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Sperm priming response to perceived mating opportunities is reduced in male guppies with high baseline sperm production

Silvia Cattelan* and Andrea Pilastro

Department of Biology, University of Padova, Padova 35131, Italy

*Address correspondence to Silvia Cattelan. E-mail: silvia.cattelan@unipd.it.

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Abstract

Producing sperm is costly and males have been selected to strategically adjust their sperm production and/or expenditure according to the fitness return associated with a specific mating. For example, males respond to fluctuations in the mating opportunities by adjusting the number of "ready" sperm. This phenomenon is known as "sperm priming" and is interpreted as a strategy to economize the investment in sperm. The cost and benefits of the sperm priming response, however, are expected to depend on a male's baseline sperm production (BSP) in the absence of females, because of the different risk of sperm depletion and the nonlinearly increasing costs of sperm production. We tested this prediction in 2 replicated lines of male guppies *Poecilia reticulata* that were artificially selected for high and low BSP. BSP has a large genetic variance and a high sire heritability in guppies, and males respond to the perceived mating opportunities by increasing the number of "ready" sperm. We investigated whether males with a different BSP differed in their sperm priming response. We found that when the perceived mating opportunities increased, males from low-sperm lines had a stronger sperm priming response than those from high-sperm lines. This result suggests that adaptive plasticity in sperm priming has the potential to evolve in response to different levels of BSP. The comparison between guppy populations with different levels of sperm production would allow to test whether the pattern reported here is also observed at the interpopulation level.

Key words: artificial selection, mate availability, *Poecilia reticulata*, sperm investment, sperm priming, sperm production.

In species where competition for fertilizing eggs is high, males invest in their ejaculate to increase their postcopulatory reproductive success, typically by increasing their sperm production (Parker and Pizzari 2010). The costs associated with sperm production have often been overlooked, but evidence that they can be substantial has accumulated over the last decades (Dewsbury 1982; Wedell et al. 2002; Hayward and Gillooly 2011). High-mating frequency and large numbers of sperm ejaculated per mating can result in the depletion of sperm reserves (Birkhead and Fletcher 1995; Matthews et al. 1997; Olsson et al. 1997; Preston et al. 2001). Males have, therefore, been selected to maximize the fitness return of their sperm investment by plastically adjusting their sperm allocation according to the mating context (Wedell et al. 2002; Kelly and Jennions 2011). A plastic sperm allocation strategy is favored because males are generally exposed to a continuous gradient of sexual conditions—such as the level of sperm competition, the number of mating opportunities, the quality of the females—all of which may change across time and space. The plasticity in sperm allocation can be in regard to sperm production, that is, the total number of sperm available in a given time-interval, and/or sperm expenditure, that is, the proportion of the sperm available that are used in a given mating (ejaculate allocation, hereafter). These 2 forms of plastic sperm allocation are qualitatively different, because changes in sperm production usually cannot be attained instantaneously. Sperm available for mating can

be increased by accelerating the last stages of sperm maturation ("sperm priming," Bozynski and Liley 2003). Adaptive sperm allocation has been documented in many species characterized by intense sperm competition (Olsén and Liley 1993; Shapiro et al. 1994; Aspbury and Gabor 2004; Scharer and Vizoso 2007; Kelly and Jennions 2011). These 2 types of plastic responses (ejaculate allocation and sperm priming) often coexist, as males may experience both instantaneous variations in the mate characteristics, such as differences in mate quality, or more gradual changes of the socio-sexual context, such as variation in the sex ratio (Kelly and Jennions 2011). For example, territorial males of the blue-headed wrasse *Thalassoma bifasciatum* show a sperm priming response over a few days when mating opportunities increase (Warner et al. 1995). At the same time, they instantaneously tailor their ejaculate allocation to female quality and/or to the presence of sneaker males (Shapiro et al. 1994).

Although phenotypic plasticity in sperm priming and ejaculate allocation has been extensively investigated (Kelly and Jennions 2011), we know very little about the underlying genetic variation of this and other forms of male sexual adaptive plasticity (Bretman et al. 2011). For instance, populations of house mice *Mus musculus domesticus* that evolved under different levels of sperm competition show a different degree of sperm allocation plasticity in response to the perception of sperm competition risk, even after 2 generations in the laboratory under the same conditions (Firman et al. 2013). This result indicates that these differences in adaptive plasticity have a genetic basis. Despite the observation that adaptive sperm allocation plasticity is nearly universal (Kelly and Jennions 2011) and that most sexual traits show genetic variability in plasticity (Hunt and Hosken 2014), the genetic basis of sperm allocation plasticity along different socio-sexual contexts have been minimally investigated.

Here, we focus on the sperm priming response of male guppies (*Poecilia reticulata*) to the perception of mating opportunities. Female guppies are highly polyandrous (Hain and Neff 2007; Neff et al. 2008) and most of the females produce multiply-sired broods (Devigili et al. 2015a). Among the traits contributing to male competitive fertilization success, the number of sperm inseminated has been identified as the most important predictor of paternity (Boschetto et al. 2011). Male guppies show large variation in the size of their sperm reserves, and the number of sperm stripped at rest (hereafter, baseline sperm production, BSP) is highly heritable and characterized by a large genetic variation (Gasparini et al. 2013). Diet restriction and inbreeding are associated with reduced sperm reserves, suggesting that sperm production is associated with significant costs (Gasparini et al. 2013; Rahman et al. 2013, 2014). Males respond to the perception of increased mating opportunities by increasing the number of their "ready" sperm (Bozynski and Liley 2003). This sperm priming response is produced within 3–7 days after males have been in visual contact with females, and has been interpreted as a strategy to reduce the cost of producing sperm in a species in which mating opportunities may vary in time due to fluctuations of population size and sex ratio (Pettersson et al. 2004). Indeed, in natural populations, guppies are exposed to frequent changes in their sex ratio, both in time and space—in particular, along gradients of predation level between upstream and downstream populations (Magurran 2005). While sperm priming is likely to reduce the risk of sperm depletion when males have high mating opportunities (i.e., when the sex ratio is female biased), it also entails costs, as sperm priming is associated with a reduced courtship rate (Devigili et al. 2015b; Cattelan et al. 2016) and may affect male longevity (Miller and Brooks 2005).

Costs and benefits associated with sperm priming may vary across male guppies with different BSP. In particular, it may be argued that a large sperm priming response may be more beneficial for males with a low BSP, which face a higher risk of sperm depletion when mating opportunities increase than males with a high BSP. In contrast, the costs of sperm priming may be higher for males that already have a high BSP, as the costs of progressively increased sperm investment are expected to grow exponentially (Kotiaho 2001). Furthermore, males with high BSP will face a lower risk of sperm depletion than males with low BSP, and the benefits of sperm priming are also expected to be lower. In order to test this prediction, we measured sperm priming in response to the perceived mating opportunities in 2 lines of males guppies that were previously artificially selected for high and low BSP (Cattelan et al. 2018). According to the above reasoning, we expected that high-BSP males should show a smaller sperm priming response associated with the perception of increased mating opportunities than their low-BSP counterparts.

Material and Methods

Experimental fish

The guppies used in this experiment were descendants of wild-caught guppies collected in 2002 from the Lower Tacarigua River in Trinidad. The fish were maintained in large stock aquaria (~100 fish/tank) at 25–27 °C temperature and a 12:12 h light:dark cycle. Fish were fed on a mixed diet of brine shrimp nauplii *Artemia salina* and commercially prepared dry food (DuplarinS). Males used in this study were 5th and 6th generation descendants of a bidirectional artificial selection experiment for high (HS) and low (LS) sperm production (Cattelan et al. 2018). Thus, our study was based on males from 2 independent lines selected for low BSP and 2 lines selected for high BSP. Males from the selection lines were raised in large tanks (115 L), each containing the same proportion of males and females (~1:1) from the same replicate and selection line. When males were 5 ± 1 months old, we haphazardly chose from the tanks 41 HS (n=24 replicate A, n=17 replicate B) males and 45 LS (n=25 replicate A, n=20 replicate B) males.

Experimental protocol

Each experimental male was individually isolated in a 3.5-L tank for 7 days (isolation period, hereafter) to standardize recent social history and acclimatize the fish to the experimental tank (Cattelan et al. 2016). After this 7-day period, males were stripped and sperm were counted. After this first stripping, half of the males were assigned to the female-present treatment for 7 days, whereas the other half were assigned to the no-female treatment. At the end of this 7-day period, males were again stripped of their sperm and sperm were counted. Males were returned to their 3.5-L tank to undergo the alternate treatment (males that were previously in the female-present treatment were isolated from females, and males from the no-female treatment were put in visual contact with females; see Figure 1). At the end of the last treatment, males were stripped from their sperm for the last time, and were photographed to measure body length. Each male, therefore, experienced both treatments, although in a different order. The time frame used to measure males' sperm priming response (7 days) has been previously shown to generate a significant sperm priming response in guppies (Cattelan et al. 2016). Moreover, this is ecologically relevant, as natural guppy populations are demographically highly dynamic and sex

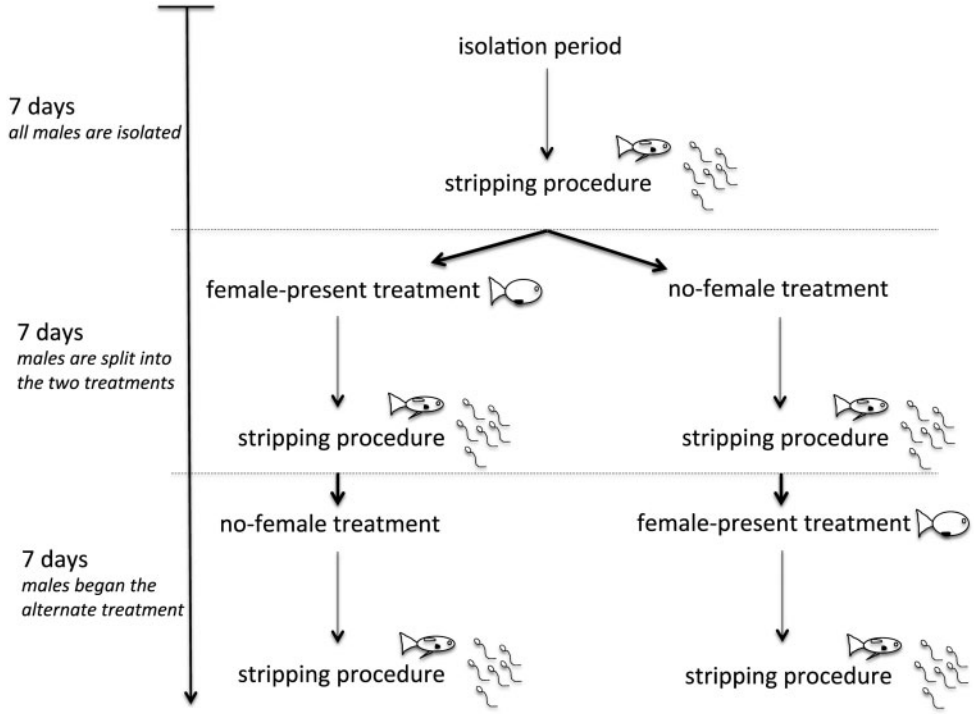

Figure 1. Schematic representation of the experimental design.

ratio can vary over a few days as a consequence of water-flow regime, and males can become temporarily isolated in pools over a period of days to weeks (Houde 1997; Magurran 2005).

The experimental tank was divided into 2 compartments by a transparent partition: 1 of the 2 compartments contained the experimental male, whereas the other contained the female when males experienced the female-present treatment and was left empty when males experienced the no-female treatment. The partition was provided with holes allowing the male in the female-present treatment to detect the olfactory cues of the female in the other compartment. We used pregnant, sexually unreceptive females to minimize possible differences among males attributable to variation in female responsiveness. Indeed virgin, receptive females undergo cyclical changes in responsiveness (Liley 1968) that could differently affect male sexual response, and postpartum females are sexually receptive usually for much less than 1 week (usually 3 days) after parturition (Liley 1966). Furthermore, significant sperm priming response toward unreceptive females has been extensively documented (Bozynski and Liley 2003; Cattelan et al. 2016).

Sperm extraction and count

In guppies, sperm are packaged in discrete bundles (spermatozeugmata) and as the number of sperm cells per bundle (~22,000) did not significantly differ between selection lines (Cattelan et al. 2018), the number of sperm stripped was derived from the number of bundles multiplied by the mean number of sperm per bundle (Cattelan et al. 2018). To collect the sperm bundles from each male, we followed an established procedure (Evans et al. 2003). Each male was anesthetized in a water bath containing 0.15 g/L tricaine mesylate (MS-222) and placed on a black slide under a dissecting microscope. A gentle pressure was then applied to the side of each male's abdomen, just anterior to the base of the gonopodium, to release sperm bundles in a drop of saline solution (NaCl 0.9%). Afterward, sperm bundles were photographed and counted from the digital images using ImageJ analysis software (http://rsbweb.nih.gov/ij/download.html).

Body length measurement

Because bigger males are expected to produce more sperm (Pitcher and Evans 2001), we measured the body length of the experimental males. After the last stripping procedure (at the end of the second treatment), anesthetized males were photographed on their left side (along with a scale for calibration). The distance between the snout and the base of the tail in millimeter, standard length (hereafter, SL), was obtained from digital images using ImageJ software.

Statistical analysis

We log-transformed (natural logarithm) sperm production to meet assumption of normality (see Table 1A, Appendix). We did not detect a statistical difference between sperm production after the isolation period and the no-female treatment (paired *t*-test, $t_{1.84} = -0.746$, $P=0.458$, see Table 2A, Appendix). Repeatability (R) in sperm production after the isolation period and the no-female treatment was estimated following Lessells and Boag (1987), and the standard error (SE) of R was calculated according to Becker (1986). Considering that sperm production was significantly repeatable (see "Results" section), we expressed BSP as the average number of sperm produced when males were isolated from females.

To test whether the 2 selection lines differed in the number of sperm stripped, we first performed an analysis of covariance (ANCOVA) in which the number of sperm stripped was the dependent variable (after $\log_n$ transformation); selection line, treatment, and treatment order were fitted as fixed effects; with the replicate as random effect and the SL as covariate. Second, we calculated the sperm priming response as the difference between the number of

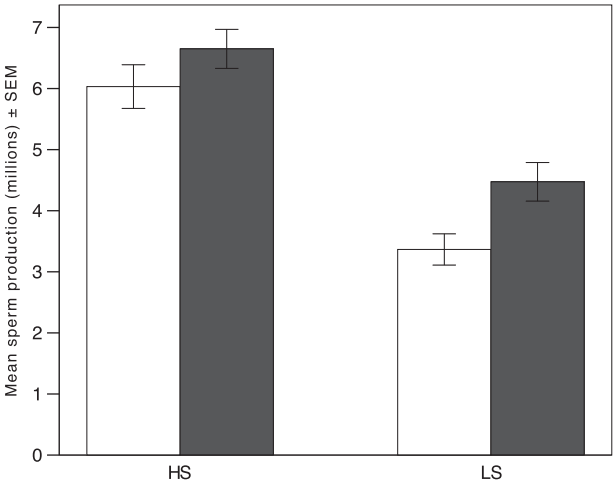

Figure 2. Mean BSP (white bars) and mean sperm production after female-present treatment (black bars) in HS and LS males. Error bars indicate the standard error of the mean.

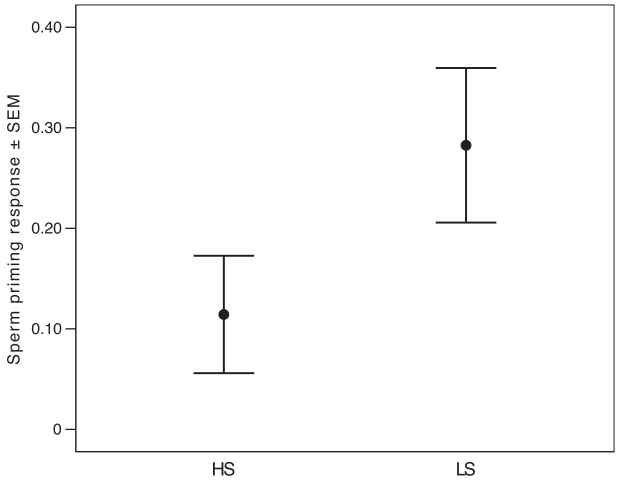

Figure 3. Sperm priming response in HS and LS expressed as delta $\log_n$ of sperm production. Error bars indicate the standard error of the mean.

Table 1. Results from ANCOVAs in which sperm-priming response (expressed as delta $\log_n$ of sperm production) was tested as the dependent variable

(a) All data				
	df	Mean square	*F*	*P*
Selection line	1, 81	0.679	3.507	0.065
Treatment order	1, 81	0.823	4.253	0.042
Replicate	1, 81	1.152	5.956	0.017
SL	1, 81	0.325	1.678	0.199
Selection line * Treatment order	2, 80	0.348	1.819	0.181
(b) Outlier removed				
	df	Mean Square	*F*	*P*
Selection line	1, 80	0.986	6.087	0.016
Treatment order	1, 80	0.714	4.408	0.039
Replicate	1, 80	0.605	3.737	0.057
SL	1, 80	0.436	2.695	0.105
Selection line * Treatment order	2, 79	0.175	1.081	0.302

sperm stripped after exposition to the females and the BSP as follows: delta($\log_n$)=$\log_n$(female-present) − $\log_n$(baseline) (following Hopkins 2000). The sperm priming response in the 2 selection lines was compared using an ANCOVA in which sperm priming was the dependent variable, the replicate was entered as random factor, selection line and treatment order as fixed factors, and the SL as covariate. Finally, for each selection line we ran a Pearson correlation's analysis between BSP and the number of sperm produced after the female-present treatment. All analyses were performed using SPSS 21.0.

Results

When males were isolated from females, the 2 measures of sperm production were significantly repeatable ($R \pm SE = 0.47 \pm 0.085$; $F_{85,\ 171} = 2.778$, $P<0.001$). BSP significantly differed between selection lines ($F_{1,\ 82} = 42.600$, $P<0.001$) and neither male SL ($F_{1,\ 82} = 2.682$, $P=0.105$) nor replicate ($F_{1,\ 82} = 1.586$, $P = 0.211$) affected BSP (Figure 2). After the female-present treatment, males from the high-sperm lines still produced significantly more sperm than their low-sperm counterparts ($F_{1,\ 82} = 21.250$, $P<0.001$, see Figure 2) and neither SL ($F_{1,\ 82} = 0.250$, $P=0.618$) nor replicate ($F_{1,\ 82} = 0.252$, $P=0.617$) affected the number of sperm stripped.

The difference in the sperm priming response was marginally not significant between selection lines, whereas treatment order was significant (Table 1a). However, following Tukey's method (Tukey 1977), we detected an outlier male (see Figure 1A, Appendix). After removing the outlier, the difference between selection lines in the sperm priming response became statistically significant and treatment order remained significant (Table 1b, Figure 3). Finally, BSP was negatively correlated with the sperm priming response (Pearson correlation, HS: $r=-0.594$, $P<0.001$; LS: $r=-0.546$, $P<0.001$; pooled: $r=-0.481$, $P<0.001$), indicating that sperm priming was strongest in males with the lowest BSP (Figure 4).

Discussion

Male guppies plastically adjust the number of "ready" sperm according to the perceived mating opportunities—a phenomenon known as sperm priming: when males are in visual contact with females, the number of "ready" sperm is higher than that observed after males are isolated from females (Bozynski and Liley 2003; Cattelan et al. 2016). In the current study, we found that this sperm priming response is reduced in males with high BSP, as compared to that observed in males with a lower BSP. The guppies used in this experiment were obtained from 2 replicated lines of males that were artificially selected for high- and low BSP (Cattelan et al. 2018). As expected, the males from the 2 artificial selection lines differed significantly in their BSP. When we compared the difference in the number of sperm stripped when males were isolated males (BSP) and when males were in the female-present treatment, we found that low-BSP males showed an increase in the number of "ready" sperm that was on average approximately 32% of their BSP, whereas the high-BSP males' increase was approximately 11% (see Figure 2). This difference was significant after the removal of an outlier, and suggests that, as expected, low-BSP males show a stronger sperm priming response to the perceived mating opportunities. Interestingly, the correlation between BSP and the sperm-priming response was negative and significant also when all males were pooled, regardless of selection line and outlier removal. This result

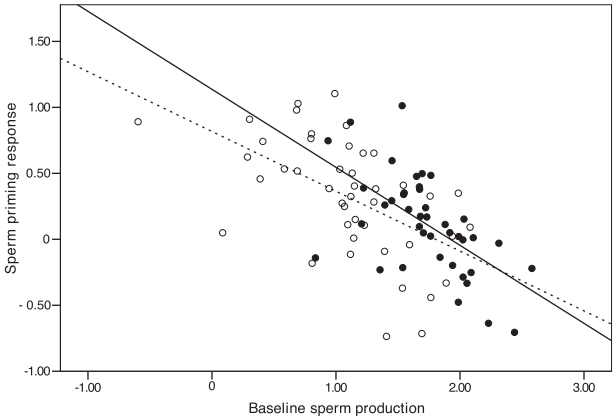

Figure 4. Correlations between sperm priming response and BSP (after natural logarithm transformation) in HS line (black circles and solid line) and LS line (open circles and dotted line).

rules out the possibility that the difference in sperm priming between selection lines was due to genetic drift and strongly suggests that males with low BSP indeed show a stronger sperm priming response.

In the guppy mating system, the capability to adjust the number of sperm available for matings is probably favored for several reasons. First, in natural populations, males experience gradients of population density and sex ratio both across time and space, resulting in large fluctuations of male mating opportunities (Grether et al. 2001; Pettersson et al. 2004). Second, sperm production is costly (Gasparini et al. 2013), yet the number of sperm transferred during copulation is the main predictor of sperm competition success (Boschetto et al. 2011). Males can deplete their sperm reserves after a few matings (Pilastro and Bisazza 1999; Pilastro et al. 2004), and require 3–7 days to replenish their depleted sperm reserves (Kuckuck and Greven 1997; Pilastro et al. 2004). Third, male-stored sperm exhibit a reduction in their swimming velocity (Gasparini et al. 2014), and, therefore, their competitive fertilization capability (Boschetto et al. 2011). Male guppies would, therefore, gain from adjusting their sperm production in response to short-term fluctuations of mating opportunities. Our results indicate that the strength of a male's priming response is negatively correlated with its BSP. However, the stronger sperm priming response of males from the artificially selected low-BSP lines did not compensate, on average, their initial disadvantage. Unpublished observations from our selection lines revealed that high-BSP males deliver a higher number of sperm during a single copulation, but used a lower proportion of their total sperm reserves, as compared to low-BSP males (Pilastro A, unpublished data), suggesting that, overall, high-BSP males have a lower risk of sperm depletion. The cost and the benefits of high and low BSP are likely to depend on the number of available mates and on the level of sperm competition (i.e., on sex ratio), which is known to show large variation in guppy populations (Jirotkul 1999; Pettersson et al. 2004). The observed capability of low-BSP males to show a stronger sperm priming response may contribute to explaining the maintenance of the large genetic variation in BSP observed in guppies (Gasparini et al. 2013).

The observed relationships between sperm priming and BPS in guppies may be a taxonomically widespread pattern. Natural populations often fluctuate in density and sex ratio (e.g. Pettersson et al. 2004; Kasumovic et al. 2008). Whenever males can use reliable cues to anticipate the level of future mating opportunities, anticipatory sperm priming is expected to evolve. Sperm depletion (e.g. Nakatsuru and Kramer 1982; Shapiro et al. 1994; Preston et al. 2001; Schütz et al. 2017) has been reported in a number of species. Because sperm depletion risks depends on BSP, a negative association between the strength of the sperm priming response and BSP could be commonly observed in other species as well.

However, a negative correlation between BSP and the strength of the sperm priming response may not be expected in response to varying levels of sperm competition. Indeed, Firman et al. (2013) found that males of house mice from a population characterized by higher levels of sperm competition (and a higher BSP) showed a greater phenotypic plasticity in sperm production in response to the perceived risk of sperm competition, when compared with males from a population characterized by a lower level of sperm competition and a lower BSP. This is probably because, in populations wherein the average level of sperm competition is high, a larger variance in the level of sperm competition is also observed (Firman et al. 2013). Males of most species experience spatial and temporal gradients of mating opportunities and sperm competition level at a fine-grained scale, that is, variations occur within a male's life. This condition is expected to favor the evolution of anticipatory phenotypic plasticity in sexual traits (Hunt and Hosken 2014). Our results indicate that, as predicted, the strength of the anticipatory plasticity in sperm determined by the perception of future mating opportunities is negatively associated with the baseline level of expression of the trait. Exploring the generality of this pattern will be an interesting avenue for future research.

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Authors' contributions

A.P. and S.C. designed the study, S.C. conducted the experiment, and S.C. and A.P. analyzed the results and wrote the manuscript.

Ethical note

The experiments were carried out in conformity with the relevant Italian laws governing the care of animals in research (D.L. 116/27-01-92, C.M.S. 8/22-04-94). The permit was approved by the ethic committee of the University of Padova (Permit no. 12/2014). The fish were fully anesthetized before sperm extraction and phenotypic measurement. Manipulation was conducted by an expert operator (S.C.) and was completed under 5 min. No mortality was recorded during the stripping procedure and, after the study, all experimental males were returned to nonexperimental tanks.

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Appendix

Table 1A. Results from the normality test (Kolmogorov–Smirnov) on sperm production, both before and after log-transformation (natural logarithm)

	Prior to transformation		After transformation	
	Test statistic	*P*	Test statistic	*P*
Isolation period	0.833	0.491	1.031	0.238
Female-present	0.755	0.619	1.22	0.102
No-female present	1.398	0.040	0.741	0.642

Table 2A. Mean number of sperm ± SEM after the isolation period and the no-female treatment in the 2 selection lines (HS and LS)

	Mean ± SEM	
	HS	LS
Isolation period	5.80 ± 0.34	3.16 ± 0.23
No-female present	6.27 ± 0.52	3.63 ± 0.37

Note: BSP of each male was calculated as the average number of sperm produced when males were isolated from females.

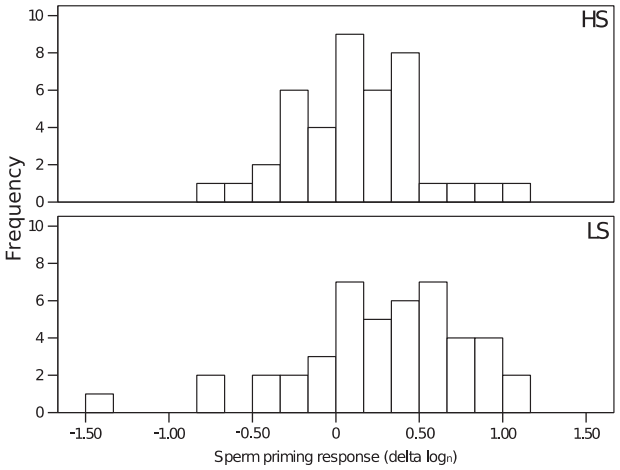

Figure 1A. Histograms showing the distribution of sperm priming response, expressed as delta($\log_n$)= $\log_n$(female-present) − $\log_n$(baseline) in the 2 selection lines.