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Possible glimpses into early speciation: the effect of ovarian fluid on sperm velocity accords with post-copulatory isolation between two guppy populations

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Abstract

Identifying mechanisms of reproductive isolation is key to understanding speciation. Among the putative mechanisms underlying reproductive isolation, sperm–female interactions (post-mating–prezygotic barriers) are arguably the hardest to identify, not least because these are likely to operate at the cellular or molecular level. Yet sperm–female interactions offer great potential to prevent the transfer of genetic information between different populations at the initial stages of speciation. Here, we provide a preliminary test for the presence of a putative post-mating–prezygotic barrier operating between three populations of Trinidadian guppies (*Poecilia reticulata*), an internally fertilizing fish that inhabits streams with different levels of connectivity across Trinidad. We experimentally evaluate the effect of female ovarian fluid on sperm velocity (a predictor of competitive fertilization success) according to whether males and females were from the same (native) or different (foreign) populations. Our results reveal the potential for ovarian fluid to act as a post-mating–prezygotic barrier between two populations from different drainages, but also that the strength of this barrier is different among populations. This result may explain the previous finding that, in some populations, sperm from native males have precedence over foreign sperm, which could eventually lead to reproductive isolation between these populations.

Introduction

Speciation starts when barriers reduce gene flow between future daughter species (Coyne & Orr, 2004; Butlin *et al.*, 2012). These barriers can be generated by divergent selection or genetic drift, which can lead to genetic and phenotypic differences among isolated populations and ultimately reduce or eliminate gene flow (i.e. speciation phenotypes; Shaw & Mullen, 2011). Following secondary contact, mating between individuals from different populations (i.e. hybridization) may then lead to the production of hybrid offspring with reduced fitness (post-zygotic isolation, Barton & Hewitt, 1985; Rundle, 2002; Nosil *et al.*, 2005; Abbott *et al.*, 2013). To avoid such costly hybridizations, selection can favour the evolution of *prezygotic barriers*, a process known as 'reinforcement' (Marshall *et al.*, 2002; Lorch & Servedio, 2007; Butlin *et al.*, 2012). Arguably, the most straightforward prezygotic barrier to gene flow operates through precopulatory recognition of, and assortative mating with, conspecifics (Seehausen *et al.*, 2008). However, in many species, conspecific recognition is impossible or mate choice is constrained, thus limiting the scope for precopulatory barriers to effectively prevent hybridization (Eady, 2001). In the absence of precopulatory barriers, hybridization can also be

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avoided through post-mating–prezygotic isolating mechanisms (Howard *et al.*, 2009), involving either ejaculate–female interactions [cryptic female choice (Eberhard, 1996; Firman *et al.*, 2017)] or intrasexual competition [sperm competition (Parker, 1970)]. Such post-mating–prezygotic barriers to hybridization are particularly common in broadcast-spawning marine invertebrates and externally fertilizing fishes, where interactions between individuals happen mainly (or exclusively) through gametes (Mendelson *et al.*, 2007; Howard *et al.*, 2009; Palumbi, 2009; Immler *et al.*, 2011; Yeates *et al.*, 2013). Similarly, in internally fertilizing species, post-mating–prezygotic barriers act to reduce interspecific hybridization, for example through cryptic female choice for conspecific sperm (i.e. conspecific sperm precedence, e.g. Birkhead & Brillard, 2007; Howard *et al.*, 2009; Manier *et al.*, 2013; Shaw & Lambert, 2014; Cramer *et al.*, 2016a,b).

Despite increasing evidence for post-mating–prezygotic barriers to gene flow, with the exception of a few externally fertilizing species (Palumbi, 2009; Kosman & Levitan, 2014), we know very little about the mechanism(s) underlying gametic isolation, particularly during the nascent phases of speciation (Gregory & Howard, 1994; Price *et al.*, 2000; Rugman-Jones & Eady, 2007; Howard *et al.*, 2009; Manier *et al.*, 2013; Tyler *et al.*, 2013; Cramer *et al.*, 2016b). This is surprising given the likely importance of gamete–gamete and gamete–female interactions in incipient speciation (Howard *et al.*, 2009). Furthermore, most of the studies revealing evidence for gametic isolation at the population level come from insects, and thus, our knowledge of these phenomena lacks taxonomic breadth. For example, although studies on insects have generally revealed post-mating–prezygotic barriers between different populations of the same species (e.g. Brown & Eady, 2001; Jennings *et al.*, 2014; Rose *et al.*, 2014; Ala-Honkola *et al.*, 2016), the role of post-mating–prezygotic barriers in driving incipient speciation among vertebrates is less clear, with some studies reporting evidence for gametic isolation (e.g. Ludlow & Magurran, 2006) and others revealing no evidence for con-population sperm precedence (Firman & Simmons, 2014; Kaufmann *et al.*, 2015). Moreover, the proximate mechanisms underlying post-mating reproductive barriers among populations of the same species remain unclear in vertebrate species, although sperm–female interactions are likely candidates (Brown & Eady, 2001; Rose *et al.*, 2014; Beirao *et al.*, 2015).

Here, we test experimentally for a potential mechanism underlying gametic isolation in the guppy, *Poecilia reticulata,* a livebearing fish native to the north-east of South America and the adjacent Antillean islands (Houde, 1997). The populations inhabiting the island of Trinidad are widely used in studies of evolutionary ecology and sexual selection (Houde, 1997; Magurran, 2005), as they exhibit extensive and well-characterized phenotypic variability in sexual coloration, behaviour and life history traits. Phenotypic diversification among guppy populations is accompanied by corresponding genetic diversification (Alexander *et al.*, 2006; Willing *et al.*, 2010; Grueber *et al.*, 2017). In particular, metapopulations of the two main drainage systems in Trinidad's Northern Range Mountains, the Caroni and Oropouche, are highly divergent from one another (Willing *et al.*, 2010), having been separated for an estimated 2 million years (Ludlow & Magurran, 2006; but see Becher & Magurran, 2000; Willing *et al.*, 2010). Despite the length of population separation, and evidence for rapid evolutionary change in this species (Endler, 1983; Ghalambor *et al.*, 2015; Gordon *et al.*, 2015), precopulatory barriers to gene flow, driven by mate recognition, are absent between populations of the two drainages (Magurran *et al.*, 1996). Mating behaviour and sexual conflict are likely to limit the opportunity for precopulatory barriers to evolve (Magurran, 1998): female choice favours rare and unfamiliar male phenotypes (Hughes *et al.*, 1999; Brooks, 2002), whereas high incidence of male sexual coercion is likely to undermine or constrain precopulatory female choice (Houde, 1997; Evans *et al.*, 2011). Importantly, despite the lack of precopulatory barriers among populations, guppies exhibit post-mating–prezygotic barriers to gene flow, which result in skewed paternity in favour of males from the same drainages over males of different drainages when sperm compete to fertilize the same set of eggs (Ludlow & Magurran, 2006).

We tested the hypothesis that female reproductive tract fluid (hereafter named ovarian fluid) acts as a mechanism through which the sperm of native (i.e. same population) and foreign (i.e. different population) males may be favoured or disfavoured, respectively, as they make their way through the female reproductive tract. To test this hypothesis, we measured sperm swimming speed in the presence of ovarian fluid obtained from females from the same or a different population to the male. The guppies originated from two Trinidadian streams (two different populations) belonging to two drainages. We then supplemented the results from these trials on two natural populations with previously collected (unpublished) data obtained using laboratory-reared descendants of wild-caught guppies originating from two different Trinidadian populations but originating from the same drainage. In the guppy, ovarian fluid affects sperm velocity (Gasparini & Pilastro, 2011; Gasparini *et al.*, 2012) and sperm viability (Gasparini & Evans, 2013), two key predictors of sperm competition success in this species (Boschetto *et al.*, 2011; Fitzpatrick & Evans, 2014), and therefore has the potential to differentially influence male fertility. Indeed, sperm–ovarian fluid interactions represent a post-mating inbreeding avoidance mechanism in guppies, as sperm swimming speed – and ultimately competitive fertilization success – is reduced when sperm are exposed to

ovarian fluid from a sister compared to an unrelated female (Gasparini & Pilastro, 2011). Recent studies highlight the importance of ovarian fluid in generating reproductive barriers across different species (Yeates *et al.*, 2013; Cramer *et al.*, 2016a). Here, we test the novel hypothesis that ovarian fluid will also mediate initial reproductive barriers at the population level by differentially affecting sperm swimming performance.

Materials and Methods

Fish populations

We tested the effect of ovarian fluid on sperm swimming speed among populations, within and across drainages, separately. For the within-drainage comparison, we used laboratory-reared fish (42 males and 42 females) descended from wild guppies caught in the Tacarigua (21 males and 21 females) and Aripo (21 males and 21 females) rivers (both in the Caroni drainage). For the comparison across drainages, we used wild-caught fish (102 males and 102 females) from the Aripo (51 males and 51 females) and the Oropouche (51 males and 51 females) rivers, which represent two distinct river drainages (the Caroni and Oropouche) in Trinidad (see Appendix S1 for details and Fig. S1a). Data collected from wild-caught and laboratory-reared individuals were analysed separately. Importantly, although our analysis is based on just three populations, these were specifically chosen for this experiment as post-mating–prezygotic barriers are present between populations from the Caroni and Oropouche drainage (Ludlow & Magurran, 2006), and hybrids generated by crosses between drainages show reduced fitness at maturity (Russell & Magurran, 2006). Specifically, in their study, Ludlow & Magurran (2006) artificially inseminated females from the Aripo and Oropouche rivers with an equal number of sperm obtained from native males (same population) or foreign males (either from same drainage but different population or from a different drainage). Ludlow & Magurran (2006) reported biased paternity in favour of males from a female's native population when the foreign competitor male was from a different drainage. However, there was no paternity bias when artificial inseminations were performed using foreign males from the same drainage (Ludlow & Magurran, 2006).

Experimental design

We used a two-by-two block design to examine how ovarian fluid influences sperm swimming performance (Fig. S1b). We tested the effect of ovarian fluid obtained from each female on the sperm of two males, one of the same population (native) and one (foreign) of another population (either from the same drainage: Aripo vs. Tacarigua, or from a different drainage: Aripo vs. Oropouche). Sperm obtained from each of these two males were tested with ovarian fluid of two different females from the native and foreign populations (Fig. S1b). Each replicate thus consisted of two males and two females from either the same or different population and/or drainage origin.

Ovarian fluid extraction and sperm velocity measurement

Ovarian fluid was extracted from anaesthetized (laboratory) or euthanized (field) females using a Drummond micropipette (Gasparini & Pilastro, 2011). Briefly, 3 μL of saline solution (0.9% NaCl) was gently injected into the female gonoduct, retrieved and then stored in a 0.5-mL tube. The operation was repeated three times, and each ovarian fluid sample (≈9 μL) was split into two aliquots and used for sperm analyses. Sperm (packaged into sperm bundles) were collected in saline solution from anaesthetized (laboratory) or euthanized (field) males, following a standard procedure (Gasparini *et al.*, 2009). Sperm were then activated with either a standard control solution (150 mM KCl solution, for wild guppies), native or foreign ovarian fluid solution (see Appendix S1 for more details). Activation in the control solution allowed us to test for intrinsic differences in sperm velocity between populations. Sperm curvilinear velocity (VCL), which predicts sperm competition success in this species (Boschetto *et al.*, 2011), was subsequently measured using a computer-assisted sperm analyser (CASA, CEROS sperm tracker Hamilton-Thorne Research, Beverly, MA, USA. See Appendix S1). Due to differences in the microscopes, cameras (e.g. final resolution/magnification) and fish origin (wild and wild descendants), we analysed data obtained from laboratory and wild populations separately.

Statistical analysis

We conducted a three-step analysis (step I within and between drainages, separately, steps II and III only between drainages) to determine:

1 Whether ovarian fluid origin (female population) has a different effect on sperm velocity, depending on ejaculate origin (male population), and whether this differential effect is present both between two populations of the same or different drainages. To address this question, we used a linear mixed-effects model with sperm velocity (VCL) as the dependent variable, male population, female population (from which sperm and ovarian fluid were obtained) and their interaction as fixed factors and male identity, female identity and replicate fitted as random effects. We tested populations of the same drainage (Aripo and Tacarigua) and populations of different

drainages (Aripo and Oropouche) with two separate models.

2 Whether sperm velocity in different wild populations (Aripo and Oropouche) is intrinsically different (i.e. in standard conditions, control solution). We used a linear model with sperm velocity (VCL) as the dependent variable and male population as fixed factors.

3 Whether sperm swim faster in ovarian fluid than in control solution (a KCl solution that activates sperm motility) in wild populations (Aripo and Oropouche), as previously demonstrated in laboratory populations (Gasparini & Pilastro, 2011). We used a linear mixed-effects model with sperm velocity (VCL) as the dependent variable, male population and treatment (ovarian fluid or control solution) and their interaction as fixed factors and male identity, female identity and replicate as random factors.

A $\log_{10}$ transformation of VCL gave a considerably better fit compared to the untransformed value (lower AIC) and was thus applied. All models with $\log_{10}$ transformation gave qualitatively similar results to the original models and yielded normally distributed residuals (i.e. model assumptions were met. Shapiro–Wilk normality test: $W > 0.9758$, $P > 0.119$). Analyses were performed in R (R Development Core Team, 2014), with the 'lmer' function of the lme4 R package (Bates *et al.*, 2017) and the 'lm' function of stats package (R Development Core Team, 2014). P values were calculated from F statistics (types II SS) with the lmerTest package. We used Satterthwaite's approximation in the 'ANOVA' function of lmerTest package to calculate the denominator degrees of freedom from the F statistics. We were unable to obtain sperm swimming speed data from two males (one from the Oropouche population and one from the Aripo population) in one of the treatment conditions (Oropouche OF and Tacarigua OF, respectively) and for two males in control treatments (one male from the Aripo and one from Oropouche). Including these males or the entire replicate in the analysis did not qualitatively change our results (data not shown). All analyses were thus performed following the removal of incomplete replicates.

Results

In our populations from the same drainage, there was no significant difference in sperm swimming velocity when ejaculates were activated with native or foreign ovarian fluid (Table 1; Fig. 1 and Table S1). Sperm velocity in native ovarian fluid was higher than in foreign ovarian fluid in 11 of 20 Aripo males (55%) and in 8 of 21 Tacarigua males (38%, see Table S1 for descriptive statistics).

In the tests performed across drainages, we found that, on average, the effect of ovarian fluid on sperm velocity was contingent on the male's population origin (significant interaction term; Table 1). Specifically, sperm from Oropouche males swam faster when activated with native ovarian fluid, whereas those from Aripo males swam similarly in native and foreign ovarian fluids (Table 1; Fig. 1 and Table S1). In 33 of 50 Oropouche males, sperm swam faster in native ovarian fluid (66%), whereas in Aripo males this was the case for only 24 of 51 males (47%). Moreover, in Oropouche ovarian fluid, sperm velocity of native males was on average 4.5% faster than that of foreign Aripo males (post hoc test: $T_{149} = 2.53$, $P = 0.012$).

Overall, we found no significant differences in sperm swimming speed between Aripo and Oropouche males when tested in the standard control solution (male population effect: $F_{1,98} = 1.01$, $P = 0.32$). Thus, we conclude that there are no intrinsic differences in sperm velocity between these populations. As expected, sperm velocity increased in ovarian fluid compared to the KCl solution (treatment, KCl/ovarian fluid: $F_{1,102.059} = 4.44$, $P = 0.04$; male population: $F_{1,50.299} = 1.92$, $P = 0.17$). This effect was similar in Aripo and Oropouche males (treatment * male population: $F_{1,99.447} = 0.08$, $P = 0.78$) and corroborates similar findings from a previous study (Gasparini & Pilastro, 2011).

Discussion

The results from our pairwise tests between two populations demonstrate that ovarian fluid differentially regulates sperm velocity of foreign and native sperm. This effect, however, was present only when the two populations originate from different drainages. Although this primary finding from our study provides possible clues into the putative mechanism(s) underlying reproductive isolation, we acknowledge that our study is based on just two natural populations, thus making generalizations about other populations premature. Surprisingly, the results from these pairwise tests indicated that the pattern was asymmetrical, occurring only in the Oropouche ovarian fluid, where velocity of native sperm was higher than velocity of foreign sperm. This finding does not reflect previous results obtained with the same two guppy populations (i.e. from Aripo and Oropouche rivers; Ludlow & Magurran, 2006), which demonstrate the presence of a symmetrical barrier between the two populations (paternity was more skewed towards native males than foreign males after artificial insemination of females). When two different populations (Tacarigua and Aripo) belonging to the same drainage were tested, we observed no differences in sperm velocity in any of the treatments. However, we cannot rule out the possibility that the absence of an effect in the within-drainage comparison was due to differences between wild and captive-reared guppy populations. Moreover, estimates of effect sizes for these comparisons between ejaculates within ovarian fluid

Table 1 Linear mixed-effects model with log10-transformed sperm velocity (VCL) as dependent variable. Male and female populations, as well as their interaction, are entered as fixed factors. Male identity, female identity and replicate are entered as random factors. Analysis of variance tables of type II SS, with Satterthwaite's approximation for degrees of freedom, are reported.

Fixed factors				Sum of squares	Mean of squares	d.f.	*F*	*P*
Same drainage	Male population			0.0007	0.0007	1/19.163	1.273	0.273
	Female population			0.0000	0.0000	1/20.262	0.102	0.915
	Male population	*	Female population	0.0001	0.0001	20.228	0.255	0.619
Different drainage	Male population			0.0017	0.0017	1/49.950	1.731	0.194
	Female population			0.0048	0.0048	1/50.196	4.959	0.030
	Male population	*	Female population	0.0049	0.0049	1/47.513	5.044	0.029

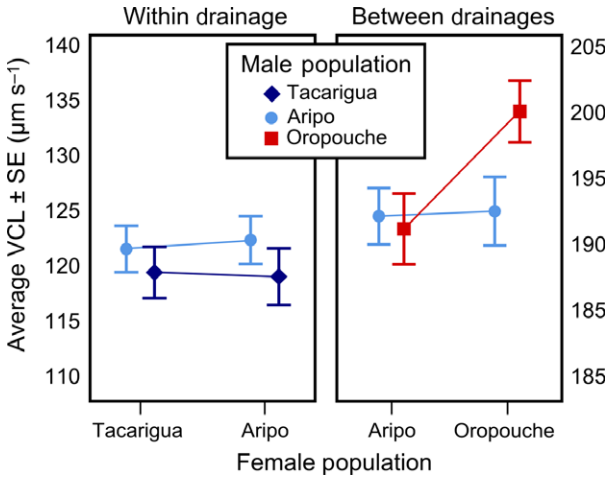

Fig. 1 Average curvilinear velocity (VCL) ± SE of sperm activated with foreign and native ovarian fluid solutions, within and across drainages. Azure circles: Aripo males. Blue diamonds: Tacarigua males. Red squares: Oropouche males.

(i.e. where sperm competition and cryptic female choice occur) were small (see Table S1), although not negligible. This suggests that more samples would be needed to make reliable comparisons. We also found that sperm swimming velocity did not differ between wild populations (Oropouche and Aripo) when measured under standard conditions (control solution). This latter result suggests that the differences in velocity found when the same sperm were activated with native or foreign ovarian fluid (in the two populations from Oropouche or Caroni drainages) were due to the characteristics of the ovarian fluids and their interaction with the ejaculate.

Taken together, these results suggest that ovarian fluid has the capacity to act as a post-mating–prezygotic barrier that limits fertilization success by Aripo males in Oropouche streams. This hypothesis, however, should not be extended to other populations without further validation (i.e. testing more than two populations, from a range of geographical origins). However, our results are in line with previous studies that evaluated the presence of similar reproductive barriers acting among (Yeates *et al.*, 2013; Cramer *et al.*, 2016a) and within species (Beirao *et al.*, 2015). Nevertheless, our findings indicate some complexity in the observed patterns at the within-species level, where the direction of the effect depended on the specific combination of populations examined. Specifically, we found that Oropouche sperm swam faster than those from heteropopulation males when tested in Oropouche ovarian fluid, but no significant change in sperm velocity between Oropouche and Aripo sperm was found when sperm were tested in Aripo ovarian fluid. Thus, the role of ovarian fluid in mediating reproductive barriers among the two guppy populations appeared to be asymmetric. Accordingly, asymmetry has been found (between species) in premating barriers (Hardwick *et al.*, 2013), post-mating–prezygotic barriers (Cramer *et al.*, 2016a) and post-zygotic barriers (Mendelson *et al.*, 2007), possibly being related to the degree of genetic diversification and thus providing scope for unidirectional introgression between isolated populations upon secondary contact.

The capacity for ovarian fluid to affect sperm swimming behaviour is widely recognized (Elofsson *et al.*, 2003; Urbach *et al.*, 2005; Gasparini & Pilastro, 2011; Gasparini *et al.*, 2012; Gasparini & Evans, 2013; Firman & Simmons, 2015; Alonzo *et al.*, 2016). In the guppy, for example, ovarian fluid affects sperm swimming speed and viability, both of which predict the competitiveness of ejaculates to fertilize eggs against rival sperm (velocity: Boschetto *et al.*, 2011; viability: Fitzpatrick & Evans, 2014). However, in guppies, ovarian fluid can *differentially* regulate the relative swimming performance of competing ejaculates, favouring sperm from unrelated males and ultimately facilitating inbreeding avoidance when ejaculates from siblings and nonsiblings compete (Gasparini & Pilastro, 2011). These 'cryptic' female preferences for unrelated mates are moderated by the differential effect of ovarian fluid on sperm swimming velocity, resulting in a *ca.* 5% increase in sperm swimming speed and a *ca.* 10% increase in paternity success in favour of unrelated males (Gasparini & Pilastro, 2011). In our study, when exposed to Oropouche ovarian fluid, sperm from Oropouche males (same drainage and same population) swam 4.5% faster

than those of Aripo males (other drainage). This difference in sperm swimming speed is therefore similar to that reported by Gasparini & Pilastro (2011) when considering sperm from related and unrelated males. Interestingly, the paternity biases in favour of same-population males (over males from other drainages) previously reported were *ca.* 15% (value obtained with GraphClick from fig. 2 in Ludlow & Magurran, 2006). This suggests, therefore, that even small female-moderated changes in sperm velocity, as reported here and by Gasparini and Pilastro, can have highly significant fitness outcomes.

Contrary to predictions, we found that ovarian fluid from Aripo females did not favour native sperm over those of males from the different populations, regardless of their origin (same or different drainage). This finding indicates that in Aripo females mechanisms other than the effect of the ovarian fluid on sperm velocity are involved in determining the observed sperm precedence in relation to population origin of the male (Ludlow & Magurran, 2006). Although we have focused here on sperm swimming velocity, guppy ovarian fluid also affects sperm viability (Gasparini & Evans, 2013), which in turn has been shown to predict competitive fertilization success in this species (Fitzpatrick & Evans, 2014). Moreover, competitive fertilization success may be influenced by MHC similarity between mates. Egg–sperm recognition often involves MHC loci (Scofield *et al.*, 1982; Wu *et al.*, 1990), and female guppies show cryptic preference for MHC-similar mates in the Tacarigua population (Gasparini *et al.*, 2015). Guppies from different drainages are significantly differentiated at this locus (Fraser *et al.*, 2010a, b), and it is therefore possible that this component of cryptic female choice (possibly independent from the effect of the ovarian fluid on sperm velocity) may contribute to the observed paternity pattern reported by Ludlow & Magurran (2006).

The asymmetrical effects detected in our study (which does not reflect previous findings) could be explained by nonadaptive processes, based on genetic drift between populations, or adaptive processes, associated with the risk of outcrossing. The historical record on guppy migration between drainages may point to an adaptive explanation for this asymmetry. In 1957, G. P. Haskins introduced *ca.* 200 guppies originating from the Caroni drainage (Aripo River) into the Oropouche drainage (Shaw *et al.*, 1992; Becher & Magurran, 2000). These individuals formed a stable population so that in the introduction site (upper Turure River) only descendants of these fish are now present, whereas downstream the original population has been progressively displaced (genetic markers from original Turure population are retained at frequencies between 2% and 9%; Becher & Magurran, 2000). Given the presence of intrinsic outcrossing/hybridization costs (Russell & Magurran, 2006), it is possible that this introduction and subsequent migratory events triggered or accelerated the evolution of post-mating–prezygotic barriers between populations of the two drainages [i.e. through a reinforcement process (Lorch & Servedio, 2007; Pfennig, 2016)] asymmetrically and potentially specifically targetted towards the Aripo population. It has to be noted, however, that although this recent unidirectional (human-mediated) migration event could have asymmetrically reinforced selection for a post-mating–prezygotic barrier, this speculation requires empirical validation and does not reflect the symmetrical results previously found on paternity bias (Ludlow & Magurran, 2006). It is interesting to note that post-mating selection preference for MHC-*similar* mates reported in guppies (in contrast to the more usual pattern of sexual selection for MHC dissimilarity; Kamiya *et al.*, 2014) has also been reported in the Atlantic salmon and has been interpreted as the result of selection against the risk of hybridization (Yeates *et al.*, 2009). These results indirectly support the view that the risk of hybridization/outbreeding may have shaped patterns of cryptic female choice in guppies from the Oropouche population (and the Caroni drainage).

To conclude, our work suggests that ovarian fluid differentially moderates sperm swimming velocity, thus offering a putative mechanistic explanation for the post-copulatory–prezygotic barrier to gene flow previously observed between guppies from Aripo and Oropouche rivers (Ludlow & Magurran, 2006). However, we acknowledge that our analyses are confined to just two natural populations, and thus, our conclusions cannot be generalized to include other populations where ongoing speciation events have been discussed (Magurran, 2005). Interestingly, ovarian fluid-moderated impacts on sperm performance can reduce the likelihood of both inbreeding and outbreeding, by disfavouring males that are at two extremes of a 'relatedness continuum' (i.e. close relatives and divergent populations), and favouring males of intermediate, optimal genetic relatedness to the female. Future experiments that focus on other guppy populations (e.g. other drainages in Trinidad and other populations outside Trinidad), and those that explore the effect of native/foreign ovarian fluid on other sperm characteristics (e.g. sperm viability), may offer a better understanding of the nature and strength of post-mating–prezygotic barriers attributable to the sperm–ovarian fluid interaction in this species.

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Sampling and experimental permits

Collections in Trinidad were carried out as approved by the Director of Fisheries, Fisheries Division, Ministry of Food Production (Trinidad and Tobago), and all animal procedures were approved by UWA's Animal Ethics Committee (Permit Number RA/3/100/513). Experiments in Padova conformed 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).

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:
Appendix S1 Supplementary Methods section: Field sampling and fish maintenance.
Table S1 Descriptive statistics (Average ± 95% C.I.) of sperm velocity of all different males (VCL, expressed in μm/s) measured in the ovarian fluids obtained from females of the tree different populations.
Figure S2 (a) Locations of northern Trinidad populations used in our study. (b) Experimental design depicting all cross combinations of OF-sperm used for the experiment.

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