings. Interestingly, presence of male com-

petitors made this pattern even more pronounced, indicating that the added competitors influenced mating intensity but not the relative use of alternative mating behaviours. Hence, our experimental design provided a precise quantification of how size variation between full siblings translates into male attractiveness and mating behaviour, adding a new way to investigate size-related effects on female choice and male mating behaviour in ectotherms (cf. Andersson 1994; Gross 1996; Jennions and Petrie 1997). In particular, the technique is likely to be a valuable tool to evaluate selection pressures in systems such as the Trinidadian guppy, where population differentiation and local dynamics (e.g., Reznick et al. 1996; Magurran 1998; Croft et al. 2003; Pettersson et al. 2004) generate size variation with potentially far-reaching effects on mating behaviour and sexual conflict (cf. Magurran 1998, 2001).

Previous investigations of female preference for male size in guppies have yielded conflicting results. Although Reynolds and Gross (1992) and Karino and Matsunaga (2002) found that females preferred larger males, Endler and Houde (1995), who examined female preference for a variety of male traits (including size) in 11 guppy populations that ranged across drainages and predation regimes, could not detect a clear trend. There was no preference with respect to size in nine of Endler and Houde's populations, though females from one locality showed a preference for smaller males and there was a preference for larger males in another. Our investigation (Experiment 1) identified a strong preference for larger males when male colour and genetic background is held constant. It would be interesting to determine whether this outcome is repeated in other populations.

Sneaky mating attempts more than doubled under competition as males devoted significantly more time to pursuing females (Experiment 2). This result is in line with Jirotkul's (1999) finding that males sneak most at intermediate sex ratios and Farr's (1976) observation that sneaky mating tends to increase under competition. Unlike Farr 1976 however, we detected no elevation in display rate when males were competing and the total time spent displaying was lower when male competitors were present. Sneaking behaviours, while generally associated with smaller male poeciliids (Hughes 1985; Farr et al. 1986; Travis and Woodward 1989; Ryan and Causey 1989) were not expected to show such a pattern in this case since it is relative size as opposed to absolute size of males that influences mating behaviour (Houde 1997). All males in this study, being individually reared, were large in comparison to stock males, so were not expected to be induced into undertaking 'small male type' behaviour. In fact it was larger siblings who invested most time in mating behaviour, including significantly more sneaky mating attempts, suggesting an additional size related factor at work. Finally, there was no interaction between male size and sneaking (or any other behaviour) indicating that brothers of different sizes respond in the same way to intrasexual competition.

Our investigation shows that phenotypic manipulation can be successfully used to investigate mate preferences and mating behaviour. Rather than altering fully developed

adult phenotypes (e.g., Andersson 1982; Karino and Matsunaga 2002), this approach benefits from involving the organism's developmental machinery and generally results in more thorough responses (Sinervo and Basolo 1996; Sinervo and Svensson 1998). As such, it adds to the growing number of study systems and topics investigated using phenotypic manipulation, e.g., life-history effects (Sinervo and Huey 1990); physiological performance (Pettersson and Brönmark 1999); growth strategies (Schmitt et al. 1999); and secondary sexual characters (Ketterson and Nolan 1999). Of course, unless clones are used, genetic differences can never be completely controlled for, even among full siblings. However, the present approach drastically reduces these effects compared to studies with unrelated individuals. By further matching sibling pairs by colour pattern, confounding influences can be kept at a minimum.

Whenever phenotypic variation is generated by the experimenter, it is important to avoid correlated effects caused by the experimental treatment (Huey and Berrigan 1996; Rose et al. 1996). In particular, early embryology may be sensitive to manipulations (Stearns and Kawecki 1994; West-Eberhard 2003). We therefore choose to keep pregnant females at an intermediate temperature until they gave birth, giving all juveniles a standardized temperature climate during embryological development (cf. Takahashi 1975; Elphick and Shine 1998; Johnston 2001). At birth, juvenile guppies are developmentally advanced (Takahashi 1975; Veggetti et al. 1993) but still retain a considerable flexibility in terms of muscle growth (Veggetti et al. 1993). Subsequent manipulation of developmental temperature primarily affected muscle growth, with secondary sexual characters (e.g., male coloration, gonopodia) responding allometrically (Pettersson and Magurran, unpublished). To avoid behavioural effects of the difference in rearing temperature, siblings were transferred to an intermediate water temperature (25°C) after both males had ceased growing. They were kept at this temperature for a minimum of 3 months to allow for as complete acclimatisation as possible (cf. Huey and Berrigan 1996). As the experimentally generated male size variation had similar influences on female choice in both cold-reared and warm-reared males, this indicated that acclimatisation had been sufficiently long and that correlated, confounding effects of the rearing regime played a negligible role in this system. It should be noted that males raised in the two different temperatures could potentially have experienced long-term effects on behaviour. However, this effect should typically have been the opposite of what we did observe in the experimental trials (e.g., Elphick and Shine 1998; O'Steen and Bennett 2003). A high temperature may induce persistent increases in ectotherm activity (Elphick and Shine 1998; O'Steen and Bennett 2003), but in the present experiment, the smaller males from the 29°C treatment were equally or less active than their larger siblings. Hence, by not disturbing critical stages of early development and by allowing for a thorough acclimatisation after experimental manipulation, our approach allowed us to generate phenotypic variation while keeping other factors controlled (cf. Sinervo and Svensson

1998), a methodology which is likely to be a valuable tool for future studies of behavioural interactions among ectotherms.

This experiment suggests that, all other things being equal, being large is a win-win situation. Not only are large males more attractive to females; they also engage in more mating activity. The latter observation may be a result of increased stamina in larger fish. In laboratory swimming trials, larger males can swim for longer period at higher water velocities (KM pers. obs.) and can presumably also maintain strenuous mating activities for longer (see Blake 1983). Nicoletto (1993) found that male display rate was correlated with sustained swimming performance. Furthermore, as Reynolds and Gross (1992) showed, larger males sire offspring with higher growth rates which results in these larger sons being preferred by females and larger daughters having increased reproductive output (Reynolds and Gross 1992), suggesting that condition dependent selection may be an important factor in male size determination. Integrating condition dependence with genetic effects will be a major challenge for future studies.

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