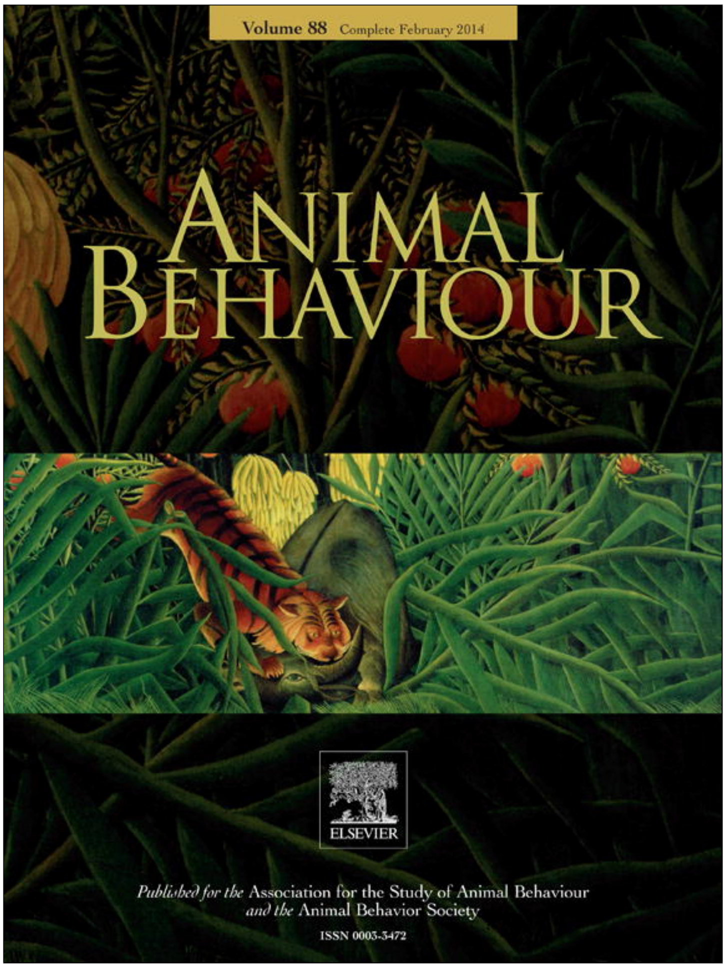

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The effect of social environment on alternative mating tactics in male Endler's guppy, *Poecilia wingei*

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Variation in sexual behaviour has both genetic (no experience or social contact needed) and environmental (based on individual experience) components. Within species, males can maximize their fitness during inter- and intrasexual selection in a variety of ways. This may lead to evolution of alternative male mating behaviours resulting in a dichotomy between courting/guarding and sneaking tactics. We used Endler's guppies to investigate (1) whether individual sexually naïve males have a preferred mating tactic (courting or sneaking), and (2) how male mating behaviour is affected by recent social history (male- or female-biased social environment). We found that individual males approached females by either courting or sneaking, even with no previous sexual experience. We further demonstrated that male sexual behaviour in standardized mating trials was strongly affected following exposure to a sex-biased social environment for 5 weeks. Males from female-biased social environments showed decreased mating effort overall compared to the baseline level (measured prior to assignment to a social environment treatment) but were more likely to court females rather than attempt sneak copulations. Males from male-biased social environments maintained a high level of mating effort and increased the rate of sneaking attempts compared to courtship displays. Our study highlights that both genetic and environmental sources of variation contribute to individual sexual behaviour. Current sexual behaviour was strongly modulated by recent social history, underpinning the importance of previous experience in the expression of sexual behaviour.

Males adopt a variety of ways to maximize their fitness during inter- and intrasexual selection, leading to high interindividual variability in sexual behaviour. Some aspects of sexual behaviour are genetically underpinned and are performed by an individual without any previous experience or social contact (Andersson, 1994). Other components of sexual behaviour and mate preference, however, may be affected by the local conditions an individual experiences. Some behaviour patterns may be learnt through observation of conspecifics or through an individual's own experience (Freeberg, 2000), while others may be influenced by the immediate social environment (Rodríguez, Rebar, & Fowler-Finn, 2013). Individuals vary in their capacity to respond to changes in their social environment, however, and in their ability to modify behaviour (Bretman, Cage, & Chapman, 2011). A degree of such plasticity determines an individual's ability to adapt rapidly to the prevailing social conditions. Indeed, the ability to respond quickly to changes in the social environment through modulation of mating behaviour and mating tactics may have profound consequences for reproductive success (Reichard, Smith, & Jordan, 2004).

Despite generally continuous variation, discrete alternative mating tactics are often readily recognized (Taborsky, 1994). The relative fitness benefit of a particular mating tactic is potentially affected by a variety of environmental, ecological and demographic factors. It has been demonstrated, for example, that predation level (Godin, 1995; Luyten & Liley, 1985), food availability and parasite load (Kolluru, Grether, Dunlop, & South, 2009), water velocity (Magellan & Magurran, 2006), population density (Jirotkul, 1999a), adult sex ratio (Magurran & Magellan, 2007), operational sex ratio (Jirotkul, 1999b; Mills & Reynolds, 2003) and breeding resource abundance (Konečná, Smith, & Reichard, 2010) may all influence the outcome of male–male competition for fertilization and hence the success of individual males and their tactics.

The most common system of alternative male mating tactic is the sneaker–guarder complex (Oliveira, Taborsky, & Brockmann, 2008). Under this system, guarder males maximize their reproductive success through investment in guarding a limiting resource, such as a breeding substrate or females, and often in performing courtship behaviour. Alternatively, males can mate as sneakers, that is, fertilizing females or ova through concealment or

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coercion, and frequently engage in sperm competition (Oliveira et al., 2008; Parker, Ball, Stockley, & Gage, 1997; Taborsky, 1998). In many species, the choice of tactic is flexible, with males switching between the two in response to local conditions (Candolin, 2004; Chapman, Morrell, & Krause, 2009; Reichard et al., 2004). However, it is less clear whether males respond directly to their current state, or whether the expression of mating behaviour is influenced by experience from previous mating attempts.

The costs and benefits of mating tactics differ and can vary across social environments. When competition is greatest, for example, it should be advantageous for at least a proportion of males to act as sneakers and force copulations. Sneaking can circumvent female choice when a particular male is unattractive, unsuccessful or not preferred (Head, Lindholm, & Brooks, 2007). Sneaking can also be less time consuming, increasing opportunities for further mate searching (Parker, 1974). Attempting to mate through cooperative, courtship-based mating will be more favourable when a male is attractive and courtship is expected to lead to successful copulation. In some mating systems at least, males transfer more sperm during cooperative mating (Pilastro & Bisazza, 1999) and there is a greater likelihood of successful female parturition after cooperative rather than forced mating (Sato, Shimoichi, & Karino, 2011).

We used *Poecilia wingei*, a small live-bearing poecilid fish with internal fertilization, to examine whether male mating behaviour is affected by social environment. This guppy is a sister species of the Trinidadian guppy, *Poecilia reticulata*, from coastal northern Venezuela (Schories, Meyer, & Schartl, 2009). The two species share many aspects of reproductive behaviour, which has been well studied and documented for *P. reticulata* (Houde, 1997), making quantification convenient. Male guppies may either court females through sigmoid displays and mate cooperatively or sneak copulate through gonopodial thrusts (Houde, 1997).

In *P. reticulata*, there is evidence for a genetic basis to male sexual behaviour (Evans, 2010), but also for an effect of different rearing environments (Evans & Magurran, 1999; Guevara-Fiore, 2012; but see Magellan & Magurran, 2009), and ecological and environmental factors (Devigili, Kelley, Pilastro, & Evans, 2012; Rodd & Sokolowski, 1995) on the adoption of particular mating tactics or mating behaviour patterns. The effect of social environment on the adoption of male mating tactics, however, remains largely untested. There is some evidence that males are able to modify mating tactics in response to their social environment (Barbosa, Ojanguren, & Magurran, 2013; Jirotkul, 1999b), but how they respond to recent social history is not clear (Evans & Magurran, 1999). The first sexual experience (Guevara-Fiore, Svensson, & Endler, 2012), contact with different numbers and quality of females (Jordan & Brooks, 2012) and comparative evaluation of potential mates (Bateson & Healy, 2005) are known to modify male sexual behaviour. Our study is novel in testing the effect of long-term exposure to biased sex ratios on male mating behaviour.

We investigated the effect of a varying social environment on adult, sexually naïve males. First, we measured whether individual, sexually naïve males had a preferred tactic, as suggested by Magurran and Magellan (2007) for *P. reticulata* and, if so, whether this tactic was modified by social experience. We examined how male mating behaviour was affected by recent social history, manipulated by housing adult males at a particular sex ratio (male- or female-biased) for a period of 5 weeks. Our specific aims were (1) to investigate whether individual sexually naïve males have a preferred mating tactic (courting or sneaking), and (2) how male mating behaviour is affected by recent social history through exposure to a male- or female-biased social environment. We predicted that individual sexually naïve males would already have a preferred mating tactic and that this would be modified by social experience. Specifically, males experiencing a male-biased social environment were predicted to mate more often through gonopodial thrusting (sneaking), as sneaking is more successful when male–male competition for copulation increases and the risk of sperm competition is high (Evans & Magurran, 1999; Mills & Reynolds, 2003). In contrast, males exposed to a female-biased environment were predicted to court females using sigmoid displays, since they had no previous experience of mating competition.

METHODS

Study Species and its Mating Behaviour

Poecilia wingei is closely related to the common (Trinidadian) guppy *P. reticulata* (Schories et al., 2009) and endemic to the Cumaná Region of Venezuela. Despite being recognized as distinct from *P. reticulata* since 1975, it has only recently been formally described (Poeser, Kempkes, & Insbrücker, 2005). The two species can be readily distinguished by morphological traits (e.g. colour pattern), structure of the gonopodium and some features of their behaviour (Houde, 1997; Poeser et al., 2005), but share most behavioural characteristics. After taking into account the behavioural differences described by Poeser et al. (2005), which reflect our own comprehensive observations, it is clear that data on *P. wingei* may be compared with studies on *P. reticulata* with confidence.

Poecilia wingei are sexually dimorphic (Poeser et al., 2005), males being brightly coloured and females dull. Males develop a gonopodium, a modified anal fin that serves to transfer sperm during insemination. Their mating system is promiscuous and nonresource-based (Poeser et al., 2005). Females are courted via a sigmoid display (Poeser et al., 2005), whereby the male arches his body into a sigmoid shape then quickly undulates his body and exposes his bright coloration and spread fins (Houde, 1997). If the male is successful, the female allows him to copulate cooperatively and enables him to insert his gonopodium into her gonopore. The pair then rotate around each other to complete insemination (Houde, 1997). Males also use sneaking tactics, whereby they chase females with their gonopodium in the upright position and try to copulate coercively, with no prior courtship (termed gonopodial thrusting). Male tactics are flexible and there is no indication of genetic determination to these mating tactics (Houde, 1997). Females can be fertilized by gonopodial thrusts at any stage of their reproductive cycle (Farr, 1980) as sperm viability in the female gonoducts is extended (Houde, 1997; López-Sepulcre, Gordon, Paterson, Bentzen, & Reznick, 2013). Behavioural elements typically preceding copulation attempts include gonopodial swings (male moves his gonopodium repeatedly up and down), apparently serving to load the gonopodium with sperm (Liley, 1966), and gonopore nipping, where the male nips the female's gonopore with his mouth. The function of gonopore nipping is unclear but it has been suggested that it allows males to assess female olfactory cues (Herdman, Kelly, & Godin, 2004). Male guppies spend most of their time following and courting females and trying to copulate either cooperatively or coercively (Magurran & Seghers, 1994).

Subjects and Housing

Wild *P. wingei* were collected in 2007 from the Laguna de Los Patos (northern Venezuela), the species' original site of occurrence. The breeding stock was kept as a large outbred population (100 adult fish) in a 120-litre tank (75 × 40 cm and 40 cm deep) in the aquarium facility at the Institute of Vertebrate Biology, Brno, Czech Republic, and fed twice each day with commercial flake food and frozen chironomid larvae. The aquarium was exposed to natural daylight for 10–14 h each day from a glass ceiling and water temperature was maintained at 25 ± 2 °C. Aquarium water was aerated continuously and its quality maintained through an air-driven foam filter and regular water changes. Live plants (*Taxiphyllum barbieri* and *Najas guadalupensis*) were provided as shelter. Over 37 days (7 March to 14 April 2011), juveniles were collected from the stock aquaria and isolated from adults in a separate 72-litre aquarium (60 × 30 cm and 40 cm deep). The juveniles were frequently checked and females (recognized by the development of a black spot around the gonopore) were moved to a separate tank from the males.

Experimental Set-up and Behavioural Assays

At the age of 3–4 months, 65 males, readily distinguished from females by the presence of a gonopodium and nuptial coloration, were placed in individual 2-litre plastic tanks (16 × 10 cm and 12 cm deep). The tanks were visually isolated using opaque barriers and exposed to a natural light regime, with additional light provided by a 40 W daylight spectrum Sun Glo fluorescent tube to maintain 12 h (0800–2000 hours) of light exposure. Water temperatures were maintained at 22–25 °C and each tank was equipped with an artificial plant as a shelter. Males were fed once each day on commercial flake food and the water was changed every 2 weeks. Males were housed under these conditions for 2 months. During this period no data on their behaviour were recorded.

The first tests of male mating behaviour were performed after 2 months. Fully sexually mature males were tested to limit any confounding effects of hormonal changes during adolescence, as adolescent individuals have been shown to be less sensitive to hormonal sex pheromones (Irvine & Sorensen, 1993). Males have been shown to modify their mating behaviour in response to female olfactory cues (Guevara-Fiore, Stapley, Krause, Ramnarine, & Watt, 2010). Sexually naïve males, with no previous experience or contact with receptive adult females, were tested to determine their baseline sexual behaviour.

Mating behaviour of sexually naïve males (Trial 1) was scored in a 6-litre tank (25 × 25 cm and 10 cm deep) with the back and sides covered with black plastic sheets to minimize disturbance. Illumination was provided by a 25 W daylight spectrum Sun Glo lamp positioned above the aquarium, ensuring even lighting throughout the tank. Water was aerated between trials, but not during the behavioural observations. Trial 1 observations were conducted when males were 5–6 months old. This observation allowed the comparison of male post-treatment behaviour using a paired design (male identity treated as random factor).

Experimental females were taken from a tank of virgin females and left for 1 day prior to experimental trials with a group of three adult nonexperimental males from the stock aquarium in order to standardize female sexual receptivity and ensure that females were nonvirgin and in the same reproductive state. We did not use virgin females, as encountering already mated females is a more realistic scenario for males under natural conditions. Male *P. reticulata* can readily distinguish between virgin and even recently mated females and modulate their mating tactics accordingly (Guevara-Fiore, Skinner, & Watt, 2009), and we anticipated that male *P. wingei* had the same capacity. Each female was tested with four experimental males. Tank water was not exchanged between replicates and olfactory cues were not considered. Focal males were not fed for 1 day prior to testing to standardize their metabolic level and sexual motivation.

After completion of each trial, focal males were photographed from both sides under standardized lighting conditions using a Canon EOS Rebel XTi camera fitted with a Sigma Macro 100 mm lens to enable identification through their unique colour patterns. During photography, fish were released into the photographic tank and gently moved to the front part of the tank using a soft sponge. The fish were gently immobilized using the sponge and two photographs were quickly taken. The fish were then returned to their original tanks. No distress was observed during photography.

Upon completion of all first trials, two recently mated females from the stock population were randomly assigned to 33 tanks containing a focal male to create a female-biased social environment consisting of one male and two females. In the remaining 32 focal male tanks, a single male and a single female from the stock population were added to create a male-biased social environment of two males and one female. Mortalities reduced the sample size to 32 males in female-biased environment and 27 males in male-biased environment.

The second male trials were completed after 5 weeks of exposure to the male- and female-biased social environments. Males had unrestricted access to females (and rivals), with full visual, physical and olfactory contact, and gained mating experience within their particular sex-biased social environment. While some focal male mortality was recorded during the treatment phase, this was not associated with the experimental treatments and simply resulted in a smaller sample size during the second trials. Trial 2 testing (after exposure to divergent sex ratios for 5 weeks) followed the same protocol, except that focal males were isolated from all conspecifics for 1.5 days prior to testing in order to enable the males replenish their sperm reserves and standardize mating effort. Previous work on *P. reticulata* suggests that 1 day should be sufficient to restore male sperm reserves (Kuckuck & Greven, 1997 in Evans, Pierotti, & Pilastro, 2003). Behavioural observations took place when males were 7–8 months old. All observations (Trials 1 and 2) were completed between 0900 and 1700 hours.

Upon completion of the second trials, males were returned to their original tanks until natural death, their survival time being recorded. Social environment treatment conditions were maintained by replacing any dead females or nonexperimental males. This procedure was imposed because there is evidence that courtship effort affects longevity (Miller & Brooks, 2005) and different mating tactics have different energetic demands (Cummings & Gelineau-Kattner, 2009).

Scoring Behaviour

A female was allowed to settle for 5 min in the test tank. A randomly chosen focal male was then gently captured, added to the test tank and left for 10 min to settle. Male mating behaviour was then scored for 15 min by a single observer using JWatcher 1.0 software for behavioural scoring (Blumstein & Daniel, 2007). The following male behaviour was scored: the number of sigmoid displays (courtship); gonopodial thrusts (sneaking); gonopodial swings (loading gonopodium with sperm); and gonopore nipping (assessment of female olfactory cues). We further quantified general male interest in the female as the time (s) devoted to interest in the female. We defined male interest as occurring when a male's head was oriented towards the female and there was a maximum distance of 10 cm between the male and female. This behaviour covered the time taken for the male to inspect the female, assess cues related to her reproductive state and her willingness to copulate, and the time taken preparing for a courtship or copulation attempt. After 15 min, the focal male was gently recaptured and returned to his original tank. Fish showed no signs of stress and no aggressive behaviour or injury was observed during the trials. All individuals were fed ad libitum after each trial.

Data Analysis

We tested all behavioural measurements separately as they describe different aspects of the male mating tactic. Given significant correlations between several behaviours, we additionally used principal component analysis (PCA) to construct composite variables. For the PCA, we did not include male interest as it is already a composite variable summing all male behaviours directed towards a female and would be redundant in the PCA. Data from both pre- and post-treatment sets were pooled in a PCA analysis as a single matrix to allow comparison of temporal changes in male behaviour. Axes with eigenvalues >1 were retained for further analysis. The analysis was performed using STATISTICA 10 software (StatSoft, Inc., Tulsa, OK, U.S.A.).

The relationship between treatment and temporal change in each male behaviour was analysed using the general linear mixed model from the nlme package (normal distribution: general male interest, exp-transformed; PCA1; PCA2), and the generalized linear mixed model in the lme4 package (Poisson distribution with log-link function: number of sigmoid displays, gonopodial thrusts, gonopodial swings and gonopore nipping per 15 min) from the R environment (R Development Core Team, 2009). This approach enabled us to account for the paired design (male identity treated as a random factor) and repeated use of the same females. The independent variables were Trial (pre- and post-treatment), Treatment (male- and female-biased environment) and their interaction. The significance of the interaction would indicate that the two treatments influenced males differently. To reveal temporal changes in behaviour within treatments, we re-ran the analyses from each treatment separately (within male- and female-biased environments) and compared behaviours before the treatment was imposed (standard social environment) against the same behaviour after exposure in female- or male-biased treatments. Finally, we compared male survival between the two treatments using the log-rank test.

Ethical Note

Fish were housed according to approved institutional and governmental experimental procedures and all authors hold a licence for conducting experimental work on vertebrates. No distress or aggression was observed over the course of the study; all behaviour concurred with natural behaviour of the study species. Mortalities were observed during the progress of the experiment but were not associated with experimental procedures; tracking the survival of experimental fish formed part of the experimental protocol. Life span in the experiment was considerably higher than in natural populations (López-Sepulcre et al., 2013). We minimized the number of fish used in the study through a trade-off between sample size and statistical power, our sample size being chosen according to comparable studies on Trinidadian guppies. The subjects came from a captive population. The treatment tank environment was enriched by the use of live and artificial plants and all fish were exposed to natural daylight and an optimum water temperature.

The original stock was collected by a conservation breeder of wild populations of poecilid fishes. Fish for our breeding stock were obtained noncommercially, directly from the collector. The fish were collected by hand net, thereby minimizing any impact on other native organisms. The fish were imported in large plastic bags at a density of no more than four fish per bag. No mortalities were recorded during import. In captivity, fish were always kept in environmentally enriched tanks (e.g. gravel, live aquatic plants) and fed ad libitum. The sexes were not separated. When moved between tanks, fish were gently captured using an aquarium hand net and placed in a plastic jar containing aquarium water and released into the new tank. Fish were observed until their natural death, which was part of the experimental protocol. The research protocol adheres to the ASAB/ABS Guidelines for the Use of Animals in Research. Experimental procedures were approved by the ethical committees of the IVB and the Ministry of Agriculture (CZ 62760203) and are in accordance with Czech legal requirements.

RESULTS

Associations between Mating Behaviours

The PCA on male behaviour data produced two axes with eigenvalues >1 (Table 1). The first (PCA 1) explained 43% of variation and was strongly negatively associated with gonopodial thrusts (sneaking attempts) and gonopodial swings (loading sperm reserves into the gonopodium). The second (PCA 2) explained 27% of variation and was strongly negatively associated with sigmoid displays (cooperative mating).

Univariate analysis of sexually naïve males (Trial 1) indicated a negative correlation between number of sigmoid displays and gonopodial thrusts (Spearman correlation: $r_S = -0.28$, $N = 59$, $P = 0.035$), suggesting that, in the absence of any sexual experience, individual males tended to approach females by either courting or sneaking, although the variation was not discrete. Males with a stronger general interest in females performed more gonopore nips (Spearman correlation: $r_S = 0.34$, $N = 59$, $P = 0.008$) and gonopodial thrusts ($r_S = 0.37$, $N = 59$, $P = 0.004$). Sigmoid displays were positively associated with gonopodial swings ($r_S = 0.59$, $N = 59$, $P < 0.001$).

After treatments were imposed (Trial 2), male interest remained positively associated with the number of gonopore nips in female-biased environments and with gonopodial thrusts in male-biased environments (Table 2). Additionally, a positive correlation between male interest and sigmoid displays was observed in female-biased environments and, in both treatments, gonopodial swings were positively associated with gonopodial thrusts rather than sigmoid displays (Table 2).

Treatment Effects on Male Mating Behaviour

The effect of social environment on male sexual behaviour was tested using general (composite variables) and generalized mixed models, where interaction between Trial and Treatment factor indicated whether social environment affected male behaviour differently. As significant interactions indicated that social environment did indeed affect male sexual behaviour (Table 3, Figs 1, 2, Supplementary Material S1), we then analysed the temporal pattern of change in all behaviours separately for each social environment (Table 3). Males from male-biased treatments showed increased attention to females, largely resulting from a high rate of gonopodial thrusting (Table 3, Fig. 1). In contrast, males from female-biased treatments showed more limited attention to females and expressed lower copulation-oriented behaviour compared to the level prior to the imposition of the experimental treatment (Table 3, Fig. 1). As a result, males from male-biased

Table 1
Proportion of variance explained (in %), eigenvalues (in parentheses) and factor loadings for the first two principal components from behavioural data (baseline and post-treatment data pooled)

	PC 1	PC 2
Variance (eigenvalues)	42.8 (1.71)	26.9 (1.08)
Gonopodial swings	**−0.760**	−0.231
Gonopore nipping	−0.568	0.001
Sigmoid displays	−0.131	**−0.953**
Gonopodial thrusts	**−0.806**	0.372

Two other components had eigenvalues <1. Significant factors are shown in bold.

Table 2
Spearman rank correlation associations between individual behaviours following five weeks of social environment treatment

	Gonopodial swings		Gonopore nipping		Sigmoid displays		Gonopodial thrusts	
Bias in environment	Female biased	Male biased	Female biased	Male biased	Female biased	Male biased	Female biased	Male biased
Male interest	0.317	0.380	**0.553**	−0.022	**0.428**	−0.098	0.373	**0.661**
Gonopodial swings			0.244	−0.366	0.216	0.260	**0.670**	**0.547**
Gonopore nipping					0.259	−0.209	0.058	0.042
Sigmoid displays							0.191	−0.130

Female-biased environment ($N = 24$ males); male-biased environment ($N = 17$ males). Significant correlations ($P < 0.05$) are shown in bold.

treatments showed higher rates in all copulation-oriented behaviours (except gonopore nipping), related to either cooperative or forced copulations (Table 3, Figs 1, 2). For composite variables (PC1 and PC2), significant variables were negatively loaded to the PCs; hence in Fig. 2 an increase denotes a decrease in the particular behaviour and vice versa.

Treatment Effect on Male Longevity

There was no significant difference in longevity between males from male-biased and female-biased treatments (log-rank test: $P = 0.836$). Median survival was 274 days and 90% survival was 451 days (Fig. 3).

DISCUSSION

We studied the effect of social environment on mating tactics of male Endler's guppies. We demonstrated that even sexually naïve males, with no prior mating experience, showed a tendency to approach females by either sneaking or courting behaviour, although variation was continuous rather than discrete. This finding corroborates previous evidence that alternative mating behaviour in guppy males has a genetic component (Evans, 2010). Although guppy males court each other in the absence of females (Field & Waite, 2004), interaction with an adult female, along with the female's response, is necessary for the adoption of the most efficient mating tactic leading to successful copulation. Indeed, inexperienced males often do not copulate successfully during their first sexual encounter (Japanese quail, *Coturnix japonica*: Cornil & Ball, 2010) and males reach their maximum sexual performance only after several copulations with females, irrespective of potential previous homosexual attempts (goat, *Capra hircus*: Imwalle & Katz, 2004). These findings demonstrate the crucial role of female behavioural cues on effective male mating behaviour in some taxa. The behaviour of sexually naïve males in the first trial, therefore, was likely to be innate, rather than learned through interactions with other males.

We found that males exhibiting greater general interest in females also performed more gonopore nipping. As gonopore nipping appears to be used for detecting olfactory cues released by females (Herdman et al., 2004), males spent an extended period assessing female reproductive state. As males can discriminate between virgin and recently mated females (Guevara-Fiore et al., 2009), they may alter their reproductive effort and mating tactic (sneaking or courting) on the basis of these female cues (Guevara-Fiore, Stapley, & Watt, 2010). Males that invested in courtship (sigmoid displays) prior to copulation frequently performed gonopodial swings, perhaps to load the gonopodium with sperm (Liley, 1966). The adoption of gonopodial swings by males may increase the amount of sperm immediately available prior to a cooperative copulation with females, enabling them to transfer a larger volume of sperm (Pilastro & Bisazza, 1999).

We experimentally demonstrated the effect of social environment on the reproductive behaviour of male *P. wingei*. Males experiencing female-biased environments, with no competition for mating from other males, decreased their mating effort in terms of all four premating behaviours measured, despite maintaining the same level of general interest in terms of time spent near the focal female. Males from the female-biased environments preferentially used sigmoid displays to court females and their attempts to sneak copulations by gonopodial thrusts were relatively rare. In contrast, males from male-biased environments used opportunities to mate by attempting sneak copulations; however, they also maintained relatively high levels of sigmoid displays.

Low interest in copulation and associated mating behaviour of males from female-biased environments was particularly strong. This finding supports theoretical predictions that males should learn to become choosy if encounter rates with females are high (Dukas, Clark, & Abbott, 2006). These males were not sperm limited, as the level of sperm competition risk has no effect on sperm production in poeciliids (Evans et al., 2003) and males had

Table 3
The effect of social treatment on male mating behaviour, analysed as the interaction between treatment level and trial (Comparison), and temporal changes in behaviour between baseline and post-treatment trials (Male-biased, Female-biased), analysed as temporal pattern within each treatment

	Comparison*			Male-biased			Female-biased		
	Statistics	P	Diff	Statistics	P	Trend	Statistics	P	Trend
Composite variables									
Male interest	2.27^F	0.140		3.08^t	0.007	Increase	0.76^t	0.457	
PCA1	9.39^F	0.004	M>F	0.35^t	0.730		4.06^t	0.001	Increase
PCA2	2.94^F	0.094	(M>F)	1.28^t	0.220		1.21^t	0.24	
Individual behaviours									
Gonopodial swings	4.81^z	<0.001	M>F	1.71^z	0.083	(Increase)	5.05^z	<0.001	Decrease
Gonopore nipping	1.27^z	0.204		0.28^z	0.778		2.38^z	0.017	Decrease
Sigmoid displays	4.77^z	<0.001	M>F	1.43^z	0.152		8.72^z	<0.001	Decrease
Gonopodial thrusts	7.15^z	<0.001	M>F	2.26^z	0.024	Increase	6.99^z	<0.001	Decrease

Test statistic (F, t or z value, as in superscript); Trend = temporal trend (increase or decrease in behaviour rate between baseline Trial 1 and post-treatment Trial 2); Diff = direction of difference in behaviour between male- and female-biased social environment treatments (M: male-biased, F: female-biased).

* For Comparison, only the interaction term is reported. For full outcomes of the statistical test, see Supplementary Material S1.

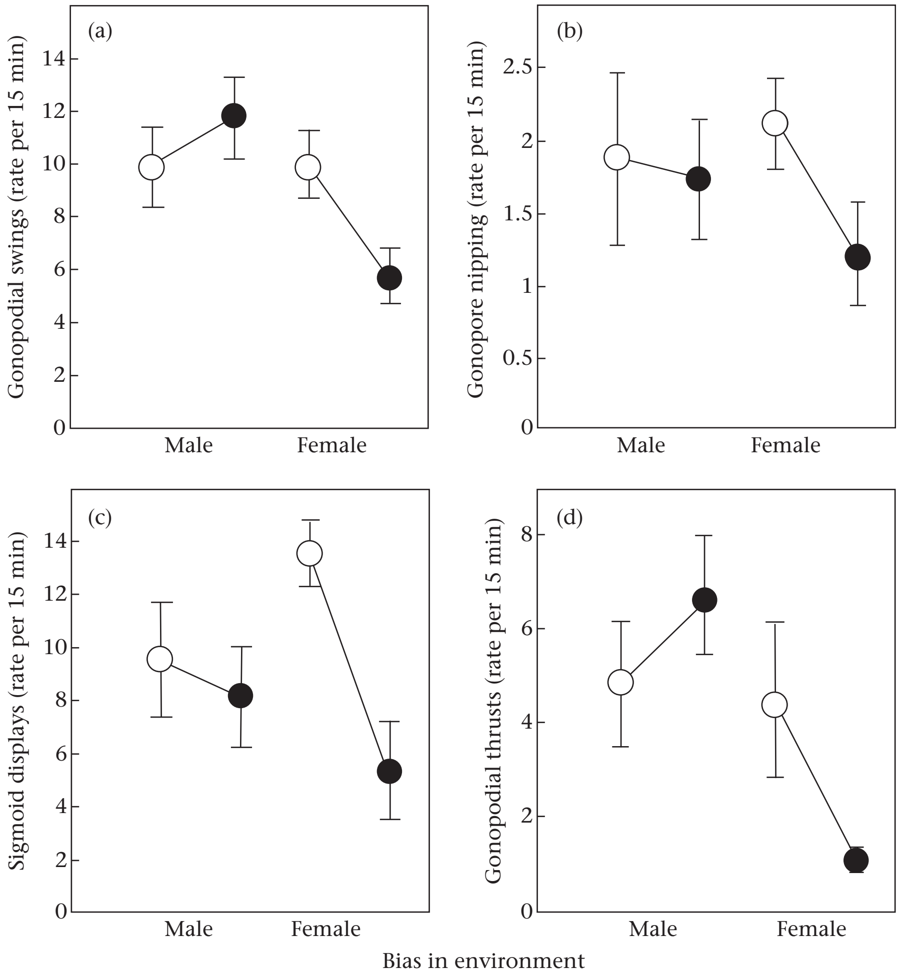

Figure 1. Temporal changes and differences between treatments in sexual behaviour trials (baseline: open circles; post-treatment: closed circles) in male- and female-biased environments. Error bars represent 1 SE. See Table 3 for the statistical significance of comparisons. Data on 'male interest' were exp-transformed prior to analysis to normalize residuals, the transformed data being presented in the figure.

sufficient time to replenish their sperm reserves prior to trials. Decreased mating effort was detected despite the use of a new, unfamiliar female, running counter to the predictions for a Coolidge effect (Dewsbury, 1981). The Coolidge effect describes a decrease in sexual interest in one female with sexual interest being revived by a new female, a process that has been demonstrated across many taxa (Koene & Ter Maat, 2007; Pizzari, Cornwallis, Løvlie, Jakobsson, & Birkhead, 2003), including fish (Kelley, Graves, & Magurran, 1999; Spence, Reichard, & Smith, 2013).

Unfortunately, such a strong decrease in mating effort was not anticipated and our experimental design did not allow for determination of potential partner compatibility related to, for example, MHC genes, which are known to play an important role in mate choice in fishes (Agbali, Reichard, Bryjová, Bryja, & Smith, 2010;

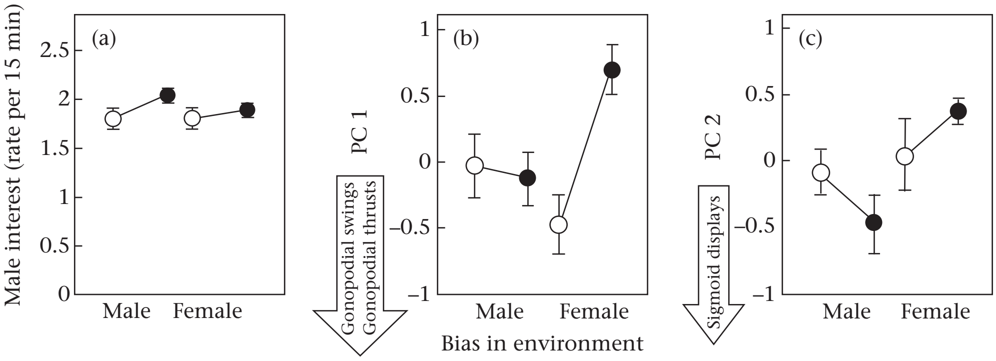

Figure 2. Temporal changes and differences between treatments in sexual behaviour trials for composite variables (baseline: open circles; post-treatment: closed circles) in male- and female-biased environments. See Table 3 for the statistical significance of comparisons. Text in arrows indicates significantly loaded variables and the direction of their association with the PC axis.

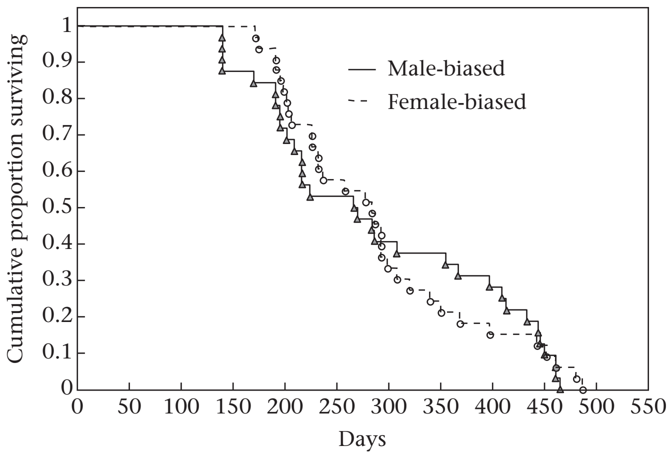

Figure 3. Survival of focal males for each experimental treatment, visualized by Kaplan–Meier estimates. Triangles: male-biased treatment; circles: female-biased treatment.

Reusch, Häberli, Aeschlimann, & Milinski, 2001). Individual guppies, however, do not discriminate between siblings and unrelated individuals during mate choice or courtship (Pitcher, Rodd, & Rowe, 2008; Viken, Fleming, & Rosenqvist, 2006), reducing the possibility of a strong mate incompatibility effect in our experiment. Males from male-biased environments exposed to strong male–male competition for access to a female used every opportunity to mate, resulting in an increased rate of sneak attempts (gonopodial thrusts), which was in accordance with our predictions.

Our study was primarily designed to compare the effects of different sex ratios on male behaviour. However, our experimental design inevitably included variation in other factors and these factors may have contributed to the results. For example, the male-biased treatment excluded all female–female interactions while the female-biased treatment excluded all male–male interactions during the 5-week period prior to testing. Furthermore, the quantitative and qualitative characteristics of male–female interactions probably varied between treatments. While this was a natural outcome of our experimental design, we did not control for their behavioural effects, for example the effect of modulation of female behaviour and its feedback to male mating behaviour (Kahn, Dolstra, Jennions, & Backwell, 2013). Males frequently modify their mating effort in accordance with female responsiveness (fish: Casalini, Reichard, Phillips, & Smith, 2013; fiddler crabs: Kahn et al., 2013). Thus, although we tested males with standardized, unfamiliar females, we acknowledge that male mating behaviour during the second assay may have been affected by all these unmeasured variables and their potential interactions.

There is clear evidence that a male's mating behaviour is influenced by its environment and previous experience (Bretman et al., 2011; Freeberg, 2000; Rodríguez et al., 2013). Our results corroborate previous findings of Jordan and Brooks (2012), who also found that guppy males modify their sexual behaviour according to previous mating experience. They found that guppy males exposed to multiple females during the conditioning period subsequently invested less effort in courting a new female than males housed with only one female at a time. Guppy males housed in male-only environments, on the other hand, have been recorded performing homosexual behaviour (courting other males and attempting to sneak copulate) that persisted even in the presence of females (Field & Waite, 2004). Similarly, Evans and Magurran (1999) found that guppy males reared in a male-biased social environment were more inclined to perform gonopodial thrusts, despite being briefly housed in an environment with an equal sex ratio. In another poecilid, *Gambusia holbrooki*, males increased their mating effort in the presence of male competitors, and the effect held even after removal of competitors (Evans et al., 2003). Our results corroborate the importance of previous experience in modulation of sexual behaviour. We hypothesize that the use of previous experience can benefit males by saving time from repeated assessments of social condition, but can become costly in a rapidly changing social environment.

Courtship effort may affect longevity (Miller & Brooks, 2005). It is energetically costly (Cady, Delaney, & Uetz, 2011; Kotiaho, 2000) and males are known to have a higher mortality during the mating season (Hunt et al., 2004; McKellar, Turcotte, & Hendry, 2009). Furthermore, males performing different mating tactics pay different energetic costs for interactions with females (Cummings & Gelineau-Kattner, 2009). We observed no effect of sex-biased environment and associated mating effort on longevity or mortality patterns. It is possible, however, that survival costs of mating effort would become detectable in more challenging (predation risk, food limitation) environments (Magnhagen, 1991), rather than under benign laboratory conditions.

In conclusion, we demonstrated that individual males displayed either sneaking or courting behaviour, even during their first mating attempts. We further established that previous experience, in the form of a sex-biased social environment, strongly affected male sexual behaviour in standardized mating trials. Males with experience of female-biased social environments decreased their mating effort considerably, but were more likely to court females rather than sneak copulations compared to males from male-biased social environments which maintained a high level of mating effort by performing frequent sneak mating attempts through gonopodial thrusts. These results underline the importance of previous experience in the expression of sexual behaviour.

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Supplementary Material

Supplementary material associated with this article is available, in the online version, at http://dx.doi.org/10.1016/j.anbehav.2013.12.010.

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