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# Female presence influences sperm velocity in the guppy

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**As sperm production is costly, males are expected to strategically allocate resources to sperm production according to mating opportunities. While sperm number adjustments have been reported in several taxa, only a few studies investigated whether sperm quality shows adaptive plasticity as well. We tested this prediction in the guppy, *Poecilia reticulata*. A total of 46 males were initially stripped of all retrievable sperm before being randomly allocated to one of two treatments simulating different levels of mating opportunities (visual contact with females or female deprived). After 3 days, males were stripped and sperm velocity was assayed using Computer Assisted Sperm Analysis. Males in the presence of females produced significantly faster sperm than their counterparts. Implications for the evolution of this ejaculate plasticity in the light of results of sperm competition studies are discussed.**

**Keywords:** sperm competition; post-copulatory sexual selection; sperm priming

## 1. INTRODUCTION

On the basis of the low unitary cost of producing sperm, it has long been assumed that sperm are cheap and males are rarely, if ever, sperm limited. In contrast to this view, there is now ample evidence that sperm production is costly (see Wedell *et al.* 2002 for a review) and that sperm production is strategically adjusted according to mating opportunities and hence, expected sperm use (Shapiro *et al.* 1994; Vermeirssen *et al.* 1997; Bozynski & Liley 2003; Aspbury & Gabor 2004). Recently, it has been recognized that not only do numbers influence fertilization success, but also sperm quality traits such as viability and velocity have a great impact on male fitness (reviewed in Snook 2005). In particular, elevated sperm velocity is often associated with high fertilization success both in presence (Birkhead *et al.* 1999; Gage *et al.* 2004; Denk *et al.* 2005) and in the absence (Froman *et al.* 1999; Levitan 2000) of sperm competition. Producing sperm with enhanced swimming performances is likely to be costly, as higher sperm velocity and mobility are associated with higher ATP content (Burness *et al.* 2004; Locatello *et al.* 2007). Recently, it has been shown that sperm quality traits are adjusted in response to the perceived level of

sperm competition (Kilgallon & Simmons 2005; Rudolfson *et al.* 2006; Simmons *et al.* 2007; but see Evans 2009) and female quality (Cornwallis & Birkhead 2007). Only one study investigated the influence of the female presence on sperm quality and the authors did not find any effects on sperm motility (Liley *et al.* 2002).

Using the guppy (*Poecilia reticulata*), a freshwater fish with internal fertilization, we investigate whether sperm velocity is associated with the presence of potential mates. We measured sperm swimming velocity in two groups of males after 3 days of treatment. During this period males were maintained either in visual contact with females or completely female-deprived. We chose a 3 day treatment as adaptive plasticity in sperm production ('sperm priming') was demonstrated over this period in guppy males (Bozynski & Liley 2003). We predict that males maintained in the presence of females will produce faster sperm than males isolated from females.

## 2. MATERIAL AND METHODS

### (a) *Experimental design*

Guppies used in this experiment were descendants of wild-caught fish from the lower part of Tacarigua River, Trinidad. Each experimental male was stripped to completely deplete sperm reserves from 'ready sperm' (see Bozynski & Liley 2003; Evans 2009) before the start of each treatment. After stripping, the male was revived in a small tank with fresh water and allowed to rest for 24 h. Each male was placed individually in a small transparent tank, right next to an identical container which either contained three females ('high mating opportunities' treatment, HM hereafter) or was empty ('low mating opportunities' treatment, LM hereafter). After 3 days of exposure, the male was stripped again and sperm were collected for subsequent velocity assay (see below). At the same time, each male was photographed with a digital camera (Nikon Coolpix 4300) and images were analyzed using IMAGE TOOL software (v. 3.0, UTHSCSA). Body size and the area of colour spots (divided in orange, black and iridescent) were measured.

### (b) *Sperm velocity assay*

Immediately after stripping, ejaculate was collected with a Drummond 3  $\mu$ l micropipette. In this species, sperm are packaged into oval-shaped bundles (i.e. spermatozeugmata). Each bundle, one at a time, was placed on a glass slide previously coated with silicone spray to reduce sperm sticking to the glass surface. Once the bundle was set, 10  $\mu$ l of activating solution (150 mM of KCl and 2 mg ml<sup>-1</sup> bovine serum albumin, see Billard & Cosson 1990) was added, and gently covered with a coverslip. Sperm were analysed using a Ceros Sperm Tracker (v. 12.3; Hamilton Thorne Research, Beverly, MA, USA). For each male, sperm velocity was measured in two different spermatozeugmata and the average was used in the analysis. Measurements of sperm velocity, taken blind of the experimental group, were based on  $178.8 \pm 13.90$  (mean  $\pm$  SE) motile sperm (range: 37–399) and include average path velocity (VAP) and curvilinear velocity (VCL). The threshold values defining static cells were predetermined at 25  $\mu$ m s<sup>-1</sup> (Locatello *et al.* 2006; Evans 2009). The within-male repeatability estimated following Becker (1984) was:  $0.70 \pm 0.08$  SE ( $p < 0.001$ ) for VAP and  $0.58 \pm 0.11$  SE ( $p < 0.001$ ) for VCL. These values are comparable to analogous studies of repeatability of sperm velocity within the same ejaculate (Locatello *et al.* 2006). Statistical analyses were performed using SPSS v. 16.0.

## 3. RESULTS

Males that were randomly assigned to one of two treatments, did not differ in any of morphological traits we considered, such as body size and coloration (ANOVA, all differences between treatments:  $p > 0.249$ ). According to prediction, males produced significantly faster sperm in the presence of females (VAP, HM males:  $121.82 \pm 3.35$   $n = 23$ ; LM males:  $108.35 \pm 3.94$ ,  $n = 23$ ;  $F_{1,45} = 6.784$ ;  $p = 0.012$ ; see figure 1; VCL,

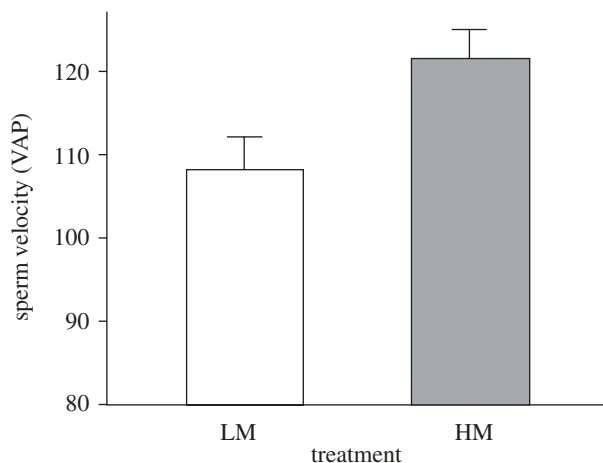


Figure 1. Sperm swimming velocity (VAP; mean  $\pm$  SE) in males maintained for 3 days in conditions simulating low (LM) and high mating opportunities (HM).

HM:  $146.76 \pm 2.61$   $n = 23$ ; LM:  $137.48 \pm 3.62$ ,  $n = 23$ ;  $F_{1,45} = 4.337$ ,  $p = 0.043$ . Sperm velocity remained significantly different between treatments also after controlling for morphological male traits (table 1).

#### 4. DISCUSSION

Our results suggest that male guppies adjust sperm velocity according to the availability of potential mates. Males which were maintained in visual contact with females produced faster sperm than males in the absence of females. Although in this study we did not assess the number of sperm produced, previous work by Bozynski & Liley (2003) and observations from the same laboratory population (C. Gasparini & A. Pilastro 2006, unpublished results) showed that males respond to female presence by increasing the number of 'ready sperm'. Taken together, these results suggest that male guppies show adaptive plasticity both in the quantity and in the quality of the 'ready sperm' in response to the perceived mating opportunities. While an increase in sperm production in the presence of females has been reported in several taxa (Olsen & Liley 1993; Vermeirssen *et al.* 1997; Aspbury & Gabor 2004; Olsen *et al.* 2006), this is the first study, to our knowledge, to demonstrate an adjustment in sperm quality in response to mating opportunities.

A previous study in the rainbow trout did not show an effect of female presence on sperm motility (Liley *et al.* 2002), while another study demonstrated that in the fowl *Gallus gallus*, males allocate faster sperm with high-quality females (Cornwallis & Birkhead 2007). An increased sperm production associated with increased mating opportunities is expected in the guppy, as males can deliver to the female, during a single copulation, up to 92 per cent of their available sperm (Pilastro & Bisazza 1999). One may wonder, however, why male guppies respond to the increased mating opportunities by increasing sperm velocity. Our result is particularly surprising, as male guppies do not adjust the number and quality of sperm that are primed in response to the perceived level of sperm competition (Evans 2009), despite sperm competition being intense in this species (Hain & Neff 2007). One explanation may be that the actual sex ratio does not predict the level of sperm competition associated with a given mating. Indeed, female guppies actively solicit mating with multiple males during the receptive period (Houde 1997). Furthermore, females are continuously exposed to forced matings, which can result in large numbers of sperm inseminated (Pilastro *et al.* 2002). Males do not associate permanently with a female, but after a mating usually turn their attention to other females (Houde 1997). Thus, males may not be able to predict whether a female will mate again (or has already mated) with other males (Engqvist & Reinhold 2005). Therefore, if most matings are associated with high levels of sperm competition (Neff *et al.* 2008), males may adjust their sperm quality in response to mating opportunities only. Experiments of artificial insemination found that both sperm number and velocity are significant predictors of sperm competition success (C. Boschetto, C. Gasparini & A. Pilastro 2006, unpublished data), suggesting that both features are important for sperm competition success in the guppy.

Interestingly, the positive association between sperm velocity (VCL) and orange coloration we found corroborates previous findings (Locatello *et al.* 2006), suggesting that colourful, high-quality males (Evans *et al.* 2004) are probably able to produce both more numerous (Pitcher & Evans 2001) and faster sperm (see also Evans *et al.* 2003). Whether sperm priming strategies of males are associated with their attractiveness represents an interesting avenue for future research.

Table 1. Results from ANCOVA for sperm swimming velocity according to experimental treatment (high versus low mating opportunities) and male phenotypic traits.

	VAP			VCL		
	F-value	p-value	$b \pm SE$	F-value	p-value	$b \pm SE$
treatment <sup>a</sup>	5.912	0.020	$-13.52 \pm 5.56$	4.247	0.046	$-9.02 \pm 4.38$
orange coloration	2.772	0.104	$1.66 \pm 1.00$	9.288	0.004	$2.39 \pm 0.78$
black coloration	0.008	0.927	$-0.41 \pm 4.42$	0.621	0.436	$-2.74 \pm 3.48$
iridescent coloration	1.266	0.268	$-0.95 \pm 0.85$	1.281	0.265	$-0.75 \pm 0.67$
body size (area)	0.106	0.747	$0.09 \pm 0.27$	0.266	0.609	$-0.11 \pm 0.21$

<sup>a</sup>HM treatment as factor's reference level.

The research was 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) and was authorized by the Istituto Superiore di Sanita (Italian National Health Institute).

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