Males Prefer Small Females in a Dichotomous Choice Test in the Poeciliid Fish *Heterandria formosa*

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Abstract

Male choice is expected to evolve when females differ in quality, even if male investment in each mating is low. The family Poeciliidae is an example of fishes in which males show little parental investment as they only provide sperm. Up until now, a preference for large females has been found in all species studied. Here we show that unexpectedly, males of the least killifish (*Heterandria formosa*) prefer to interact with small instead of large females in a dichotomous male choice test, even though large females are more fecund. During a free-swimming choice experiment, males did not discriminate between females based on their size. We suggest that this unique preference for small females, or the lack of preference for large females, results from strong first male sperm precedence in this species. Smaller females are younger and therefore more likely to be virgin, which probably makes them more profitable mates for males. When presented with a virgin and a mated female of similar size, males showed no preference for either type. This suggests that males do not use pheromone cues to assess female mating status but that they are likely to use female size as a proxy for it.

Introduction

Male choice is much less studied than female choice (Andersson 1994). However, males are expected to exhibit choice over mating partners when females differ in fecundity, the operational sex ratio is female biased or the risk of sperm competition differs between females (Parker 1983; Andersson 1994). Most commonly observed male mating preferences are those that maximize a male's expected fertilization success in each mating (Bonduriansky 2001). Such preferences tend to favour female phenotypes associated with high fecundity or reduced sperm competition intensity (Bonduriansky 2001). Male choosiness is expected to increase when male parental investment increases (as shown by Simmons 1992 and Gwynne 1993). However, if variance in female quality is large, males can be choosy even if

their investment in each mating is insignificant compared to the female investment (Parker 1983).

The family Poeciliidae is an example of fishes, in which males exhibit low parental investment by only providing sperm at mating (Farr 1989). Despite this, male preference for large females has been found in all species studied in this respect (Table 1). In poeciliid fishes, as in fish in general, fecundity increases with size (Bagenal & Braum 1978; Travis et al. 1990; Herdman et al. 2004), and thus males mating with large females could gain benefits in terms of increased offspring number.

In addition to fecundity, males may differentiate among females based on their expected success in sperm competition as first demonstrated by Schwagmeyer & Parker (1990) in 13-lined ground-squirrels (*Spermophilus tridecemlineatus*). Dosen & Montgomerie (2004b) showed that male guppies (*Poecilia reticulata*)

Table 1: Studies of male mate choice for female size and reproductive state in poeciliid fishes

Study	Species	Experimental set-up	Preference (measure)
Farr & Travis (1986)	*Poecilia latipinna,* sailfin molly	No choice	For postpartum vs. gravid and for virgin vs. gravid (following time, no. copulation attempts, no. gonoporal nips and no. courtship displays)
Ptacek & Travis (1997)	*P. latipinna*	Free-swimming choice test	For large vs. small (no. copulation attempts and no. gonoporal nips)
Gabor (1999)	*P. latipinna*	Dichotomous choice test	For large vs. small (time close to female)
Bisazza et al. (1989)	*Gambusia holbrooki,* eastern mosquitofish	Dichotomous choice test AND Free-swimming choice test	For large vs. small (time close to female) For postpartum vs. gravid (no. copulation attempts)
McPeek (1992)	*G. holbrooki*	Dichotomous choice test AND Free-swimming choice test	No size preference, no preference for post-partum vs. gravid females (time close to female) No size preference (no. copulation attempts and no. of chases)
Hoysak & Godin (2007)	*G. holbrooki*	Dichotomous choice test AND Free-swimming choice test	For large vs. small (time close to female) For large vs. small (no. copulation attempts, *but no difference in* time close to female)
Basolo (2004)	*Brachyrhapsis rhabdophora*	Dichotomous choice test	Large males prefer large females, small males prefer small females (time close to female)
Dosen & Montgomerie (2004a)	*Poecilia reticulata,* guppy	Dichotomous choice test	For large vs. small (time close to female)
Herdman et al. (2004)	*P. reticulata*	Dichotomous choice test AND Free-swimming choice test	No size preference (time close to female) For large vs. small (no. gonoporal nips, no. approaches, but *no difference in* no. copulation attempts and no. courtship displays)
Guevara-Fiore et al. (2009)	*P. reticulata*	Free-swimming choice test AND Dichotomous choice test with visual cues only AND Dichotomous choice test with olfactory cues only	For virgin vs. mated (following time, no. gonoporal nips, no. copulation attempts, no. copulations, but *no difference in* time courting) No preference (time close to female) For virgin vs. mated (time close to olfactory cues)
Plath et al. (2006)	*Poecilia mexicana,* Atlantic molly	Dichotomous choice test AND Free-swimming choice test	For large vs. small (time close to female) For large vs. small (no. gonoporal nips, no. copulation attempts)
Deaton (2008)	*Gambusia affinis,* western mosquito fish	No choice free swimming	For large vs. small (no. copulation attempts)
Tudor & Morris 2009	*Xiphophorus malinche,* swordtail	Free-swimming choice test AND Dichotomous choice test	For large vs. small (time close to female, no. displays, no. approaches) No preference (time close to female). See ref. for the effect of male isolation time on preference

are sensitive to the risk of sperm competition and prefer to associate with females that have not been recently inseminated and male mosquitofish (*Gambusia holbrooki*) also react to the perceived risk of sperm competition (Wong & McCarthy 2009). In insects, males have been shown to prefer virgin (Lewis & Iannini 1995: *Tribolium castaneum*; Carazo et al. 2004: *Tenebrio molitor*) or young (Simmons et al. 1994: *Requena verticalis*) females to maximize their share of paternity. In poeciliids, however, the effect of female mating status on male mate choice has not been much studied. To our knowledge, a preference for virgin females has been previously examined only in the sailfin molly (*Poecilia latipinna*) and the

guppy (*P. reticulata*), in which such preferences have been found (Farr & Travis 1986; Guevara-Fiore et al. 2009).

Heterandria formosa (Agassiz), the least killifish, is a small live-bearing poeciliid found in a variety of habitats in the coastal plain of the south-eastern United States (Martin 1980). Fertilization is internal and females are matrotrophic, providing embryos with resources through a placenta-like structure (Grove & Wourms 1991). *Heterandria formosa* females superfetate (simultaneously carry broods of embryos at different stages of development, Fraser & Renton 1940), so they gestate throughout their reproductive life and give birth to several young every few days. *Heterandria formosa* has a resource-free mating system and the reproductive behaviour of males consists mainly of forced matings, so called gonopodial thrusts (Farr 1989; Bisazza & Pilastro 1997). Here we show that unlike in many other poeciliids, *H. formosa* males prefer to interact with small instead of large females in a dichotomous mate choice test, even though large females are more fecund (Schrader & Travis 2008). When males were presented a choice between a virgin and a mated female of similar size, males showed no preference for either.

Methods

All experimental animals were aquarium-born offspring of fish collected from the Otter Creek River, Florida, United States, in 2002. For the experiments, we selected males that were actively following females in stock tanks to be sure that all experimental males were sexually active. Females develop a black spot around their gonopore and on their anal fin as they mature (Fraser & Renton 1940), and we only used clearly mature females in the experiments. Male mate preferences were first measured in a dichotomous male mate choice test, immediately followed by a free-swimming choice test. The tests were performed between 9 AM and 3 PM.

Dichotomous Male Mate Choice Test

We slightly modified a method commonly used for studying female mate choice in Poeciliidae (Ryan & Wagner 1987; Houde 1997). The trials were conducted in an aquarium ($40 \times 20 \times 25$ cm, water level 12.5 cm, covered with a dark plastic foil on three sides except for the front) that was divided into three equally large sections with opaque removable dividers. Two small transparent plastic containers ($8 \times 7 \times 8$ cm, Junior bottom filter; Hagen, West Yorkshire, UK) housing the test females were placed inside the end compartments. To allow possible olfactory cues, the lid was patterned with small slits (width 1 mm) covering about 40% of the lid area.

The focal male was introduced into the middle section of the aquarium. All fish were left to acclimatize for 5 min. After this, the dividers were removed and the male was free to swim anywhere in the aquarium, except inside the small containers housing the females. Male behaviour was observed for 2×15 min. After the first observation period the male was again enclosed in the middle section and the positions of the female containers were switched. This was done in order to control for possible side preferences of males. After 5 min acclimatization, another 15 min of male behaviour was observed.

Male mate preference was defined as the time a male was interacting with the female. Interactions consisted of the male (1) orienting towards the female less than one body length apart, (2) if the female was not stationary, following female movements less than one body length apart and (3) making courtship-like glides towards the female, with arched body and erect fins. Courtship behaviour has not been described in the least killifish (Farr 1989), but Aspbury & Basolo (2002) reported 'courtship-like' behaviour in this species. We summed up the time (in seconds) that the male spent engaged in the above-mentioned interaction types and used that as a measure for preference for a given female.

Free-Swimming Choice Test

Immediately after the dichotomous male mate choice test, we performed a free-swimming choice test to compare the results of these two experimental set-ups. Association tests have been shown to predict actual mating preferences of guppy females (Kodric-Brown 1993), but Gabor (1999) argued that association preferences are not always sexually motivated. Thus the females were set free from their compartments and the behaviour of the male was recorded for 30 min. In the 'virgin vs. mated' treatment this ensured that males could use possible chemical cues to identify female mating status (Crow & Liley 1979; Bisazza et al. 1989). During the free-swimming experiment, the number of copulations, which are called gonopodial thrusts (male approaches the female from behind and thrusts his gonopodium towards the female's genital pore), and copulation attempts (an initiation of a gonopodial thrust that does not reach the female) was counted. The sum of these was used as a measure of copulation attempts,

as in practice it is very difficult to judge whether the gonopodium of a male actually touches the female gonopore or not. We also recorded the time a male spent following the females (following time), which is when the male follows the female as she moves around the tank or, if the female is stationary, when the male orients himself towards the female. If the two females were swimming close to each other, we recorded that as time 'following both females'.

Small vs. Large Females Experiment

For this experiment, two females and a male were haphazardly chosen from different 40 l mixed-sex stock tanks each, so that the male and the females were not familiar with each other. All the females were very likely to be pregnant and most of them had a clearly distended abdomen. Males were tested only once whereas females were returned to their original stock tanks after the test. It is therefore possible that some females were tested twice, although this is unlikely, as there were over 50 females per stock tank. Mean male standard length (SL) was 13.7 mm (SD = 0.5, n = 14). The SL of large females varied from 19.2 to 25.9 mm (x ± SD, 23.2 ± 2.2, n = 14) and that of small females from 12.6 to 19.2 mm (16.0 ± 1.6, n = 14). These sizes are within the range found in nature (Lindström, K., pers. obs.). The average difference between large and small females was 7.2 mm (SD = 1.9) and statistically significant (paired t = 14.16, df = 13, p < 0.001). The trials were conducted in February and March 2004.

Virgin vs. Mated Females Experiment

Virgin females were collected from a tank holding virgin females only and mated females were collected from stock tanks. We size-matched 15 pairs of females by allowing a maximum 0.5 mm difference in SL within a pair. Males were collected from stock tanks, but a male and a mated female within a triplet of fish never originated from the same stock tank. None of the mated females were visibly pregnant and thus males could not use the distended abdomen of females as a cue for virginity. To distinguish virgin and mated females, we marked slightly sedated (with benzocaine solution) females with a dot of either reddish or greenish nail polish on top of their head before the experiment. Females were allowed to recover before the trials. The colours were randomly assigned to female types within pairs. Each fish was used only once in this experiment. Mean male SL was 13.4 mm (± 0.4, n = 15), virgin

female SL varied from 10.1 to 15.3 mm (12.4 ± 1.5, n = 15) and mated female SL from 10.1 to 15.2 mm (12.3 ± 1.4, n = 15). There was no difference in the size of virgin and mated females within pairs (paired t = 0.66, df = 14, p = 0.52). These trials were conducted in June 2004.

Statistical Analyses

We used pair-wise tests to compare the behaviour of males towards the two types of females they interacted with, as the interactions of a given male with the two females are not independent. When the pair-wise differences of the variable in question were normally distributed based on Kolmogorov–Smirnov test, we used paired t-tests. Otherwise we used the nonparametric equivalent, Wilcoxon paired-sample test. All tests were two-tailed. The analyses were performed with SYSTAT 9 (SPSS Inc., Chicago, IL, USA).

Results

Males showed a preference for small females (Fig. 1) (paired t = 3.78, df = 13, p = 0.002), but showed no preference for virgin or mated females in the dichotomous choice test (Fig. 2) (paired t = 0.17, df = 14, p = 0.87). During the free-swimming experiment, males followed the small female for 435 s (±389) (x ±SD), the large female for 375 s (±315) and both females for 381 s (±461). There was no significant difference in the time males followed
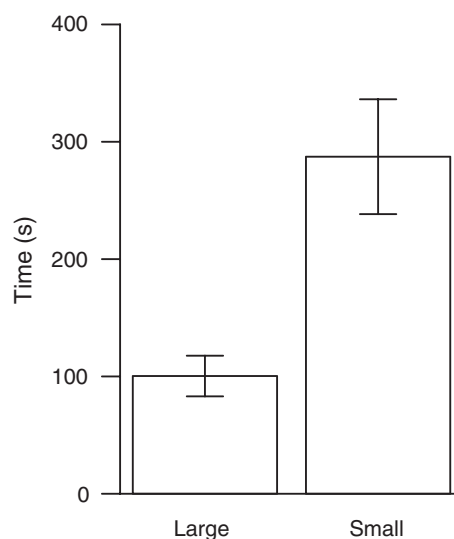
Fig. 1: Mean times (±SE) males interacted with large and small females in a dichotomous male mate choice test. The sample size is 14.
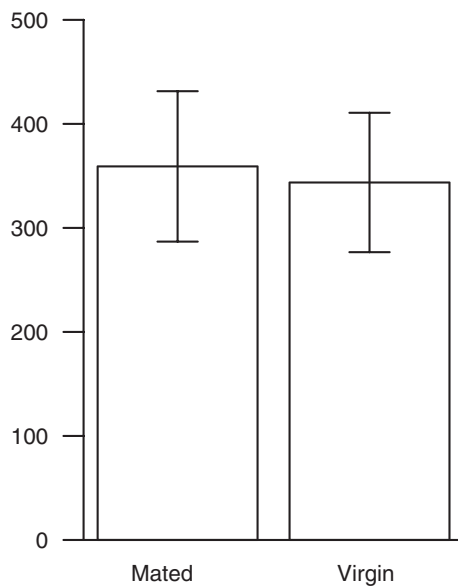
Fig. 2: Mean times (±SE) males interacted with mated and virgin females in a dichotomous male mate choice test. The sample size is 15.

small and large females (paired t = 0.38, df = 13, p = 0.71) or in the number of copulation attempts males performed towards small (8.4 ± 8.2) (x ± SD) and large (11.6 ± 8.1) females (paired t = 1.46, df = 13, p = 0.17). There was also no significant difference in the time males followed virgin (219 s ± 173) and mated (309 s ± 202) females (paired t = 1.18, df = 14, p = 0.26). Males followed both of these females when they swam together for 366 s (SD = 381). There was no significant difference in the amount of copulation attempts that males performed towards virgin (2.1 ± 2.1, n = 15) and mated females (1.3 ± 1.4) (Wilcoxon signed ranks Z = −1.11, p = 0.27, n = 15).

Discussion

We found that *H. formosa* males prefer small instead of large females in a dichotomous mate choice test even though large females are more fecund (Schrader & Travis 2008). This is a unique finding in Poeciliidae, as in other species males have been shown to prefer large females (Table 1). When we simultaneously presented a virgin and a mated female to males, they did not show a preference for either type. We suggest that the preference for small females could result from strong first male sperm precedence in *H. formosa*. When virgin females are mated sequentially to two males, the first male usually has sperm precedence

the extent of which depends on the time interval between the matings (Ala-Honkola 2009; Ala-Honkola et al. 2009). This is different from the guppy, which shows second male sperm precedence (Evans & Magurran 2001). In nature, multiple paternity in *H. formosa* is lower than in other poeciliids but it increases with population density (Soucy & Travis 2003), which suggests that in low density populations females may be in contact with few males only. Thus, at least in low density populations, intervals between matings with different males may be so long that first male sperm precedence prevails. As smaller females would often be younger and therefore more likely to be virgin, we suggest that they are more profitable mates for males. It was therefore somewhat unexpected that males were unable to identify virgin females.

In Poeciliids, larger females are more fecund (Travis et al. 1990; Herdman et al. 2004). *Heterandria formosa* differs from those poeciliids in which males prefer large females (Table 1), in that it superfetates (females simultaneously carry broods of embryos at different stages of development). In species with superfetation, the size–fecundity relationship is not as steep as in species that do not superfetate (Reznick & Miles 1989). Therefore, fecundity difference between small and large females in *H. formosa* may not be as large as in species that do not superfetate. This may be another reason why male preference for large females has not evolved.

During the free-swimming experiment, males did not follow the small females for longer periods of time than the large ones, or try to copulate more often with small females. Even though dichotomous choice tests have been criticized that they may reflect other than sexual interest (Gabor 1999), the difficulty of free-swimming experiments is that the interactions between stimulus females may also affect male behaviour. In our study the females often swam together which prevented scoring which female the male was following. The time the male used following both females as these were swimming together was as long as the time he followed each of the females separately. Thus, we were unlikely to detect significant differences in following times unless the difference would have been extremely large.

The success rate of copulation attempts in *H. formosa* is very low (4–20%, Bisazza & Pilastro 1997; Aspbury & Basolo 2002). Hence it is possible that a male has to use every opportunity to make a copulation attempt whenever a female is in a suitable position. It is also possible that copulation attempts are

so cheap that it does not pay the male to discriminate between two simultaneously presented females. However, our earlier results show that the number of copulation attempts correlates with paternity success (Ala-Honkola et al. 2009), which suggests that mating activity correlates with fitness. Both Plath et al. (2006) and Hoysak & Godin (2007) found that males preferred larger females in a dichotomous choice test and also attempted more copulations with them in a free-swimming choice test (see Table 1). We found that males attempted to copulate on average 8.4 times with small females and 11.6 times with large females. Thus one may argue that males preferred large females in this experiment, but that we were not able to detect the effect as our sample size was low. At a power level 80%, the minimum detectable difference at $\alpha = 0.05$ is 6.7 in this experiment (see Zar 1999, p. 107 for calculations). Thus we would only have been able to detect the difference in copulation attempts if males directed about 50% more copulations to one female type than the other. On the other hand, males did not follow the large females more than the small ones, which suggests that there is no clear indication that males would have preferred large females in the free-swimming choice test. One aspect of possible preference that we did not collect data on was which female the male tried to copulate with first.

An alternative explanation for the observed preference for small females in the dichotomous choice test could be a male shoaling preference for similar sized fish (Hoare et al. 2000). The males were always closer in size to the small than the large female in a pair. It is possible that males prefer to shoal with similar sized fish in order to minimize predation risk by avoiding the so-called 'oddity effect' (Landeau & Terborgh 1986; Theodorakis 1989). However, during the free-swimming choice test there was no indication that males would prefer to shoal with the small females.

In some species of poeciliids, for example the sailfin molly (Ptacek & Travis 1997) and the guppy (Herdman et al. 2004; Guevara-Fiore et al. 2009), males exhibit gonopore nipping behaviour. This behaviour is not shown by *H. formosa* (O. Ala-Honkola, pers. obs.). Gonopore nipping could be a way for males to assess female sexual state in order to detect when females are sexually receptive. As *H. formosa* superfetates, it does not have a post-partum non-pregnant state as non-superfetating poeciliids do. Therefore, there may not have been selection on males to develop the ability to assess female mating status and hence not the ability to distinguish virgin from non-virgin females. Instead, *H. formosa* males may use female size as a proxy for virginity.

Males are expected to invest more in matings with high-quality females, even if their investment in each mating is small (Parker 1983). The trait signalling high female quality may be for example her size, age or probability of sperm competition. How males assess these qualities depends on the mating system and natural history of the species in question. In contrast to earlier studies on Poeciliidae, male preference for large females was not found in *H. formosa*, but males preferred to interact with small females in a dichotomous male mate choice test. As we only used fish from one population in this study, it is possible that male preference for small females is unique to this population. However, we have evidence from another population (Saint Johns River system, FL, USA) that males increase the time they spend in sneaking position (waiting for a suitable moment for a sneak copulation) as female size decreases (see Table 2 in Ala-Honkola et al. 2010). Thus the preference for small female size may be common in this species.

Acknowledgements

Literature Cited

Ala-Honkola, O.. 2009. Pre- and post-copulatory sexual selection in the least killifish, *Heterandria formosa*. PhD Thesis, University of Helsinki, Helsinki. http://ethesis.helsinki.fi.

Ala-Honkola, O., Uddström, A., Diaz Pauli, B. & Lindström, K. 2009: Strong inbreeding depression in male mating behaviour in a poeciliid fish. J. Evol. Biol. **22**, 1396—1406.

Ala-Honkola, O., Tuominen, L. & Lindström, K. 2010: Inbreeding avoidance in a Poeciliid fish (*Heterandria formosa*). Behav. Ecol. Sociobiol. In press. DOI 10.1007/s00265-010-0955-7.

Andersson, M. 1994: Sexual Selection. Princeton Univ. Press, Princeton, NJ.

Aspbury, A. S. & Basolo, A. L. 2002: Repeatable female preferences, mating order and mating success in the poeciliid fish, *Heterandria formosa*. Behav. Ecol. Sociobiol. **51**, 238—244.

Bagenal, T. B. & Braum, E. 1978: Eggs and early life history. In: Methods for Assessment of Fish Production in Fresh Waters (Bagenal, T. B., ed.). Blackwell Scientific, Oxford, pp. 165—201.

Basolo, A. L. 2004: Variation between and within the sexes in body size preferences. Anim. Behav. **68**, 75—82.

Bisazza, A. & Pilastro, A. 1997: Small male mating advantage and reversed size dimorphism in poeciliid fishes. J. Fish Biol. **50**, 397—406.

Bisazza, A., Marconato, A. & Marin, G. 1989: Male mate preferences in the mosquitofish *Gambusia holbrooki*. Ethology **83**, 335—343.

Bonduriansky, R. 2001: The evolution of male mate choice in insects: a synthesis of ideas and evidence. Biol. Rev. **76**, 305—339.

Carazo, P., Sanchez, E., Font, E. & Desfilis, E. 2004: Chemosensory cues allow male *Tenebrio molitor* beetles to assess the reproductive status of potential mates. Anim. Behav. **68**, 123—129.

Crow, R. T. & Liley, N. R. 1979: Sexual pheromone in the guppy, *Poecilia reticulata* (Peters). Can. J. Zool. **57**, 184—188.

Deaton, R. 2008: Factors influencing male mating behaviour in *Gambusia affinis* (Baird & Girard) with a coercive mating system. J. Fish Biol. **72**, 1607—1622.

Dosen, L. D. & Montgomerie, R. 2004a: Female size influences mate preferences of male guppies. Ethology **110**, 245—255.

Dosen, L. D. & Montgomerie, R. 2004b: Mate preferences by male guppies (*Poecilia reticulata*) in relation to the risk of sperm competition. Behav. Ecol. Sociobiol. **55**, 266—271.

Evans, J. P. & Magurran, A. E. 2001: Patterns of sperm precedence and predictors of paternity in the Trinidadian guppy. Proc. R. Soc. Lond. B **268**, 719—724.

Farr, J. A. 1989: Sexual selection and secondary sexual differentiation in poeciliids: determinants of male mating success and the evolution of female choice. In: Ecology & Evolution of Livebearing Fishes (Poeciliidae) (Meffe, G. K. & Snelson, F. F., eds). Prentice Hall, Englewood Cliffs, NJ, pp. 91—123.

Farr, J. A. & Travis, J. 1986: Fertility advertisement by female sailfin mollies, *Poecilia latipinna* (Pisces, Poeciliidae). Copeia **1986**, 467—472.

Fraser, E. A. & Renton, R. M. 1940: Observation on the breeding and development of the viviparous fish, *Heterandria formosa*. Q. J. Microsc. Sci. **81**, 479—516.

Gabor, C. 1999: Association patterns of sailfin mollies (*Poecilia latipinna*): alternative hypotheses. Behav. Ecol. Sociobiol. **46**, 333—340.

Grove, B. D. & Wourms, J. P. 1991: The follicular placenta of the viviparous fish, *Heterandria formosa*. I. ultrastructure and development of the embryonic absorptive surface. J. Morphol. **209**, 165—284.

Guevara-Fiore, P., Skinner, A. & Watt, P. J. 2009: Do male guppies distinguish virgin females from recently mated ones? Anim. Behav. **77**, 425—431.

Gwynne, D. T. 1993: Food quality controls sexual selection in mormon crickets by altering male mating investment. Ecology **74**, 1406—1413.

Herdman, E. J. E., Kelly, C. D. & Godin, J. G. J. 2004: Male mate choice in the guppy (*Poecilia reticulata*): do males prefer larger females as mates? Ethology **110**, 97—111.

Hoare, D. J., Krause, J., Peuhkuri, N. & Godin, J. G. J. 2000: Body size and shoaling in fish. J. Fish Biol. **57**, 1351—1366.

Houde, A. E. 1997: Sex, Color, and Mate Choice in Guppies. Princeton Univ. Press, Princeton, NJ.

Hoysak, D. J. & Godin, J. J. 2007: Repeatability of male mate choice in the mosquitofish, *Gambusia holbrooki*. Ethology **113**, 1007—1018.

Kodric-Brown, A. 1993: Female choice of multiple male criteria in guppies – interacting effects of dominance, coloration and courtship. Behav. Ecol. Sociobiol. **32**, 415—420.

Landeau, L. & Terborgh, J. 1986: Oddity and the confusion effect in predation. Anim. Behav. **34**, 1372—1380.

Lewis, S. M. & Iannini, J. 1995: Fitness consequences of differences in male mating-behavior in relation to female reproductive status in flour beetles. Anim. Behav. **50**, 1157—1160.

Martin, F. D. 1980: *Heterandria formosa* Agassiz, least killifish. In: Atlas of North American Fishes (Lee, D. S., Gilbert, C. R., Hocutt, C. H., Jenkins, R. E., McAllister, D. E. & Stauffer, J. R., Jr, eds). North Carolina State Museum of Natural History, Raleigh, NC, pp. 547.

McPeek, M. A. 1992: Mechanisms of sexual selection operating on body size in the mosquitofish (*Gambusia holbrooki*). Behav. Ecol. **3**, 1—12.

Parker, G. A. 1983: Mate quality and mating decisions. In: Mate Choice (Bateson, P., ed.). Cambridge Univ. Press, New York, NY, USA, pp. 141—164.

Plath, M., Seggel, U., Burmeister, H., Heubel, K. U. & Schlupp, I. 2006: Choosy males from the underground: male mating preferences in surface- and cave-dwelling atlantic mollies (*Poecilia mexicana*). Naturwissenschaften **93**, 103—109.

Ptacek, M. B. & Travis, J. 1997: Mate choice in the sailfin molly, *Poecilia latipinna*. Evolution **51**, 1217—1231.

Reznick, D. N. & Miles, D. B. 1989: Review of life history patterns in poeciliid fishes. In: Ecology & Evolution of Livebearing Fishes (Poeciliidae) (Meffe, G. K. &

Snelson, F. F., eds). Prentice Hall, Englewood Cliffs, NJ, pp. 125—148.

Ryan, M. J. & Wagner, W. E. 1987: Asymmetries in mating preferences between species – female swordtails prefer heterospecific males. Science **236**, 595—597.

Schrader, M. & Travis, J. 2008: Testing the viviparity-driven-conflict hypothesis: parent–offspring conflict and the evolution of reproductive isolation in a poeciliid fish. Am. Nat. **172**, 807—817.

Schwagmeyer, P. L. & Parker, G. A. 1990: Male mate choice as predicted by sperm competition in 13-lined ground squirrels. Nature **348**, 62—64.

Simmons, L. W. 1992: Quantification of role reversal in relative parental investment in a bush cricket. Nature **358**, 61—63.

Simmons, L. W., Llorens, T., Schinzig, M., Hosken, D. & Craig, M. 1994: Sperm competition selects for male mate choice and protandry in the bush cricket, *Requena verticalis* (orthoptera, tettigoniidae). Anim. Behav. **47**, 117—122.

Soucy, S. & Travis, J. 2003: Multiple paternity and population genetic structure in natural populations of the poeciliid fish, *Heterandria formosa*. J. Evol. Biol. **16**, 1328—1336.

Theodorakis, C. W. 1989: Size segregation and the effects of oddity on predation risk in minnow schools. Anim. Behav. **38**, 496—502.

Travis, J., Trexler, J. C. & Mulvey, M. 1990: Multiple paternity and its correlates in female *Poecilia latipinna* (poeciliidae). Copeia **1990**, 722—729.

Tudor, M. S. & Morris, M. R. 2009: Variation in male mate preference for female size in the swordtail *Xiphophorus malinche*. Behaviour **146**, 727—740.

Wong, B. B. M. & McCarthy, M. 2009: Prudent male mate choice under perceived sperm competition risk in the eastern mosquito fish. Behav. Ecol. **20**, 278—282.

Zar, J. H. 1999: Biostatistical Analysis, 4th edn. Prentice-Hall, Upper Saddle River, NJ.