

RESEARCH PAPERS

*Department of Psychology and Department of Biology, University of Padova,
Padova*

Sexual Competition, Coercive Mating and Mate Assessment in the One-Sided Livebearer, *Jenynsia multidentata*: Are They Predictive of Sexual Dimorphism?

Angelo Bisazza, Silvia Manfredi & Andrea Pilastro

Bisazza, A., Manfredi, S. & Pilastro, A. 2000: Sexual competition, coercive mating and mate assessment in the one-sided livebearer, *Jenynsia multidentata*: are they predictive of sexual dimorphism? *Ethology* **106**, 961–978.

Abstract

We investigated the mechanisms of sexual selection operating on body size in the one-sided livebearer (*Jenynsia multidentata*), a small fish characterized by male dwarfism. Mating in the one-sided livebearer is coercive: males approach females from behind and try to thrust their copulatory organ at the female genital pore. Females counter males' mating attempts by either swimming away or attacking them. We tested the hypothesis that the components of sexual selection favouring small size in males (sexual coercion) were more effective than those favouring a large size (male competition and mate choice). When alone, small males had a significantly higher success in their mating attempts than large males. The proportion of successful attempts was also positively correlated with female size. When two males competed for the same female, the large male had a significant mating advantage over the small one. With a 1 : 1 sex ratio, the large-male mating advantage vanished because each male tended to follow a different female. Large males, however, preferentially defended large females, thus compelling small males to engage with smaller, less fecund females. Males did not discriminate between gravid and non-gravid females, but preferred mating with larger females. This preference disappeared when males were much smaller than the female, probably in relation to the risk for the male of being eaten or injured by the female. In a choice chamber, male-deprived females that had their sperm storage depleted remained close to males and showed a preference for large individuals, a behaviour not observed in non-deprived females. Nonetheless, when placed with males in the same aquarium, all females showed avoidance and aggression. Struggling may represent a way by which the female assesses the skill and endurance of males.

Corresponding author: A. Pilastro, Dipartimento di Biologia, Università di Padova, via U. Bassi 38/B, I-35131 Padova, Italy. E-mail: pilastro@civ.bio.unipd.it

Introduction

Sexual coercion is one of the potential outcomes of the conflicts between males and females that arise in sexual selection. In some animal species this mechanism can be as important as mate choice or male competition in determining reproductive success in the two sexes (Berry & Shine 1980; Thornhill & Alcock 1983; Westneat et al. 1990; Smuts & Smuts 1993). Interest in the costs borne by females and in strategies adopted to counteract male coercion is growing (Magurran & Nowak 1991; Clutton-Brock & Parker 1995).

Clutton-Brock & Parker (1995) recognized three main forms of sexual coercion. A male can use its physical strength either to force copulation, to harass the female until she mates or to intimidate the female that refuses to mate. Greater size and physical strength is not essential for mating with unwilling females. In birds, males can unite to overcome female resistance (Castro et al. 1996). In fishes with external fertilization, males can employ inconspicuousness or female mimicry to approach mating pairs and release their sperm at the time of spawning (Gross 1984; Taborsky 1994).

Atherinomorphs are a relatively small group of fishes inhabiting a great variety of marine inshore, brackish and freshwater habitats. They are mostly egg-laying, but internal fertilization evolved independently in at least nine instances, and four families consist almost entirely of livebearers (Parenti 1981; Nelson 1994). Given the great number of different reproductive adaptations shown by fishes of this group, they provide a unique opportunity to study the relation between fertilization mode and sexual selection mechanisms.

Coercive mating appeared in at least four of the nine groups with internal fertilization. In the Phallostetidae (order Atheriniformes), males clasp females and physically force them to mate (Breder & Rosen 1966; Mok-Eym 1997). Conversely, in the Poeciliidae and Anablepidae (order Cyprinodontiformes) and in *Horaichthys setnai* (order Beloniformes) males evolved a totally different way of obtaining copulations, by-passing female consent (Kulkarni 1940; Farr 1989). The male approaches a female, usually from behind, and tries to thrust its copulatory organ into the female's gonoduct or to attach sperm near her genital pore, a tactic commonly called gonopodial thrusting. In poeciliid fishes using this tactic, body size is negatively correlated with mating success (Bisazza & Marin 1995; Bisazza & Pilastro 1997; Pilastro & Bisazza 1999). Small males appear to be favoured both because they are less conspicuous and because they manoeuvre better when trying to insert the gonopodium into the female's genital tract (Pilastro et al. 1997). Within this fish family, males court females and obtain copulations with their consent, or adopt gonopodial thrusting in case of female unwillingness. In some poeciliids, coercive copulation (gonopodial thrusting) is the only mating tactic adopted

by males, whereas in other species both tactics (courtship and gonopodial thrusting) can be adopted (reviewed in Bisazza 1993).

It was suggested (Farr 1989; Bisazza 1993) that interspecific variation in both male sexual ornamentation and body size is the consequence of the different weight of various components of sexual selection in different species. Gonopodial thrusting requires agility and inconspicuousness and therefore favours male dwarfism and drabness. On the other hand, both an active role of the female through mate selection and a strong influence of male competition on mating success generally favour larger, ornamented males (Farr 1989). Male mate preferences could also affect sexual selection and hence dimorphism. A special preference for particular females, for example females which are close to fertilize a new batch of eggs, may lead to an extremely male-biased operational sex ratio with consequent intensification of both male competition and intrasexual selection (Bisazza & Marin 1995; Bisazza et al. 1996). A preliminary test of these hypotheses by comparison of data available in the literature, suggested that among poeciliids, the relative size of males and their degree of ornamentation correlated significantly and positively with the relative importance of female mate choice and male competition, and drabness and dwarfism were significantly associated with a predominant role of sexual coercion (Bisazza 1993). The conclusions of this comparative analysis remain tentative since it was based on a limited number of species and used anecdotal data to a large extent. Further tests of this hypothesis require the study of sexual selection mechanisms in other fishes with internal fertilization and a similar insemination mechanism.

An opportunity for such a test is represented by fishes of the family Anablepidae. The family is represented by two genera of livebearers, *Jenynsia* and *Anableps*, and by one egg-laying species, *Oxyzygonectes dovii*. Anablepidae are closely related to Poeciliidae but they evolved internal fertilization and viviparity independently, from substrate-spawning ancestors (Parenti 1981; Novarini 1994). Sexes are monomorphic in coloration, but differ in body size. In the one-sided livebearer *Jenynsia multidentata*, in particular, males are much smaller than females, and this species shows probably the most extreme example of size dimorphism in the atherinomorphic fishes (Meyer et al. 1985; Ghedotti & Weitzman 1996). Sexual selection and mating ecology has never been investigated in this fish family, and only a few accounts of mating behaviour exist for these fishes (Mattig & Greven 1994). Mating in the one-sided livebearer is coercive: males approach females from behind and try to thrust their copulatory organ at the female genital pore. Females counter males' mating attempts by either swimming away or attacking them. Courtship display was never observed in this species (Bisazza, unpubl. obs.).

We studied the mechanisms of sexual selection in *J. multidentata*, in order to understand the occurrence of male dwarfism and lack of male sexual ornaments in this species. Studies on the closely related poeciliids showed that small males are at an advantage in coercive mating, whereas female choice and male-male competition generally favour large males (Hughes 1985; Bisazza et al. 1996; Bisazza & Pilastro 1997; Ptacek & Travis 1997; Pilastro et al. 1997). Bisazza (1993, 1997) has proposed that variation in sexual dimorphism within Atherinomorpha is mainly

determined by sexual selection and that reverse sexual size dimorphism arises whenever the advantage of small males in sexual coercion exceeds the advantage of large size in female choice and male–male competition. If this hypothesis is true, in the one-sided livebearer we expect that: (i) male success in gonopodial thrusting increases with the size difference between male and female; (ii) male size is a weak predictor of success in male competition for females; and (iii) female one-sided livebearers do not base their mate choice on male size.

In a series of laboratory experiments, we first measured the success of coercive mating attempts as a function of male and female body length. Secondly, we estimated the influence of body size on male–male competition. Thirdly, we investigated female preference for males of different size. Finally, four experiments were designed to assess whether males show any mating preferences in relation to female reproductive status and body size, as observed among poeciliids (Bisazza et al. 1989).

Methods

Study Species

South-American fishes of the genus *Jenynsia* occur in Atlantic coastal drainages from the Rio Negro Province (Argentina) to the city of Rio de Janeiro (Brazil). The one-sided livebearer, *Jenynsia multidentata* (formerly *J. lineata*; see Ghedotti & Weitzman 1996) is the most widely distributed species. It is a bottom dwelling fish that feeds on algae and small invertebrates. At the onset of maturity, the anal fin of males develops into a tubular copulatory organ, the gonopodium. The gonopodium can be brought forward on one side only, and left and right males are found in approximately the same proportion. Contrary to what is commonly stated in aquarist textbooks the female's sexual opening appears to be perfectly symmetrical, so males can mate indifferently with all the females of the population (Ghedotti & Weitzman 1996; Bisazza, unpubl. data).

As in poeciliids (Constantz 1989; but see Snelson 1982), males stop growing when they become sexually mature, whereas females continue to grow during their entire life. The standard length of adult males ranges between 16 and 34 mm. Females mature sexually with a standard length usually greater than 25 mm, and then grow up to over 70 mm.

After about 40 d of pregnancy, females give birth to up to 40 young, with large females being more fecund than small ones (Bisazza, unpubl. data). In both *Anableps* and *Jenynsia*, females separated from males before parturition are usually able to undergo a new pregnancy, but never a second one (Bisazza, unpubl. data). In this respect, Anablepidae differ from most poeciliids, whose females can store sperm for much longer time (Constantz 1984).

The fish used in this study were F1 generation of 20 wild caught individuals captured in the Rodrigo de Freitas lagoon, near Rio de Janeiro (Brazil). Fish were maintained in groups of 10–15 individuals (at approximate 1 : 1 sex ratio) in 150 l aquaria, and fed ad libitum with brine shrimp, live fruitflies and dry food for tropical fish. Aquaria were kept at $24 \pm 1^\circ\text{C}$, and the photoperiod was 05:00 to 21:00 h.

Fish used in the experiments were sexually mature and aged 6–12 months. Most tests were carried out in small aquaria (60 × 36 cm) filled with water up to 30 cm and fitted with rocks and plants on the bottom and abundant floating vegetation. The experiment on male–male competition was performed in aquaria of larger size (69 × 69 cm). At the end of each test, males and females were anaesthetized with MS222 and standard length was taken to the nearest 0.5 mm.

If not otherwise stated, means \pm SD are given. Data were checked for normality and, when two or more groups were compared, for equality of variances. When these assumptions were not met, correspondent non-parametric tests were used. All probabilities are two-tailed. Data were analysed with the SPSS statistical package.

Body Size and Success of Coercive Copulation through Gonopodial Thrusting

In this experiment we measured the success of coercive mating attempts in relation to the length of the male and the female. A female was introduced into the experimental aquarium and allowed to settle for 2 d. In the evening of the day before the beginning of the experiment, one male was also introduced and allowed to settle overnight. Observations were carried out the following morning (09:00 to 13:00 h). For each replicate, male and female behaviour was observed for four periods of 15 min, each separated by at least 30 min. In a few cases, one to three additional 15-min observations were made until a minimum of 40 attempts per male had been recorded.

As an estimate of mating success, we counted the number of mating attempts ending with contact between genitalia. In studies with poeciliids (Hughes 1985; Yan 1987) this criterion has been used often as an estimate of a successful copulation, and the reliability of this method to estimate sperm transfer was recently demonstrated for the eastern mosquitofish *Gambusia holbrooki* (Giacomello 1995; Pilastro et al. 1997). We calculated mating success as the proportion of contacts over the total number of coercive copulations (gonopodial thrusts) attempted.

In one trial a male of 18 mm housed with a female of 67 mm disappeared during the night and, the day after, the female clearly showed a swollen belly. A similar event was recorded during preliminary experiments, indicating that large females can eat small males if confined in a small aquarium. In the remaining tests, males of 19 mm or smaller were therefore not used. Male and female standard length averaged 26.6 ± 4.6 mm (range 19.5–32 mm) and 43.8 ± 12.1 mm (range 31–67 mm), respectively.

Male–Male Competition

Two males were allowed to compete for one ($n = 19$) or two females ($n = 14$) in a large aquarium (69 × 69 cm). Females were introduced into the aquarium 2 d before the beginning of the experiment, and two males were introduced the evening before the test day. The two males differed in size, the smaller male being around 80% the standard length of the larger one (one-female trials: $81.6 \pm 7.4\%$, range 64–90%; two-females trials: $75.7 \pm 6.4\%$, range 62.5–86%). During the fol-

lowing morning (09:00 to 12:00 h), the number of attacks and the number of mating attempts performed by each male was recorded during three 10-min periods separated by at least 15 min. The bottom of the aquarium was divided into a 23×23 cm grid made with thin blue plastic sticks. During tests with two females, the position of the females was recorded using the grid as a reference in order to measure the shoaling tendency of females. Females used in these trials differed in size, the standard length of the smaller being on average $79.8 \pm 0.08\%$ of the larger one.

Females Choosing between Males of Different Length

In this experiment we used females that had been deprived of males for 60 d (standard length = $42.1 \text{ mm} \pm 6.4$, $n = 18$) and females kept with males for the same period (standard length = $44.4 \text{ mm} \pm 7.2$, $n = 16$). Each test aquarium was divided length-wise into two unequal halves with a transparent glass divider. The smaller half was further subdivided into two compartments by a 12×8 cm filter placed in the middle (Fig. 1). A fluorescent lamp was set over the two male compartments, whereas a black screen prevented the female compartment from being illuminated directly. Thus, males were in full light and the female remained in the shade.

One male was introduced into each of the two small compartments and allowed to settle for 24 h before the test. A single female was then placed in the centre of the female compartment and observations started as soon as the female resumed normal swimming. The test lasted for 30 min. At 30-s intervals the position of the female was recorded. In particular, we recorded: (i) which of the two halves of the compartment, in relation to the males' compartment, the female was occupying; (ii) whether the female was closer or further than one body length from

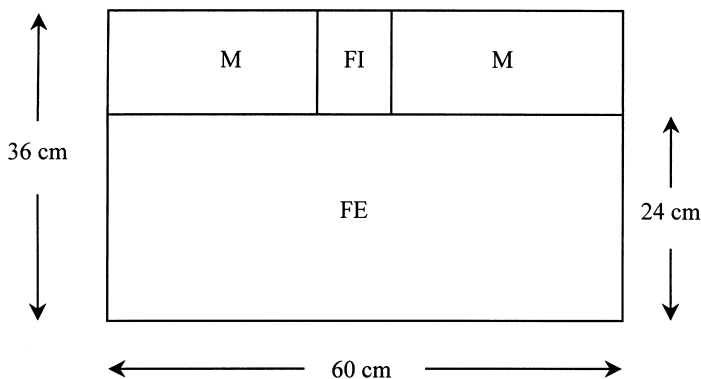


Fig. 1. Apparatus used for female choice test (M = male compartment, FE = female compartment, FI = filter)

the glass dividing her compartment from that of the male; and (iii) the number of observed attempts to enter one or the other male's compartment. The sizes of the large and small males in the two experiments averaged $29.9 \text{ mm} \pm 2.5$ standard length and $20.2 \text{ mm} \pm 1.6$ standard length (male-deprived females), and $28.5 \text{ mm} \pm 1.8$ standard length and $19.8 \text{ mm} \pm 2.3$ standard length (females kept with males), respectively.

Male Choosing between Gravid and Non-Gravid Females

This experiment was aimed at testing whether males of *J. multidentata* show any preference for male-deprived, virgin or postpartum females over gravid females previously kept with males.

Male-deprived females

Ten females were deprived of males for 80 days. During the last 30 days, females were kept under observation to ensure that none was pregnant. Each deprived female was then paired with a gravid female (distinguishable because of her more prominent belly) of similar size ($\pm 5\%$ standard length), but which had been previously maintained with males. The two females were introduced into one experimental aquarium and allowed to settle for 2 d. One male, previously deprived of females for 24 h, was then introduced into the aquarium. Male mating attempts were scored for 60 min starting from the first attempt (usually within 20 s after the male was introduced). Sometimes females reacted to male attempts by attacking or chasing the male. The number of aggressive acts by the females towards the male was also scored.

Virgin females

The previous experiment was repeated with the same set-up but using 10 virgin females instead of male-deprived ones.

Post-partum females

The preference of males for postpartum females was tested with a slightly different set-up. Females which are close to parturition are distinguishable from the other gravid females because of their particularly prominent belly. We used six dyads of females composed of one female close to the parturition paired with a similar-sized female which was gravid but not close to parturition. For each test day, a different male randomly taken from a stock of 30 males, was introduced into the aquarium containing the two females. On each day until 6 d after parturition we scored the number of male mating attempts and female aggressive acts towards the male for 60 min after the first male's attempt. We considered in the analyses the observations carried out on the nine consecutive days, starting 2 d before and ending 6 d after parturition. At the end of the test, the male was released back into the stock aquarium, and he might therefore have been used more than once.

Males Choosing between Females of Different Length

Two gravid females of different sizes were introduced into the experimental aquarium and allowed to settle for 2 d. Large females averaged 53.6 ± 8.7 mm standard length (range 41–67 mm); small females averaged 36.4 ± 5.8 mm standard length (range 30–46 mm). On average, the standard length of the small female was $68.3 \pm 6.6\%$ of the large one. One male, previously deprived of females for 48 h, was then introduced into the aquarium and the number of his mating attempts with the two females was counted for 30 min after the first attempt; 20 replicates were made. The average standard length of males was 25.2 ± 3.7 mm (range 21–33 mm).

Six additional very small males (mean standard length = 21.4 ± 0.92 mm; range 20–22.5 mm) were tested using pairs of females that were smaller than those of the previous tests. In these replicates, large and small females averaged 33.3 ± 2.4 mm (range 30–37.5 mm) and 25.8 ± 1.9 mm (range 23.5–28.5 mm), respectively. In this case, the average standard length of the small female was $77.6 \pm 4.4\%$ of that of the large one.

Results

Body Size and Success of Coercive Copulation through Gonopodial Thrusting

The sexual behaviour of males is stereotyped and consists exclusively of coercive copulatory attempts through gonopodial thrusting. When a male meets a female, he moves immediately behind her and then darts forward along the side of the female corresponding to the side he is able to bring forward his gonopodium. When his snout is at the level of the female's tail, he rotates the gonopodium forward and, keeping his body in close contact with that of the female, tries repeatedly to insert his gonopodium into her gonopore. Females usually swim away, trying to avoid copulation. Often, especially under intense harassment, females react by turning and attacking the male.

The effect of male and female length on the frequency of successful copulation attempts over the total attempts was tested using a multiple regression analysis. Both male size and female size were significantly correlated with mating success, although the correlation with the latter was much higher (female size: partial $r = 0.68$, $p < 0.001$; male size: partial $r = -0.36$, $p < 0.05$; $F = 15.0$, $df = 2.29$, $p < 0.001$, $n = 32$). Male relative length (male standard length/female standard length) was a good predictor of his mating success (Fig. 2): the relatively smaller the male, the greater his success (Pearson correlation coefficient $r = -0.70$; $p < 0.001$, $n = 32$). The relationship appeared to be linear, and log transformations did not modify the results ($r = -0.71$; $p < 0.001$, $n = 32$).

Male Competition

When two males competed for the same female, the larger male made significantly more intrasexual attacks than the smaller male (Table 1). Nonetheless, in some trials dominance was not so clear-cut, and the smaller male made nearly the

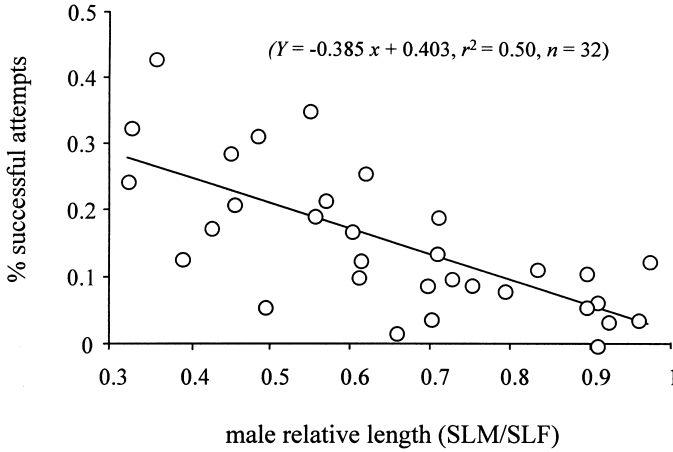


Fig. 2: Relation between male relative length (male standard length/female standard length) and male mating success. Mating success was expressed as the frequency of copulatory attempts (gonopodial thrust) ending with a contact between genitalia, over the total number of attempts

same number of attacks as the larger one. The proportion of attacks carried out by the large male was not correlated with the difference in length between the two males (Pearson correlation coefficient $r = 0.02$, ns, $n = 19$), nor with the size of the female ($r = 0.23$, ns, $n = 19$). Large males made significantly more mating attempts than small ones (Table 1), but variance was large and in five trials small males showed more attempts than large ones. Overall, the proportion of mating attempts and dominance, i.e. proportion of aggressive attacks, were positively correlated with each other ($r = 0.60$, $p = 0.006$, $n = 19$).

Table 1: Number of male copulatory attempts and attacks towards the other male in relation to male length (mean \pm SD) and sex ratio. For behavioural data, median and inter-quartile range are given

Sex ratio	No. of attacks	No. of copulatory attempts
2 males, 1 female ($n = 19$)		
Larger male (SL) (28.2 mm \pm 2.3)	17 (6–25)	42 (23–52)
Smaller male (SL) (23.0 mm \pm 2.4)	1 (0–4)	15 (9–21)
Wilcoxon signed-ranks test	$z = 2.98$, $p = 0.002$	$z = 3.83$, $p < 0.001$
2 males, 2 females ($n = 14$)		
Larger male (SL) (28.3 mm \pm 3.1)	11.5 (7.5–15.5)	42 (37.5–52.3)
Smaller male (SL) (21.3 mm \pm 1.8)	0 (0–2)	42 (36.5–56)
Wilcoxon signed-ranks test	$z = 3.30$, $p = 0.001$	$z = 0.16$, ns

In the replicates with two females, females showed little tendency to shoal and during $79.1 \pm 7.8\%$ (range 70–93.3%) of the observation periods they were swimming in two different squares of the grid and were usually followed by one male at a time. Fights between males occurred almost exclusively when both males were following the same female. Large males still made significantly more attacks than small ones (Table 1). There was a tendency for reduced aggressiveness of the small males in trials with two females but the difference was non-significant ($z = 1.48$, $p = 0.15$, $n = 14$, Mann–Whitney test). With a 1:1 sex ratio, the number of mating attempts carried out by the small male was nearly identical to that performed by the large male (Table 1). The proportion of mating attempts carried out by the large male in these replicates was significantly lower than that observed in the replicates with two males and one female ($t = 3.76$, $p = 0.001$, $n = 33$). Large males did most of their mating attempts with the large female, whereas small males did most of their mating attempts with the small female (repeated measure ANOVA, $F = 58.3$, $df = 3,39$, $p < 0.001$, Fig. 3).

Females Choosing between Males of Different Length

Male-deprived females were observed more frequently than non-deprived females to stay within one body length from the glass dividing their compartment from that of males ($z = 4.77$, $p < 0.001$, $n = 34$, Mann–Whitney test), and to attempt to enter their compartments ($z = 3.21$, $p = 0.001$, $n = 34$, Fig. 4). Male-deprived females showed a preference for the larger male as they were observed more frequently close to it ($z = 2.181$; $p = 0.027$, $n = 18$) and made more attempts

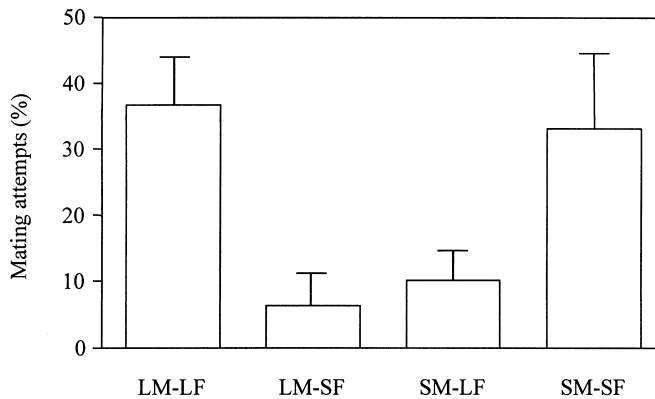


Fig. 3: Mean frequency of male mating attempts (\pm SD) in relation to male and female standard length. Observations are grouped into four groups: large male (LM) with large female (LF); large male with small female (SF); small male (SM) with large female; small male with small female. The frequency of copulatory attempts differed significantly amongst all four groups in pairwise comparisons at $p < 0.05$ (t -test), with the exception of LM–LF vs. SM–SF. Differences remained significant after Bonferroni correction for multiple comparisons

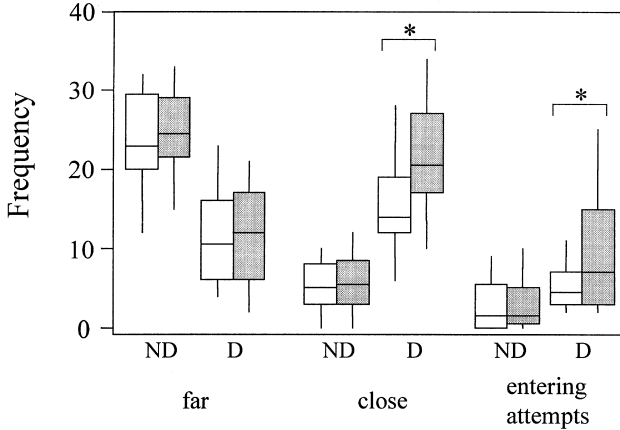


Fig. 4: Females choosing between males of different size. Number of times which females were observed, respectively, far, close (i.e. within one body length) and attempting to enter the compartment of the large male (shaded box) and small male (open box). Results for male-deprived ($n = 18$) and non-deprived females ($n = 16$) are shown (D = male-deprived; ND = non-deprived). An asterisk indicates a significant preference ($p < 0.05$). Line, box and bar represent median, interquartile range and extreme values, respectively (BOXPLOT procedure, SPSS)

to enter his compartment ($z = 2.177$; $p = 0.027$, $n = 18$, Wilcoxon signed-ranks test). In contrast, non-deprived females did not show any preference (all $p > 0.05$, $n = 16$).

Males Choosing between Gravid and Non-Gravid Females

Male-deprived females

Males did not show any preference for gravid or deprived females, as the number of mating attempts directed towards females did not differ between the two groups ($z = 0.53$, $p = 0.63$, Wilcoxon signed-ranks test, Table 2). Deprived females were less aggressive towards males than gravid females, although not significantly so ($z = 1.56$, $p = 0.16$, $n = 10$, Wilcoxon signed-ranks test).

Virgin females

The number of male mating attempts towards virgin females did not differ from that towards gravid females ($z = 0.36$, $p = 0.75$, $n = 10$, Wilcoxon signed-ranks test, Table 2). Virgin females were less aggressive than gravid ones, although not significantly so ($z = 1.25$, $p = 0.24$, $n = 10$, Wilcoxon signed-ranks test). One male in this experiment made an exceptionally high number of attempts (308) and received a proportionally high number of attacks from females (109). Conclusions did not change after removal of this outlier.

Table 2: Standard length (mean \pm SD), number of male copulatory attempts, and attacks by the female in relation to the female's reproductive condition. For behavioural data, median and interquartile range are given

Experiment	<i>n</i>	Female	SL (mm)	No. of copulatory attempts	No. of attacks towards male
Male-deprived	10	Male-deprived	44.0 \pm 5.1	52 (46.5–70.3)	2 (0–3.75)
		Gravid	44.5 \pm 4.5	52.5 (47.5–66.5)	4.5 (1.75–6.0)
Virgin	10	Virgin	41.7 \pm 2.8	59 (51–64)	4 (1–15.5)
		Gravid	40.5 \pm 7.1	43.5 (35–88.8)	16.5 (2.5–34)
Post-partum	6	Post-partum ^a	49.2 \pm 7.7	27 (21.5–33.5)	6.5 (0–9.75)
		Gravid	49.6 \pm 7.4	56 (43.8–58.5)	1.75 (0.38–6.13)

^a Average of the first 2 d after parturition. SL, standard length.

The proportion of male mating attempts towards gravid females was not lower than that towards virgin and deprived females even when the latter were pooled ($z = 0.12$, $p = 0.91$, $n = 20$, Wilcoxon signed-ranks test). Males did not show any mate preference even if only the first 30 or 10 min of observation were considered ($z = 0.36$, $p = 0.52$; $z = 0.02$, $p = 0.99$, respectively, Wilcoxon signed-ranks test). When data of virgin and deprived females were pooled, non-gravid females were significantly less aggressive than gravid females ($z = 1.92$, $p = 0.05$, $n = 20$, Wilcoxon signed-ranks test). The proportion of attacks performed by the two types of females was not significantly correlated with the proportion of male mating attempts received ($r = -0.18$, $n = 20$, ns).

Post-partum females

The results of this experiment showed that females close to parturition were preferred over normal gravid females, whereas the latter were preferred to those females that had recently delivered young. Two days after parturition any preference disappeared and male mating attempts towards postpartum females became again close to 50% (Fig. 5). The frequency of male mating attempts towards postpartum females differed significantly between days ($\chi^2 = 22.4$, $df = 8$, $p < 0.004$, Kruskal–Wallis analysis of variance). When the number of attempts towards the two females is compared, males made significantly more mating attempts towards the female close to parturition ($z = 1.99$, $p = 0.046$, $n = 6$; $z = 2.03$, $p = 0.042$, $n = 6$, Wilcoxon signed-ranks test). On the 1st day after parturition, males made significantly more mating attempts towards the other, gravid female ($z = 2.20$, $n = 6$, $p = 0.028$, Wilcoxon signed-ranks test). A similar trend was also observed on the day of parturition ($z = 1.78$, $p = 0.075$, $n = 6$, Wilcoxon signed-ranks test), whereas in the other days no significant difference emerged (Fig. 5). The total number of attacks towards males, carried out by the two females did not differ between days

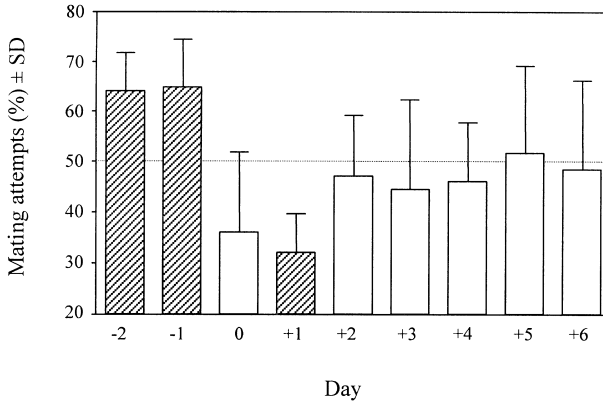


Fig. 5: Males choosing between gravid and postpartum females: Proportion of the copulatory attempts (% with SD bars) made by males towards the postpartum female in relation to the gravid female, against the female's reproductive cycle (0 = d of parturition; $n = 6$). Hatched bars indicate a proportion significantly different from 50% ($p < 0.05$, Wilcoxon signed-ranks test)

($\chi^2 = 3.22$, $df = 8$, $p = 0.92$, Kruskal–Wallis analysis of variance) and in none of the test days did the two females differ in the proportion of attacks towards males at $p < 0.05$ (Wilcoxon signed-ranks test).

Males Choosing between Females of Different Length

Large and small males showed an opposite tendency in their preference for females of different sizes. Males longer than 23 mm made more attempts towards the larger of two females, whereas males 23 mm or shorter selected the smaller one (Table 3). The proportion of mating attempts towards the larger female was significantly different between the two groups of males ($t = 5.44$, $p < 0.001$, $n = 20$). This proportion was significantly and positively correlated with both the absolute size of the male ($r = 0.70$, $p < 0.01$, $n = 20$) and his size relative to the size of the larger female ($r = 0.58$, $p < 0.01$, $n = 20$).

The behaviour of small males observed in this test could be due either to a preference for small females or avoidance of very large females. We tested six additional small males (standard length < 23 mm) with two females whose difference in size was similar to the previous replicates but whose absolute size was smaller. Here, small males showed a statistically significant preference for the larger of the two females (Table 3).

Discussion

Traditionally, sexual selection is considered to be the main evolutionary force determining a sexual size dimorphism (Darwin 1871). When male–male competi-

Table 3: Male choosing between two females of different length: mean \pm SD number of male copulatory attempts in relation to male and female standard length

	n	Copulatory attempts		Frequency of copulatory attempts with the large female (%)
		Larger female	Smaller female	
Larger female > 40 mm				
Large males (24–33 mm)	11	43.3 \pm 34.6	15.1 \pm 3.9	75.1 \pm 17.9 ^a
Small males (< 23 mm)	9	17.1 \pm 9.6	31.7 \pm 9.5	34.5 \pm 14.8 ^b
Larger female < 40 mm				
Small males (< 23 mm)	6	54.8 \pm 20.8	18.3 \pm 8.5	74.1 \pm 10.7 ^a

^a Observed frequency that differed significantly from 0.5 (one-sample t-test, $p < 0.01$).

^b Observed frequency that differed significantly from 0.5 (one-sample t-test, $p < 0.05$).

tion and female choice favour large males, sexual selection generally results in males being larger than females (Andersson 1994). A possible exception, among fishes, may be represented by livebearing fishes of the order Cyprinodontiformes. Sexual selection, in particular the small-male advantage in gonopodial thrust, a form of coercive copulation, has been proposed as an explanation for male dwarfism and drabness commonly observed in these fishes (Bisazza 1993). Interspecific variation in sexual dimorphism could be then the result of variation in the relative importance of the different components of sexual selection in different species (Bisazza et al. 1996; Bisazza 1997; Bisazza & Pilastro 1997), a hypothesis supported by a comparative analysis of fishes of the family Poeciliidae (Bisazza 1993).

Here we studied a close relative of poeciliids, the one-sided livebearer, a species with a reverse sexual size dimorphism even more pronounced than in poeciliids and without any obvious male sexual ornamentation. Fertilization mode is similar to that of poeciliids (internal fertilization by means of the anal fin transformed into an intromittent organ) but has evolved independently (Parenti 1981; Novarini 1994). We tested the hypothesis that one of the evolutionary causes of the reverse sexual size dimorphism in this species is a mating system dominated by sexual coercion and its relation with male body size.

In agreement with our hypothesis, we found a negative correlation between male size and male mating success in gonopodial thrusting. Large males were weakly favoured in male–male competition only when two males competed for the same female. However, contrary to our expectation, female choice seemed to favour large males, at least in some conditions, since we found a preference for large males in females that were previously male-deprived. Finally, males preferred large females to some extent, but apparently they did not discriminate postpartum

or virgin females from gravid females. In summary, we found that the effects of male body size on sexual selection mechanisms were similar to those found in the eastern mosquitofish, which shows an analogous mating system dominated by sexual coercion (Bisazza & Marin 1991, 1995).

In particular, the effect of male and female body size on the success of gonopodial thrusting in the one-sided livebearer appears to be the same as that found in *G. holbrooki* (Bisazza & Marin 1995; Pilastro et al. 1997) and in four other poeciliid species (Bisazza & Pilastro 1997; Pilastro & Bisazza 1999): the frequency of successful mating attempts was positively correlated with female size and negatively with male size. The reasons for the latter correlation are probably the same as for poeciliids, i.e. a greater manoeuvrability and inconspicuousness of small males (Pilastro et al. 1997).

When two males with different size were experimentally forced to compete for the same female, the large male did a larger part of mating attempts, although he was not able to prevent the small male from doing a notable proportion of mating attempts. With a sex ratio of 1 : 1, competition for females was reduced and small males had the same opportunity as larger males to attempt copulations. Among poeciliids, females tend to shoal and a dominant male may be equally able to monopolize one or many females (Bisazza & Marin 1995). Both field observations (Bleher, pers. comm.) and the results of competition experiments in the present study indicate that female one-sided livebearers forage individually and have a scarce tendency to aggregate, which may be responsible for the inability of males to monopolize more than one mate at time. From these results we expect that large males will be at a competitive advantage only at high population densities or with highly male-biased sex ratios.

We did not expect to find female mate choice since female *J. multidentata* never overtly co-operate with males to achieve copulation. However, evidence has been recently found that eastern mosquitofish females deprived of males for some time tend to approach males, thus increasing the probability of being inseminated (Pilastro et al. 1997; Bisazza et al. 2000). These results provide interesting new hints concerning the sexual dynamics generated by the evolution of coercive mating. In fact, one-sided livebearer females showed apparently inconsistent behaviour: in the female mate-choice experiment they actively sought (large) male vicinity, but when in the same aquarium with males (in male choice experiments), they showed avoidance and aggression towards males. One possible explanation is that if multiple matings are costly to females (Magurran & Seghers 1994), they may tolerate association with a male if he repels copulatory attempts by other males (Arnqvist 1989). In the Italian populations of *G. holbrooki*, for example, females never obviously co-operate with males during matings, but they actively seek the proximity with the largest, dominant male in their group. The dominant male interferes with the sexual activity of subordinates, reducing it to less than 10%. By doing this he also reduces the time available for his own sexual activity. Thus, females are less harassed by a group of males than by a single male (Bisazza & Marin 1995). The above interpretation is consistent with the traditional view about species with male coercive mating tactics. Female struggling is viewed as the

manifestation of an asymmetry in mating interests. Males try to obtain as many matings as possible, whereas females attempt to avoid or reduce costs associated with multiple and indiscriminate mating (Clutton-Brock & Parker 1995 and references therein). Conversely, in some cases, females may resist male attempts in order to select mates showing strength and endurance (Thornhill 1980; Arnqvist 1992; Allen & Simmons 1996). Under this view, female *J. multidentata*'s reluctance to copulate may be a way to select males in perfect physical conditions, able to inseminate unwilling females.

Males apparently did not discriminate females according to the phase of their reproductive cycle. However, these experiments were based on small sample sizes and would need to be confirmed by a larger data set. Conversely, males made significantly more mating attempts with larger, more fecund, females. Interestingly, we found that in small males, mate preference could be reversed if they were confronted with very large females. Anecdotal evidence (see Methods) suggests that large females can prey on small males. The same phenomenon was observed in *G. holbrooki*, and it was suggested that this may set a lower limit to selection for small size of males (Bisazza 1993).

Other possible selective factors determining the observed reversed sexual size dimorphism, other than a small male advantage in coercive mating, should be considered. Possible alternatives include the fecundity advantage of large females (Ralls 1976), the advantage of an early maturation in males (Bisazza 1993), a reduced growth rate of males as a consequence of the intense sexual activity, and an energetic advantage of small males in locomotion (Blanckenhorn et al. 1995). As suggested by Clutton-Brock & Parker (1995), large size in females may also be favoured because it reduces sexual harassment by males. These alternative hypotheses are not mutually exclusive.

In conclusion, the results of this study suggest that coercive mating may have an important role in sexual selection mechanisms in fishes with internal fertilization, and may be responsible for reverse sexual size dimorphism. This mating tactic has been traditionally considered an alternative tactic adopted by competitively inferior males, doing the best of a bad job. However, our results suggest that this is not necessarily so, and that, most importantly, the female's role is not a passive one. Conflict between the two sexes can give rise to an intricate set of different sexual selection mechanisms (Henson & Warner 1997), whose outcome, in terms of sexual dimorphism, are not easily predictable.

Acknowledgements

We would like to thank George Barlow, Guglielmo Marin, Giuliano Matessi, Michael Taborsky and two anonymous referees for their comments on various versions of the manuscript. This study was partially supported by a 60% grant from the Ministero per l'Universita' e la Ricerca Scientifica e Tecnologica to A. B.

Literature Cited

Allen, G. R. & Simmons, L. W. 1996: Coercive mating, fluctuating asymmetry, and male mating success in the dung fly *Sepsis cynipsea*. *Anim. Behav.* **52**, 737–741.

- Andersson, M. 1994: Sexual Selection. Princeton Univ. Press, Princeton, New Jersey.
- Arnqvist, G. 1989: Multiple mating in a water strider: mutual benefit or intersexual conflict? *Anim. Behav.* **38**, 749—756.
- Arnqvist, G. 1992: Pre-copulatory fighting in a water strider: inter-sexual conflict or mate assessment? *Anim. Behav.* **43**, 559—567.
- Berry, J. F. & Shine, R. 1980: Sexual dimorphism and sexual selection in turtles (order Testudines). *Oecologia* **44**, 185—191.
- Bisazza, A. 1993: Male competition, female mate choice and sexual size dimorphism in poeciliid fishes. In: *Behavioural Ecology of Fishes* (Huntingford, F. A. & Torricelli, P., eds). Harwood Academic Press, Chur, Switzerland, pp. 257—286.
- Bisazza, A. 1997: Sexual selection constrained by internal fertilization in the livebearing fish *Xenotoca eiseni*. *Anim. Behav.* **54**, 1347—1355.
- Bisazza, A., Marconato, A. & Marin, G. 1989: Male mate preference in the mosquitofish *Gambusia holbrooki*. *Ethology* **83**, 335—343.
- Bisazza, A. & Marin, G. 1991: Male size and female mate choice in the eastern mosquitofish. *Copeia* **1991**, 730—735.
- Bisazza, A. & Marin, G. 1995: Sexual selection and sexual size dimorphism in the eastern mosquitofish *Gambusia holbrooki* (Pisces Poeciliidae). *Ethol. Ecol. Evol.* **7**, 169—183.
- Bisazza, A., Novarini, N. & Pilastro, A. 1996: Male body size and male—male competition: Interspecific variation in poeciliid fishes. *Ital. J. Zool.* **63**, 365—369.
- Bisazza, A. & Pilastro, A. 1997: Small male mating advantage and reversed size dimorphism in poeciliid fishes. *J. Fish. Biol.* **50**, 397—406.
- Bisazza, A., Vaccari, G. & Pilastro, A. 2000: Female mate choice in a mating system dominated by male sexual coercion. *Behav. Ecol.*, in press.
- Blanckenhorn, W. U., Preziosi, R. F. & Fairbairn, D. J. 1995: Time and energy constraints and the evolution of sexual dimorphism – to eat or to mate? *Evol. Ecol.* **9**, 369—381.
- Breder, C. M. & Rosen, D. E. 1966: *Modes of Reproduction in Fishes*. Natural History Press, Garden City, NY.
- Castro, I., Minot, E. O., Fordham, R. A. & Birkhead, T. R. 1996: Polygynandry, face-to-face copulation and sperm competition in the Hihi *Notiomystis cincta* (Aves: Meliphagidae). *Ibis* **138**, 765—771.
- Clutton-Brock, T. H. & Parker, G. A. 1995: Sexual coercion in animal societies. *Anim. Behav.* **49**, 1345—1365.
- Constantz, G. D. 1984: Sperm competition in Poeciliid fishes. In: *Sperm Competition and the Evolution of Animal Mating Systems* (Smith, R. L., ed.). Academic Press, Orlando, pp. 465—485.
- Constantz, G. D. 1989: Reproductive biology of Poeciliid fishes. In: *Ecology and Evolution of Livebearing Fishes (Poeciliidae)* (Meffe, G. K. & Snelson, F. F., eds). Prentice Hall, Englewood Cliffs, pp. 33—50.
- Darwin, C. 1871: *The Descent of Man and Selection in Relation to Sex*, 2nd edn. Murray, London.
- Farr, J. A. 1989: Sexual selection and secondary differentiation in poeciliids: determinants of male mating success and the evolution of female choice. In: *Ecology and Evolution of Livebearing Fishes (Poeciliidae)* (Meffe, G. K. & Snelson, F. F., eds). Prentice Hall, Englewood Cliffs, pp. 91—123.
- Ghedotti, M. J. & Weitzman, S. H. 1996: A new species of *Jenynsia* (Cyprinodontiformes: anablepidae) from Brazil with comments on the composition and taxonomy of the genus. *Univ. Kansas. Nat. Hist. Mus. Occ.* **179**, 1—25.
- Giacomello, E. 1995: Dimorfismo maschile e successo riproduttivo in *Gambusia holbrooki* (Pisces, Poeciliidae). PhD Thesis, Univ. of Padova, Padova, Italy.
- Gross, M. R. 1984: Sunfish, salmon and the evolution of alternative reproductive strategies and tactics in fishes. In: *Fish Reproduction: Strategies and Tactics* (Wootton, R. & Potts, G., eds). Academic Press, London, pp. 55—75.
- Henson, S. A. & Warner, R. R. 1997: Male and female alternative reproductive behaviors in fishes: a new approach using intersexual dynamics. *Ann. Rev. Ecol. Syst.* **28**, 571—592.
- Hughes, A. L. 1985: Male size, mating success, and mating strategy in the mosquitofish *Gambusia affinis* (Poeciliidae). *Behav. Ecol. Sociobiol.* **17**, 271—278.

- Kulkarni, C. V. 1940: On the systematic position, structural modification, bionomics and development of a remarkable new family of cyprinodont fishes from the province of Bombay. *Rec. Indian. Mus.* **42**, 379—423.
- Magurran, A. E. & Nowak, M. A. 1991: Another battle of the sexes: The consequences of sexual asymmetry in mating costs and predation risk in the guppy, *Poecilia reticulata*. *Proc. R. Soc. Lond. B.* **246**, 31—38.
- Magurran, A. E. & Seghers, B. H. 1994: Sexual conflict as a consequence of ecology: Evidence from guppy, *Poecilia reticulata*, populations in Trinidad. *Proc. R. Soc. Lond. B.* **255**, 31—36.
- Mattig, S. & Greven, H. 1994: Beobachtungen zur Balz und Paarung des Vieraugenfisches *Anableps anableps* (Linné, 1758) (Anablepidae, Cyprinodontiformes). *Z. Fischk.* **2**, 167—192.
- Meyer, M. K., Wischnath, L. & Foerster, W. 1985: Lebendgebärende Zierfische: Arten der Welt. Mergus-Verlag, Melle, Germany.
- Mok-Eym, & Munro, A. D. 1997: Some anatomical and behavioural aspects of reproduction in members of an unusual teleost Family: the Phallostethidae. *J. Nat. Hist.* **31**, 739—778.
- Nelson, J. S. 1994: *Fishes of the World*. Wiley, New York.
- Novarini, N. 1994: Filogenesi molecolare ed evoluzione della fecondazione interna e della viviparità negli Atherinomorpha. PhD Thesis, Univ. of Padova, Padova, Italy.
- Parenti, L. R. 1981: A phylogenetic and biogeographic analysis of cyprinodontiform fishes (Teleostei, Atherinomorpha). *Bull. Amer. Mus. Nat. Hist.* **168**, 335—557.
- Pilastro, A. & Bisazza, A. 1999: Insemination efficiency of two alternative male mating tactics in the guppy (*Poecilia reticulata*). *Proc. R. Soc. Lond. B.* **266**, 1867—1872.
- Pilastro, A., Giacomello, E. & Bisazza, A. 1997: Sexual selection for small size in male mosquitofish (*Gambusia holbrooki*). *Proc. R. Soc. Lond. B.* **264**, 1125—1129.
- Ptacek, M. B. & Travis, J. 1997: Mate choice in the sailfin molly, *Poecilia latipinna*. *Evolution* **51**, 1217—1231.
- Ralls, K. 1976: Mammals in which females are larger than males. *Q. Rev. Biol.* **51**, 245—276.
- Smuts, B. B. & Smuts, R. W. 1993: Male aggression and sexual coercion of females in nonhuman primates and other mammals: evidences and theoretical implications. *Adv. Study. Behav.* **22**, 1—63.
- Snelson, F. F. 1982: Indeterminate growth in males of the sailfin molly, *Poecilia latipinna*. *Copeia* **1982**, 296—304.
- Taborsky, M. 1994: Sneakers, satellites, and helpers: parasitic and cooperative behaviour in fish reproduction. *Adv. Stud. Behav.* **23**, 1—100.
- Thornhill, R. 1980: Rape in *Panorpa* scorpionflies and a general rape hypothesis. *Anim. Behav.* **28**, 52—59.
- Thornhill, R. & Alcock, J. 1983: *The Evolution of Insect Mating Systems*. Harvard Univ. Press, Cambridge.
- Westneat, D. F., Sherman, P. W. & Morton, M. L. 1990: The ecology and evolution of extra-pair copulations in birds. *Curr. Ornithol.* **7**, 331—369.
- Yan, H. Y. 1987: Size at maturity in male *Gambusia heterochir*. *J. Fish. Biol.* **30**, 731—741.

Received: February 4, 1999

Initial acceptance: June 11, 1999

Final acceptance: January 24, 2000 (M. Taborsky)