Evolutionary biology

Sexual conflict and the function of genitalic claws in guppies (*Poecilia reticulata*)

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Poeciliid fish, freshwater fish with internal fertilization, are known for the diversity of structures on the male intromittent organ, the gonopodium. Prominent among these, in some species, is a pair of claws at its tip. We conducted a manipulative study of these claws in the guppy, *Poecilia reticulata*, to determine if these aid in transferring sperm to resistant females. We compared the sperm transfer rates of clawed versus surgically declawed males attempting to mate with either receptive or unreceptive (i.e. resistant) females. Our analyses demonstrate that the gonopodial claws function to increase sperm transfer to unreceptive females during uncooperative matings but not during receptive matings. Up to threefold more sperm were transferred to unreceptive females by clawed than declawed males. These data suggest that the claw is a sexually antagonistic trait, functioning to aid in transferring sperm to resistant females, and implicate sexual conflict as a selective force in the diversification of the gonopodium in the Poeciliidae.

1. Introduction

Genitalia are often strikingly complex, diverse and rapidly evolving [1–3]. Although there is increasing support for sexual selection in driving this diversification, the mechanism of selection, whether pre- or post-copulatory, is less well understood [1–3]. Post-copulatory selection, either through sperm competition or some form of female biasing of sperm use, has received some support [3–5]. Pre-copulatory sexual selection has received less attention but may occur in cases when genital structures function as grasping devices and sexual conflict over mating rate is implicated [5–7].

Although most of the evidence we have for the role of sexual selection in genital evolution tends to be correlative [3], a few recent studies provide more direct evidence from the experimental evolution of genitalia [8–10] or phenotypic manipulations of genital features [6,7,10–15]. These latter ‘phenotypic engineering’ studies are particularly informative, because they focus directly on function and offer the promise of finely distinguishing among alternative mechanisms of selection, unimpeded by unmeasured correlated traits. Here, we use this manipulative approach to distinguish among alternative mechanisms of selection on a genital structure in the Trinidadian guppy, *Poecilia reticulata*.

In the Poeciliidae, the gonopodium, the modified anal fins that serve as intromittent organs, are remarkably diverse. Much of the divergence among species is in structures at the distal tip, which can include claws, hooks, serrae and spines [16] (figure 1a). Previous studies have focused on interspecific variation in these structures [16–18] and, for a few species, including *P. reticulata*, interpopulation variation [19–23]. Here, we use phenotypic engineering to explore the function of the genitalic claws in *P. reticulata* (figure 1a), a structure that varies among populations [22,23], and for which there has been much speculation about its function [16,18,22,23].
insemination, removing the claw would reduce sperm transfer irrespective of the female status.

2. Material and methods

Guppies were laboratory-reared descendants of fish from a natural Trinadian population, the Oropuche River [24]. For the declawed treatment, genital claws were removed with a scalpel under a dissection microscope (figure 1b). For the control treatment, the gonopodium was manipulated in a similar manner without removing the claws and, instead, most of the spines in the two dorsal fin rays in the caudal fin were removed. All males were anaesthetized with MS-222.

To determine the function of the claws, an individual clawed or declawed male was placed with a virgin female in an observation aquarium and their reproductive behaviours were assessed. Receptivity of females was determined post hoc. Females were considered receptive if cooperative copulation(s) occurred during their trial, or unreceptive if only sneak copulation attempts occurred. Trials were terminated when at least two cooperative copulations or 20 sneak copulation attempts occurred or, failing that, at 2 h. Females were then removed from the observation aquarium and euthanized with MS-222. Sperm were extracted by injecting 100 μl of physiological solution (0.9% NaCl) with a micropipette into the female genital pore, which was then drawn up and reinserted into the female five times, and then transferred to an Eppendorf tube. To break apart sperm bundles, each sample was drawn up and expelled five more times with a Pipetman. Finally, 10 μl of the sample was transferred onto each of two counting chambers in an Improved Neubauer Haemocytometer.

For further details on fish, sperm transfer, mating trials and reproductive behaviours, see electronic supplementary material.

(a) Statistical methods

All sperm counts were square-root transformed prior to analysis. There was a large difference in the amount of sperm transferred to receptive and unreceptive females (11 orders of magnitude; electronic supplementary material table S1) and some observer effects were also detected, resulting in a significant difference in variance (i.e. heteroscedasticity; Fligner-Killeen: d.f. = 7, med $\chi^2 = 57, p < 0.001$). To address these statistical issues, we standardized the data for receptive and unreceptive females by both their respective means and observer:

$$\frac{X_{\text{sperm transferred}}}{C_2} = \frac{X_{\text{sperm transferred}}}{C_2} = \frac{1}{\text{med}}$$

Note that this standardization removes any main effect of receptivity (i.e. $X_{\text{receptive}} = 1$ versus $X_{\text{unreceptive}} = 1$; $p = 1.000$). The transformed-standardized data showed no significant difference in variance (Fligner-Killeen: d.f. = 1, med $\chi^2 = 2.74, p = 0.098$). To determine the effects of male treatment and its interaction with female receptivity on sperm transfer, an ANOVA was then performed. All analyses were performed in the statistical software R v. 2.14.2 [26].

3. Results

Claw removal had no effect on males’ behavioural interactions with females (see electronic supplementary material). The ANOVA of the standardized values of sperm transferred to females revealed a significant male treatment effect and strongly suggested an interaction between male treatment and female receptivity (table 1). Post hoc Tukey tests revealed that the effects resulted from a significant positive effect of the claw in unreceptive females ($p = 0.012$) but no claw effect in receptive females (figure 2). Therefore, the claw aids in transfer of sperm to unreceptive females but not to receptive
females. Given that claw removal did not significantly affect sperm transfer to receptive females, this allows us to reject the alternative hypothesis that the claws are required to retain sperm at the tip of the gonopodium.

4. Discussion

Our phenotypic manipulation of the gonopodium has allowed us to distinguish between two alternative hypotheses for claw function: securing sperm at the gonopodial tip and aiding in the transfer of sperm to resistant females. The claw plays a very clear role in sperm transfer to resistant females; up to threefold more sperm were transferred when males had intact claws compared with those with claws removed (table 1 and figure 2). These data suggest that the claw is a sexually antagonistic trait, functioning as a device to grasp resistant females, as originally hypothesized by Rosen & Gordon [16] and Chambers [18]. It is also possible that the claws serve to stimulate females to retain sperm (i.e. post-copulatory selection). For example, females mating with clawed males may be less likely to dump sperm [22] or more likely to use sperm for fertilization [27,28]. However, preliminary assays by Cheng [22] suggested no paternity biasing towards clawed males. Therefore, sexual conflict appears to be playing a selective force in the diversification of this trait in the Poeciliidae.

Our study joins a very few ‘phenotypic engineering’ studies of genitalia [6,7,10–15]. These studies have alternately supported a pre- and/or post-copulatory function of the manipulated structures. Much like our own study, laser ablation of the male genital spines in \textit{Drosophila bicepistata} [6] and \textit{Drosophila ananassae} [7] resulted in reduced copulatory success. The authors suggested that spines function in the mechanical coupling of the genitalia and that resistance of females may favour their elaboration. In the case of guppies, we have been able to demonstrate that resistance itself favours the claws of the gonopodia: the claws played no role in sperm transfer when females were receptive to mating. A comparative framework will be required to determine the extent to which sexually antagonistic selection can account for the diversification of gonopodia in the Poeciliidae. Functional studies, such as we have reported here, will be critical in informing future comparative studies.

All procedures involving live animals described in this manuscript were reviewed and approved by the University Animal Care Committee at the University of Toronto under animal use protocol number 20008230. All procedures were conducted in accordance with appropriate national and provincial guidelines and regulations.

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