

## Original Article

## Coevolution of male courtship and sexual conflict characters in mosquitofish

Silu Wang, Molly Cummings, and Mark Kirkpatrick

Department of Integrative Biology, University of Texas, Austin, TX 78712, USA

Received 31 May 2014; revised 5 February 2015; accepted 7 March 2015.

Coerced copulations cause sexual conflict: they enhance male fitness at a potential cost to female fitness. In contrast, male display allows female mate choice and stimulates cooperation, and so is thought to be an alternative to coercion. Here, we investigate the evolutionary relationship between coercion and display in *Gambusia*, a genus of poeciliid fish. We used a novel Markov chain method to quantify the transition rate from a male display to a copulatory thrust (the display-to-thrust [DT] rate), which we use to measure the importance of male display to copulation. We assess the potential for male coercion and the female response to coercion based on morphology. We found positive relationships between DT rate and 2 morphologies that are indicative of coercion: male gonopodium shape and the male morphological mating advantage (the ratio of gonopodium shape to female papilla size scores). We suggest that male display can cause the evolution of traits involved in sexually antagonistic coevolution, or conversely that display can evolve as a byproduct of sexual antagonism.

**Key words:** coercion, display, female choice, sexual selection, sexually antagonistic coevolution.

## INTRODUCTION

Sexually antagonistic selection has profound impacts on diverse aspects of mating systems, including reproductive behavior and sexual morphologies (Parker 1979; Gavrillets 2000; Wachtmeister and Enquist 2000). In the coevolution of males and females, advantages achieved by one sex generate selection for counteradaptations by the other sex (Rice 1996; Holland and Rice 1999; Arnqvist and Rowe 2002; Lessells 2006). The resulting sexually antagonistic coevolution (SAC) has been studied in the context of several morphological characters (Rice 1996; Civetta and Clark 2000; Gavrillets 2000; Arnqvist and Rowe 2005).

SAC is most often associated with taxa in which male–male competition drives an arms race between males and females over mating rate, mate quality, outcrossing, and other fitness interests (Ross and Crews 1977; Rice 1996; Arnqvist and Rowe 2002; Parker 2006). It frequently results in male coercion, a suite of behavioral and morphological traits that enhance male fitness but often undermine or manipulate female mate choice (Clutton-Brock and Parker 1995). Male display, on the other hand, is commonly associated with taxa in which females exert control over mating rate and paternal identity via female choice (Lenz 1994; Gamble et al. 2003; Parker 2006; Hebets et al. 2011; Oliver and Lobel 2013). Male display may be beneficial to females as it can enable mate assessment

(Trivers 1972; Andersson 1994), minimize manipulation of females (Moore et al. 2001), stimulate cooperation during mating (Farr 1980), lead to parental investment after mating (Armstrong 1963; Servedio et al. 2013), and prime reproductive physiology (Lehrman 1959; Lehrman 1964; Bastock 1967).

Here, we examine the coevolutionary relationship between display and morphologies that are indicative of male coercion, and female response to them, in a genus of poeciliid fishes. This family may be particularly prone to sexual conflict because they have internal fertilization and 20–30 days of gestation, and indeed sexual conflict has been well documented in them (Parzefall 1973; Constantz 1989; Greven 2005; Langerhans 2011; Kwan et al. 2013). Poeciliid mating systems are generally characterized by male reproductive behavior—gonopodial thrusting only (coercion) versus gonopodial thrusting and courtship display (Rosen and Tucker 1961; Martin et al. 2010; Langerhans 2011). In general, poeciliid courtship involves males approaching females slowly from the front and displaying immediately before copulation attempts (Liley 1966; Farr 1976; Farr 1980; Constantz 1989; Ryan and Causey 1989; Godin 1995). Courtship copulation attempts (male display followed by gonopodial thrusting) often follow a female receptivity response (exposed genital pore), leading some poeciliid researchers to assign a cooperation-facilitating role to courtship (Farr 1980). Male coercion, on the other hand, involves males approaching females swiftly from behind and performing gonopodial thrusts without a display (Baerends et al. 1955; Rosen and Tucker 1961; Parzefall 1969; Itzkowitz 1971; Peden 1972b; Constantz 1975; Martin 1975; McPhail 1978; Farr 1989).

Address correspondence to S. Wang, who is now at Department of Zoology, University of British Columbia, Vancouver, BC V6T 1Z4, Canada. E-mail: silu\_wang@hotmail.com.

In poeciliid fishes, there is a mounting evidence that morphological traits in males and females experience SAC (Peden 1972a, 1972b; Constantz 1989; Langerhans 2011; Kwan et al. 2013). During copulation, males transfer sperm with the gonopodium, which is a modified anal fin. The length of gonopodia varies across poeciliids, and species with longer gonopodia tend to lack courtship displays (Rosen and Tucker 1961; Langerhans 2011). Genera with male displays have shorter gonopodia, and phylogenetic analysis suggests that the evolution of shorter gonopodia precedes the evolution of courtship (Martin et al. 2010). Interestingly, genera with shorter gonopodia also tend to have a greater number of accessory structures (Langerhans 2011). Gonopodial accessory structures include serrated tips, hooks or claws—features that are thought to facilitate coercive copulation (Rosen and Gordon 1953; Rosen and Tucker 1961; Reynolds et al. 1993; Greven 2005; Langerhans 2011; Kwan et al. 2013). These gonopodial structures can cause female vaginal bleeding and injuries (Clark et al. 1954; Kadow 1954; Rosen and Tucker 1961), and these injuries may decrease the probability that the female will remate (Constantz 1989). The most definitive evidence for an SAC role of gonopodial anatomy is from the guppy, *Poecilia reticulata*. Experimental manipulation demonstrated that the gonopodial claw increases sperm transfer to unreceptive females (Kwan et al. 2013).

Female reproductive morphology appears to coevolve with the male gonopodium in poeciliids. The female genital papilla is a pad-like structure at the opening of the oviduct that is thought to deflect the gonopodium and give females more control over copulations (Parzefall 1973; Constantz 1989). An enlarged urogenital aperture may reduce contact with the gonopodium and so reduce female vaginal injuries (Langerhans 2011). It may also enhance female cryptic choice by allowing females to flush out sperm with later inseminations (Pilastro and Bisazza 1999). The papilla and urogenital aperture both tend to be larger in species in which males have a more pointed gonopodium (Peden 1972a; Langerhans 2011).

To examine the relationship between sexual conflict and display, we investigate the coevolution between male display and morphologies associated with SAC in a genus of poeciliids. In the genus *Gambusia*, mating is often coercive (Peden 1972b; Constantz 1975; Farr 1989). Some species do show male display (Rosen and Tucker 1961; Langerhans 2011), however, and female receptive mating sometimes occurs (Peden 1970; Hughes 1984). We study the relationship between male display behavior and 2 morphological features: male gonopodium shape and female papilla size. Following Arnqvist and Rowe (2002), we assess the male morphological advantage using a ratio of the scores for those 2 structures.

To quantify the contribution of male display to mating, we analyzed data on behavioral sequences using a new method that

calculates the rate of transition from a display to a copulatory thrust. A high transition rate occurs when display is closely followed by copulatory thrust, with few other intervening behaviors, similar to species where display is predominant (Liley 1966; Constantz 1989). We interpret this as representing a situation where male display plays an important role in mating. Our results suggest that male displays are more important when males have a morphological advantage over females that facilitates forced copulations.

## METHODS

### Male mating behavior

We obtained data on the mating behavior of 10 *Gambusia* species from the PhD thesis of Peden (1970). His estimates of the frequencies of transitions between pairs of behaviors are shown in Table 1. Full descriptions of the behaviors are given in the thesis. Briefly, interactions between pairs of males and females were recorded for each species. Pairs were separated in a 51 × 21 × 16-cm aquarium by a divider, which was removed on the day before the observations. Behavior sequences were recorded for 10 or 15 min (Peden 1970).

To quantify the contribution of male display to mating, we determined the rate at which males proceed from a display (frontal and lateral displays; see Table 1) to a copulatory thrust. We first calculated the mean number of transitions between different behaviors that occur between a display and a copulatory thrust. The calculation, which is based on a novel analysis using Markov chain theory, is described in Supplementary Appendix 1. It accounts for all of the infinite possible sequences of behaviors. A key assumption is that the transitions are independent: the probabilities of transitions to the next behavioral state are influenced by the current state but not by previous states. We then take the reciprocal of the mean number of transitions. We refer to this statistic as the display-to-thrust rate, or DT rate.

The DT rate measures the speed with which males pass from the display to a copulatory thrust. We interpret it to be a proxy for the relative importance of display to the copulation. A high DT rate suggests that there is a strong connection between display and copulation (Liley 1966). A low DT rate, on the other hand, says that many behaviors intervene between a display and a copulation attempt, suggesting that display is less important.

The calculation of the DT rate was modified for 4 species. *G. atrova* has 2 types of displays, and for this species, we averaged the DT rates starting with each of the displays. *G. vittata*, *G. gaigei*, and *G. nobilis* were never observed to make a male display, and for these species, we assigned a DT rate of 0 because clearly display is not important to copulation.

**Table 1**  
***Gambusia* male mating behaviors (Peden 1970). Behaviors in bold are the starting and ending states used to calculate the DT rate**

Behavior	Description
<b>1. Display</b>	Side facing female, fins partly folded (frontal display); or swims parallel to female, fins extended (lateral display)
2. Others	Nonmating behaviors (e.g., feeding, sparring)
3. Orient	Faces toward female, straight bodied, 2–10 cm from the female
4. Approach	Moves toward female from behind
5. Nibble	Gently contacts female body with mouth
6. Examine	Frequent pauses head at less than 1 cm from female genital opening
7. Swing	Swings gonopodium forward to a precopulatory position
<b>8. Thrust</b>	Propels body upward and directs gonopodium toward female genital area

## Female and male reproductive morphologies

We estimated the mismatch between female and male reproductive morphologies. We assessed male gonopodium shape using the categories defined by Peden (1972a, 1972b) and Langerhans (2011): 0 = rounded, 1 = broadly acute, 2 = acute. We likewise used their categories for papilla size: 1 = papilla absent and aperture completely covered by tissue protuberance, 2 = papilla absent and aperture partially enclosed by external tissue, 3 = papilla small or absent, and aperture moderately sized, 4 = papilla large and aperture large. Images of these morphologies can be found at <http://www.fishesoftexas.org/taxon/gambusia-affinis/>, Hendrickson and Cohen (2012) and Peden (1972a).

Our measure of male morphological advantage is the gonopodium-to-papilla ratio, or GP ratio, which we define as gonopodium shape score/papilla size score. A low GP ratio suggests that females have relatively more morphological control over copulations, whereas a large ratio suggests that males may be more effective at forcing copulations.

In addition, we measured gonopodium length of 6 *Gambusia* species available in Texas Natural History Collection (*G. affinis*, *G. georgei*, *G. atrora*, *G. geiseri*, *G. heterochir*, and *G. nobilis*). We supplemented these measurements with data on *G. holbrooki* gonopodium length from Kahn et al. (2009).

## Statistical methods

To assess the coevolution of the DT rate with the morphological traits, we controlled for phylogenetic effects by first calculating phylogenetically independent contrasts for each of the measures (Felsenstein 1985) using the ape package (Paradis et al. 2004) in the R language (R Core Team, 2012). We then calculated the correlation between the contrasts for DT rate and contrasts for each morphological trait (gonopodium length, gonopodium shape, papilla size, and GP ratio). To test the null hypothesis that these correlations are zero, we constructed the null distribution for  $r$  by randomly permuting the data values using the independence\_test function of the coin package (Hothorn et al. 2008) in R with 10000 samples.

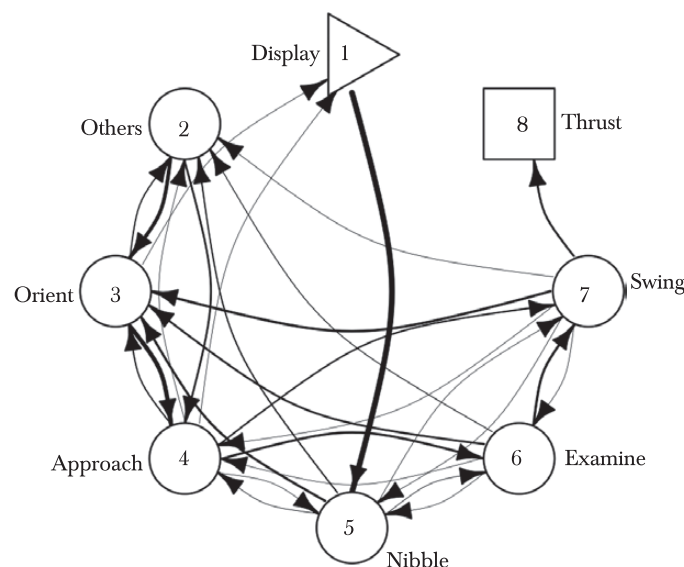
We calculated the independent contrasts using the phylogeny of Langerhans (2011). One node of the phylogeny is a polytomy of 4 species, which can be resolved into a fully bifurcating tree in 15 ways. We calculated the  $P$  value for each of these resolutions using the permutation method described above; the results for all 15 trees are shown in Supplementary Appendix 2. Finally, we obtained an overall significance level by taking the average of the 15 values for each of the correlations of interest.

## RESULTS

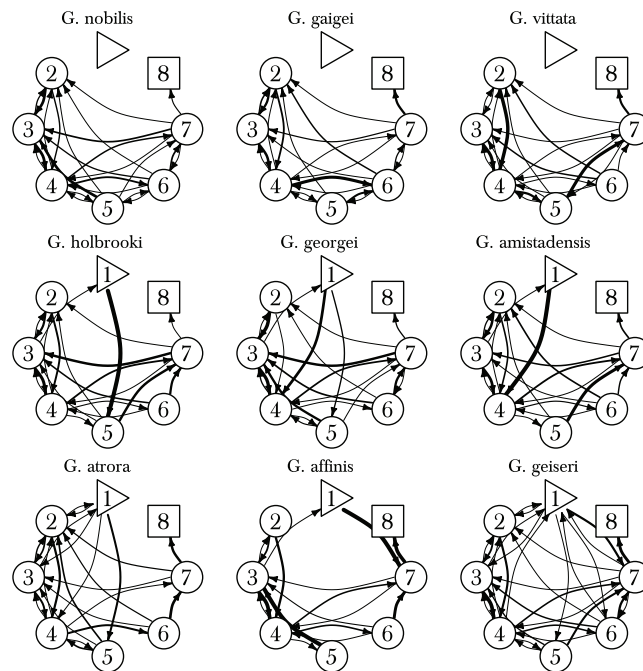
Figure 1 shows Peden's (1970) transition matrix for *G. heterochir* in graphical form. Each node corresponds to a behavior listed in Table 1. The weight of an arrow connecting 2 nodes is proportional to the frequency of the transition out of the preceding behavior to the succeeding behavior. The DT rate is the average speed of progressing from a display (the triangular node) to a copulatory thrust (the square node). The DT rate of *G. heterochir* is near the median DT rate of the 10 species.

Figure 2 shows the graphs for the other 9 species, ordered from low to high DT rate. The first 3 species (*G. nobilis*, *G. gagei*, and *G. vittata*) have a DT rate of 0 because display was never observed. The highest DT rate is found in *G. geiseri*. It has a DT rate of 0.18, which implies that males pass through an average of  $1/0.18 = 5.56$  transitions from a display to a copulatory thrust. The DT rates, gonopodium shapes, papilla sizes, and GP ratios for all 10 species are given in Table 2.

The DT rate is negatively correlated with gonopodium length ( $r = -0.68$ ) (Figure 3 A) and positively correlated with gonopodium shape ( $r = 0.65$ ), gonopodium length ( $r = 0.60$ ), and the GP ratio ( $r = 0.63$ ) (Figure 3B–D) across the species before phylogenetic corrections are applied. To understand the role that phylogenetic effects might play, we first mapped the DT and GP ratios on the phylogeny (Figure 4). We then assessed the significance of the correlations after correcting for phylogenetic effects as described in Methods. We find significant positive relations between the independent contrasts of DT rate and 2 morphological measures: gonopodium shape ( $P = 0.04$ ) and the GP ratio ( $P = 0.05$ ). The



**Figure 1**  
Mating behavior transition network of *Gambusia heterochir* based on observations of 86 pairs (from Peden 1970).



**Figure 2**

Male mating behavior transition rates for the other 9 *Gambusia* species (data from Peden 1970). The numbers of pairs observed were: 9 in *G. affinis*, 15 in *G. holbrooki*, 21 in *G. georgei*, 12 in *G. gaigei*, 4 in *G. amistadensis*, 10 in *G. atrora*, 14 in *G. geiseri*, 31 in *G. nobilis*, and 13 in *G. vittata*.

**Table 2**

**The DT rate (the speed of transition from display to thrust) and morphological data for the ten *Gambusia* species**

Species	DT rate	G shape	Papilla	GP ratio	G length	<i>N</i>
<i>Gambusia nobilis</i>	0	0	3	0.00	8.06	5
<i>Gambusia gaigei</i>	0	1	3	0.33	—	
<i>Gambusia vittata</i>	0	0	1	0.00	—	
<i>Gambusia holbrooki</i>	0.028	2	4	0.50	6.84	
<i>Gambusia heterochir</i>	0.050	0	2	0.00	7.44	5
<i>Gambusia georgei</i>	0.055	1	3	0.33	7.39	5
<i>Gambusia amistadensis</i>	0.069	1	3	0.33	—	
<i>Gambusia atrora</i>	0.088	1	3	0.33	6.80	5
<i>Gambusia affinis</i>	0.131	2	4	0.50	6.84	5
<i>Gambusia geiseri</i>	0.176	2	4	0.50	6.84	4

The gonopodium shape score (G shape) and papilla size score (Papilla) are from Langerhans (2011). The GP ratio is the gonopodium shape score over papilla size score. Gonopodium length (G length) is from our measurements on *N* individuals, except data for *G. holbrooki* is from Kahn et al. (2009)

correlations were not significant between the independent contrasts of DT rate and gonopodium length ( $P = 0.15$ ) and between DT rate and papilla size ( $P = 0.12$ ). (Supplementary Appendix 2 gives statistics associated with each of the 15 resolutions of the phylogeny.)

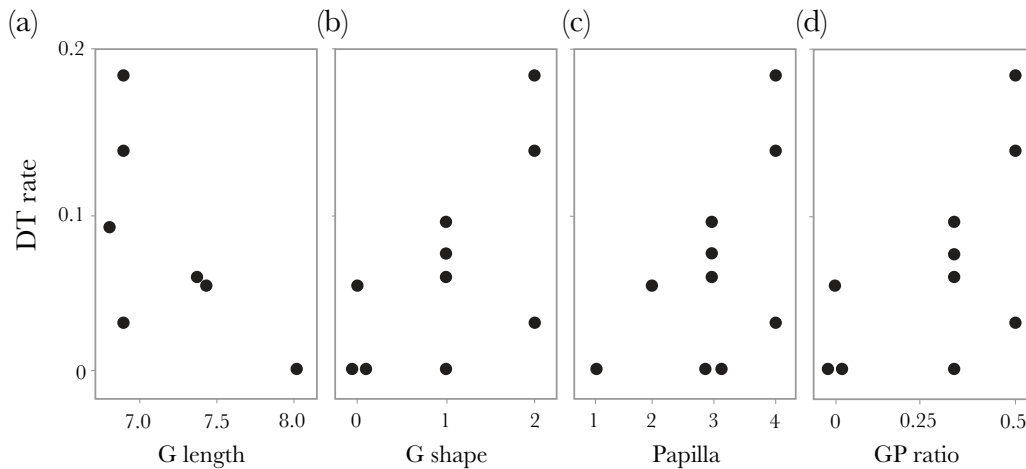
## DISCUSSION

We find a significant positive relationship between male display and morphological traits involved in sexual conflict. Specifically, there is a significant positive correlation between phylogenetically independent contrasts for the transition rate from display-to-copulatory thrust (the DT rate) and 2 morphological measures associated with male coercion: gonopodium shape and the male morphological advantage over females (measured as the ratio of the gonopodium shape score to the female papilla size, the GP ratio). We propose 2 hypotheses to explain these findings: display might drive these

correlations, or the evolution of morphologies involved in SAC might lead to the evolution of display.

### Display as an escalation of SAC

Male display may drive the positive correlation between DT rate and the morphological signatures of SAC (male gonopodial score and the GP ratio). Under this hypothesis, male display evolves to exploit a female receptivity response, which then causes the mating rate for females to reach a level above their optimum. This could explain the fact that display is sometimes deployed with coercion in a strategic combination for male mating success (Brennan and Prum 2012). That situation leads to the evolution of female behavioral or morphological defenses, followed by the evolution of greater male investment in morphological offenses. This idea is essentially the “chase-away selection” hypothesis (Holland and Rice 1999; Arnqvist and Rowe 2002). To date, there is little direct

**Figure 3**

Scatterplots of DT rate (the speed of transition from display to thrust) against male gonopodium length (a) and gonopodium shape (b), papilla size (c), and the GP ratio (gonopodium shape score over papilla size score) (d).

behavioral evidence for the evolution of female resistance to male displays. In poeciliids fishes, females of species with highly developed display (e.g., *Xiphophorus nigrensis*) spend more time associating with courting males than do females from species with males that show display less frequently (e.g., *G. affinis*) (Lynch et al. 2012).

A second possibility is that male display may serve to communicate with potential rivals and to mediate male–male competition. High levels of behavioral male–male competition may predict continuous competition for fertilization opportunity with the ova after mating (often be associated with sperm competition), which in many circumstances causes elevated sexual conflict (Simmons 2001; Birkhead and Pizzari 2002). In other species, male courtship displays share elements with threat display (Berglund et al. 1996; Borgia and Presgraves 1998), suggesting that male display may have evolved from a competitive threat display. In the poeciliid *Xiphophorus birchmani*, the erect dorsal fin of the male lateral display is directed at competitors rather than females (Fisher and Rosenthal 2007). In Endler’s guppy (*Poecilia wingei*), males experienced with high competition environments resort to more force copulations than display, whereas males experienced with female-biased environments show the reversed tendency (Rezucha and Reichard 2014). It is not yet known whether intersexual interaction or male–male competition drives male display behavior in *Gambusia*.

### Display as a de-escalation of SAC

The positive correlation between DT rate and morphologies involved in SAC may have evolved because display results from SAC rather than drives SAC. Under this hypothesis, male display serves to de-escalate SAC. The harm to females caused by coercive mating may indirectly decrease the fitness of their mates. Poeciliids have internal fertilization. They store sperm for days to weeks before fertilization (Kobayashi and Iwamatsu 2002), and gestate for 20–30 days (Greven 2011). Consequently, there is abundant opportunity for injuries to decrease female reproduction. That outcome would also decrease male fitness, which selects for them to reduce conflicting armaments (Parker 1979, 2006).

Male mating displays could lead to cooperative mating that alleviates the cost to both partners. Indeed, guppy strains that showed the highest display rate and lowest forced copulation rate had higher reproductive success than alternative mating patterns (Farr 1980). Such de-escalation or conflict resolution has been predicted

as a possible alternative outcome in SAC systems (Chapman 2006; Lessells 2006) with adaptations that lead to reduction in collateral harm (Lessells 2006).

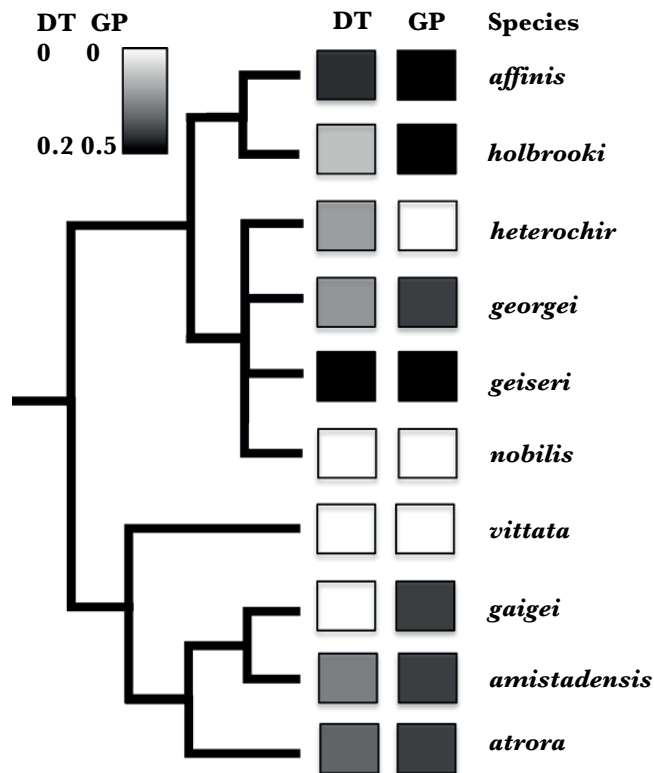
Display may benefit males for 3 reasons. First, it can enhance the fertilization rate by either priming the female’s reproductive physiology (Lehrman 1964; Bastock 1967) or inducing female cooperation (Farr 1980; Condon and Wilson 2006). More sperm are transferred in matings that are cooperative than forced (Zimmerer and Kallman 1989; Pilastro and Bisazza 1999); and this can result in higher reproductive success for males (Farr 1980). Second, display may increase male fitness by decreasing the harm to their mates. In some cases, harmful male traits can benefits males (Lessells 1999; Johnstone and Keller 2000; Morrow et al. 2003), but in others male coercion is selected against because it decreases male as well as female fecundity (Parker 1979, 2006). Third, display might increase male reproductive success in the postcopulatory phase (Thornhill 1983; Farr 1989). Female poeciliids are most responsive to male display when their ova are most fertile (Kadow 1954; Franck 1964; Liley 1966; Farr 1989). Hence, male display should also be selected if it increases male fertilization success (Thornhill 1983; Farr 1989).

Male display might benefit females as well as males. Females assess potential mates over male display and subsequently gain nuptial gifts (Lewis et al. 2004) and/or additive genetic benefits via inheriting “good genes” for their offspring (Petrie 1994). *Gambusia* females have mating preferences for male traits that may correlate with male quality. These traits include dark pigmentation (Gould et al. 1999), which is associated with male performance in male–male competition (Warburton et al. 1957; McAlister 1958; Hubbs and Peden 1969; Peden 1973).

### The lock-and-key hypothesis

Langerhans (2011) proposed that male gonopodial shape and female papilla size have coevolved by selection against hybridization, as suggested by the “lock-and-key” hypothesis of postzygotic isolation (Dufour 1844; Shapiro and Porter 1989). Consistent with that idea, both female papilla size and male gonopodium shape showed greater difference between sympatric than allopatric *Gambusia* populations (Langerhans 2011).

If selection against hybridization drives the evolution of these genital morphologies, our result suggests that male display coevolves in response to them. In that case, display may provide pre-mating



**Figure 4**  
The phylogeny of the 10 *Gambusia* species (from Langerhans 2011) showing their DT rates (the speed of transition from display to thrust) and GP ratios (gonopodium shape score over papilla size score).

isolation that reinforces morphological isolation. Features that are prominent during male displays, such as body shape and gonopodium size, vary among *Gambusia* populations and contribute to reproductive isolation (Langerhans 2010). Display is expected to provide greater contribution to reproductive isolation in species that have weak morphological isolating mechanisms. Consistent with this prediction, we find that display is more important (the DT rate is high) in species in which males may be more able to force copulate (a high GP ratio) and so have weak morphological barriers to hybridization.

However, the lock-and-key hypothesis still lacks empirical support. In particular, the female papilla has not been shown to impede hybridization with sympatric heterospecific males (Langerhans 2011). Future empirical test of this prediction will be needed. If this did occur, the next step is to figure out the mechanistic linkage of display and tightness of genital lock-and-key in a *Gambusia* species.

The lock-and-key hypothesis and sexual conflict hypothesis are not mutually exclusive. Sexual conflict has been recognized as “an engine of speciation” (Rice 1996; Rice and Holland 1997). SAC was predicted to give rise to reproductive isolation both in sympatry (Gavrilets 2000) and allopatry (Gavrilets and Waxman 2002). A scenario for how this might play out in *Gambusia* starts with evolution of the female reproductive aperture and papilla evolved to deflect the male gonopodium. The gonopodium shape of conspecific males then evolves in response via SAC, but the gonopodium of heterospecific males does not. The outcome is that structures in males and females that originally evolved under SAC ultimate function as lock-and-key isolation mechanisms.

## The evolution of display in poeciliids

Previous investigations found a negative relationship between male display and gonopodial length in poeciliids (Rosen and Tucker 1961; Martin et al. 2010; Langerhans 2011; but see Ptacek and Travis 1998 for a reverse pattern). Given those earlier findings, our results reveal 2 surprising patterns. First, we find a positive coevolution between display and sexual conflict morphologies. Second, we find that gonopodium shape but not length coevolve with display. These results do accord with pattern found within a smaller comparative study of 3 species in the genus *Poecilia*. Their the single display species investigated had longer male gonopodia than 2 species that do not display, suggesting that greater male coercion efficiency corresponds with the presence of courtship display (Ptacek and Travis 1998). The fact that gonopodium shape, instead of gonopodium length, predicts display evolution in *Gambusia* suggests that coercion have different relationships with display at different evolutionary scales. Earlier researchers noted that poeciliid genera with the shortest gonopodia (*Poecilia*, *Xiphophorus*, *Gambusia*, and *Belonesox*) all contained some species with male display (Rosen and Tucker 1961). These researchers hypothesized that gonopodial accessory structures evolved with shorter gonopodia to provide tactile assistance in stabilizing the gonopodium for copulatory contact, whereas species with longer gonopodia relied on vision to guide gonopodial interactions. According to this argument, elaborate displays evolved in these genera as a means to increase the effectiveness of sperm transfer with shorter gonopodia (Rosen and Tucker 1961). More recent research has found phylogenetic support for the negative relationship between poeciliid gonopodial length and accessory structures (Langerhans 2011). Here, looking within one of those genera, we find a positive relationship between gonopodial accessory structure shape and male display across 9 species of *Gambusia*. This pattern links display to sexually antagonistic structures but also suggests that genera with shorter gonopodia may experience greater sexual conflict.

Although our study implicates sexual conflict’s role in increasing male display across *Gambusia* species, there are other factors that might influence this pattern. For example, female sensory biases may be selecting for greater display features in males. Recent research demonstrates that female *G. affinis* show stronger neuro-molecular responses to ornamented courting males from a different genus (*Poecilia latipinna*) than to conspecific males, indicating a pre-existing female bias for courting male stimuli in this genus (Wang et al. 2014). Natural selection may also be driving the evolution of male display in *Gambusia*. For instance, *Poecilia* and *Gambusia* color elaboration tends to be more intense in populations under lower predation pressure (Godin and Briggs 1996; Martin et al. 2013). Ecological factors surrounding poeciliid populations may explain the variable strength of selection for male display within and between lineages. Numerous factors may be driving selection for more elaborate male display, and further testing is required to understand their relative contributions.

## SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.behco.oxfordjournals.org/>

## FUNDING

This research was supported by National Science Foundation of United States (NSF IOS-0843000 to M.C. and NSF DEB-0819901 to M.K.).

We thank Zhiheng Li for conducting gonopodium length measurements. Chad Brock, Gina Calabrese, Laura Crothers, Ian Etheredge, Trevor Price, Mary Ramsey, Luke Reding, Kat Ruddick and Michael Ryan for helpful comments. We thank Minh Pham for data processing assistance.

**Handling editor:** John Fitzpatrick

## REFERENCES

- Andersson M. 1994. Sexual selection. Princeton (NJ): Princeton University Press.
- Armstrong EA. 1963. A study of bird song. London: Oxford University Press.
- Arnqvist G, Rowe L. 2002. Antagonistic coevolution between the sexes in a group of insects. *Nature*. 415:787–789.
- Arnqvist G, Rowe L. 2005. Sexual conflict. Princeton (NJ): Princeton University Press.
- Baerends GP, Brouwer B, Waterbolk HT. 1955. Ethological studies on *Lebistes reticulatus*. *Behaviour*. 8:249–334.
- Bastock M. 1967. Courtship: an ethological study. Chicago (IL): Aldine.
- Berglund A, Bisazza A, Pilastro A. 1996. Armaments and ornaments: an evolutionary explanation of traits of dual utility. *Biol J Linn Soc*. 58:385–389.
- Birkhead TR, Pizzari T. 2002. Post-copulatory sexual selection. *Nat Rev Gen*. 3:262–273.
- Borgia G, Presgraves DC. 1998. Coevolution of elaborated male display traits in the spotted bowerbird: an experimental test of the threat reduction hypothesis. *Anim Behav*. 56:1121–1128.
- Brennan LRP, Prum RO. 2012. The limits of sexual conflict in the narrow sense: new insights from waterfowl biology. *Phil Trans R Soc B*. 367:2324–2338.
- Chapman T. 2006. Evolutionary conflicts of interest between males and females. *Curr Biol*. 16:744–754.
- Civetta A, Clark AG. 2000. Correlated effects of sperm competition and postmating female mortality. *Proc Natl Acad Sci USA*. 97:13162–13165.
- Clark E, Aronson LR, Gordon M. 1954. Mating behavior patterns in two sympatric species of xiphophorin fishes: their inheritance and significance in sexual isolation. *Bull Am Mus Nat Hist*. 103:135–226.
- Clutton-Brock TH, Parker GA. 1995. Sexual coercion in animal societies. *Anim Behav*. 49:1345–1365.
- Condon CHL, Wilson RS. 2006. Effect of thermal acclimation on female resistance to forced matings in the eastern mosquitofish. *Anim Behav*. 72:585–593.
- Constantz GD. 1975. Behavioral ecology of mating in the male gila topminnow *Poeciliopsis occidentalis* (Cyprinodontiformes: Poeciliidae). *Ecology*. 56:966–973.
- Constantz GD. 1989. Reproductive biology of poeciliid fishes. In: Meffe GK, Snelson FF, editors. *Ecology and evolution of livebearing fishes (Poeciliidae)*. Englewood Cliffs (NJ): Prentice Hall. p. 33–50.
- Dufour L. 1844. Anatomie générale des diptères. *Ann Sci Nat*. 1:244–264.
- Farr JA. 1976. Social facilitation of male sexual behavior, intrasexual competition, and sexual selection in the Guppy, *Poecilia reticulata* (Pisces: Poeciliidae). *Evolution*. 30:707–717.
- Farr JA. 1980. Social behavior patterns as determinants of reproductive success in the guppy, *Poecilia reticulata* (Pisces: Poeciliidae). *Behaviour*. 74:1–2.
- Farr JA. 1989. Sexual selection and secondary sexual differentiation in poeciliids: determinants of male mating success and the evolution of female choice. Englewood Cliffs (NJ): Prentice Hall.
- Felsenstein J. 1985. Phylogenies and the comparative method. *Am Nat*. 125:1–15.
- Fisher HS, Rosenthal GG. 2007. Male swordtails court with an audience in mind. *Biol Lett*. 3:5–7.
- Franck D. 1964. Vergleichende Verhaltensstudien an lebendgebarenden Zahnkarpfen der Gattung *Xiphophorus*. *Zool J Physiol*. 71:117–170.
- Gamble S, Lindholm AK, Ender JA, Brooks R. 2003. Environmental variation and the maintenance of polymorphism: the effect of ambient light spectrum on mating behaviour and sexual selection in guppies. *Ecol Lett*. 6:463–472.
- Gavrilets S. 2000. Rapid evolution of reproductive barriers driven by sexual conflict. *Nature*. 403:886–889.
- Gavrilets S, Waxman D. 2002. Sympatric speciation by sexual conflict. *Proc Natl Acad Sci USA*. 99:10533–10538.
- Godin JGJ. 1995. Predation risk and alternative mating tactics in male Trinidadian guppies (*Poecilia reticulata*). *Oecologia*. 103: 224–229.
- Godin JGJ, Briggs SE. 1996. Female mate choice under predation risk in the guppy. *Anim Behav*. 51:117–130.
- Greven H. 2011. Gonads genitals and reproductive biology. In: Evans JP, Pilastro A, Schlupp I, editors. *Ecology and evolution of poeciliid fishes*. Chicago and London: The University of Chicago Press. p. 3–17.
- Greven H. 2005. Structural and behavioral traits associated with sperm transfer in Poeciliinae. Homestead (FL): New Life Publication. p. 145–163.
- Gould JL, Elliott SL, Masters CM, Mukerji J. 1999. Female preferences in a fish genus without female mate choice. *Curr Biol*. 9:497–500.
- Hebets EA, Stafstrom JA, Rodriguez RL, Wilgers DJ. 2011. Enigmatic ornamentation eases male reliance on courtship performance for mating success. *Anim Behav*. 81:963–972.
- Hendrickson DA, Cohen AE. 2012. Fishes of Texas Project and Online Database Texas Natural History Collection, a division of Texas Natural Science Center, University of Texas at Austin [cited 2015 April 10]. Available from: [www.fishesoftexas.org](http://www.fishesoftexas.org).
- Holland B, Rice WR. 1999. Experimental removal of sexual selection reverses intersexual antagonistic coevolution and removes a reproductive load. *Proc Natl Acad Sci USA*. 96:5083–5088.
- Hothorn T, Hornik K, van de Wiel MA, Zeileis A. 2008. Implementing a class of permutation tests: the coin package. *J Stat Softw*. 28:1–23.
- Hubbs C, Peden AE. 1969. *Gambusia georgei* sp. nov. from San Marcos Texas. *Copeia*. 1969:357–364.
- Hughes AL. 1984. Seasonal trends in male life history strategies in the mosquitofish *Gambusia affinis* and their adaptive significance [PhD Dissertation]. [Bloomington (IL)]: Indiana University.
- Itzkowitz M. 1971. Preliminary study of the social behavior of male *Gambusia affinis*. *Chesapeake Sci*. 12:219–224.
- Johnstone RA, Keller L. 2000. How males can gain by harming their mates: sexual conflict, seminal toxins, and the cost of mating. *Am Nat*. 156:368–377.
- Kadow P. 1954. An analysis of sexual behavior and reproductive physiology in the guppy *Lebistes reticulatus* (Peters) [PhD Dissertation]. [New York]: New York University.
- Kahn AT, Mautz B, Jennions MD. 2009. Females prefer to associate with males with longer intromittent organs in mosquitofish. *Biol Lett*. 6:55–58.
- Kobayashi H, Iwamatsu T. 2002. Fine structure of the storage micropocket of spermatozoa in the ovary of the guppy *Poecilia reticulata*. *Zool Sci*. 19:545–555.
- Kwan L, Cheng YY, Rodd FH, Rowe L. 2013. Sexual conflict and the function of genitalic claws in guppies (*Poecilia reticulata*). *Biol Lett*. 9:20130267.
- Langerhans BR. 2010. Predicting evolution with generalized models of divergent selection: a case study with poeciliid fish. *Integr Comp Biol*. 50:1167–1184.
- Langerhans BR. 2011. Genital evolution. In: Evans J.P. Pilastro A, editors. Schlupp I. *Ecology and evolution of poeciliid fishes*. Chicago (IL): University of Chicago Press. p. 228–240.
- Lehrman DS. 1959. Hormonal response to external stimuli in birds. *Ibis*. 101:478–497.
- Lehrman DS. 1964. The reproductive behavior of ring doves. *Sci Am*. 211:48–54.
- Lenz N. 1994. Mating-behavior and sexual competition in the regent bowerbird, *Sericulus chrysocephalus*. *Emu*. 94:263–272.
- Lessells CM. 1999. Sexual conflict in animals. In: Keller L, editor. *Levels of selection in evolution*. Princeton (NJ): Princeton University Press. p. 75–99.
- Lessells CM. 2006. The evolutionary outcome of sexual conflict. *Phil Trans R Soc Lond B Biol Sci*. 361:301–317.
- Lewis SM, Cratsley CK, Rooney JA. 2004. Nuptial gifts and sexual selection in *Photinus* fireflies. *Integr Comp Biol*. 44:234–237.
- Liley NR. 1966. Ethological isolating mechanisms in four sympatric species of poeciliid fishes. *Behav Suppl*. 13:1–197.
- Lynch KS, Ramsey ME, Cummings ME. 2012. The mate choice brain: comparing gene profiles between female choice and male coercive poeciliids. *Genes Brain Behav*. 11:222–229.
- Martin RG. 1975. Sexual and aggressive behavior density and social structure in a natural population of mosquitofish *Gambusia affinis holbrooki*. *Copeia*. 3:445–454.
- Martin SB, Albert JS, Leberg PL. 2010. The evolution of poeciliid gonopodium: integrating morphological and behavioral traits. In: Uribe, MC, Grier HJ, editors. *Viviparous fishes II*. Homestead (FL): New Life Publications.
- Martin RA, Riesch R, Heinen-Kay JL, Langerhans RB. 2013. Evolution of male coloration during a post-Pleistocene radiation of Bahamas mosquitofish (*Gambusia hubbsi*). *Evolution*. 68:397–411.

- McAlister WH. 1958. The correlation of coloration with social rank in *Gambusia hurtadoi*. *Ecology*. 39:477–482.
- McPhail JD. 1978. Sons and lovers: the functional significance of sexual dichromisms in a fish *Neoheterandria tridentiger* (Garman). *Behaviour*. 62:329–339.
- Moore AJ, Gowaty PA, Wallin WG, Moore PJ. 2001. Sexual conflict and the evolution of female mate choice and male social dominance. *Proc Roy Soc Lond B*. 268:517–523.
- Morrow EH, Arnqvist G, Pitnick S. 2003. Adaptation versus pleiotropy: why do males harm their mates? *Behav Ecol*. 14:802–806.
- Oliver SJ, Lobel PS. 2013. Direct mate choice for simultaneous acoustic and visual courtship displays in the damselfish *Dascyllus albisella* (Pomacentridae). *Environ Biol Fish*. 96:447–457.
- Paradis E, Claude J, Strimmer K. 2004. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* 20:289–290.
- Parker GA. 1979. Sexual selection and reproductive competition in insects. New York: Academic Press.
- Parker GA. 2006. Sexual conflict over mating and fertilization: an overview. *Phil Trans R Soc B*. 361:235–259.
- Parzefall J. 1969. On comparative ethology of various *Mollinesia* species inclusive of a cave form of *M. sphenops*. *Behaviour*. 33:1–38.
- Parzefall J. 1973. Attraction and sexual cycle of poeciliids. In: Schroder JH, editor. *Genetics and mutagenesis of fish*. New York: Springer Verlag.
- Peden AE. 1970. Courtship behavior of *Gambusia* (Poeciliidae) with emphasis on isolation mechanisms [PhD Dissertation]. [Austin (TX)]: University of Texas.
- Peden AE. 1972a. Differences in the external genitalia of female *Gambusia* fishes. *Southwest Nat*. 3:265–272.
- Peden AE. 1972b. The function of gonopodial parts and behavioural pattern during copulation by *Gambusia* (Poeciliidae). *Can J Zool*. 50:955–968.
- Peden AE. 1973. Virtual extinction of *Gambusia amistadensis* a poeciliid Fish from Texas. *Copeia*. 1973:210–221.
- Petrie M. 1994. Improved growth and survival of offspring of peacocks with more elaborate trains. *Nature*. 371:598–599.
- Pilastro A, Bisazza A. 1999. Insemination efficiency of two alternative male mating tactics in the guppy (*Poecilia reticulata*). *Proc R Soc B*. 266:1887–1891.
- Ptacek MB, Travis J. 1998. Hierarchical patterns of covariance between morphological and behavioural traits. *Anim Behav*. 56:1044–1048.
- R Core Team. 2012. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.
- Reynolds JD, Gross MR, Coombs MJ. 1993. Environmental conditions and male morphology determine alternative mating behavior in trinidadian guppies. *Anim Behav*. 45:145–152.
- Rezucha R, Reichard M. 2014. The effect of social environment on alternative mating tactics in male Endler's guppy, *Poecilia wingei*. *Anim Behav*. 88:195–202.
- Rice WR. 1996. Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. *Nature*. 381:232–234.
- Rice WR, Holland B. 1997. The enemies within: intergenomic conflict, interlocus contest evolution (ICE), and the intraspecific Red Queen. *Behav Ecol Sociobiol*. 41:1–10.
- Rosen DE, Gordon M. 1953. Functional anatomy and evolution of male genitalia in poeciliid fishes. *Zoologica*. 38:1–47.
- Rosen DE, Tucker A. 1961. Evolution of secondary sexual characters and sexual behavior patterns in a family of viviparous fishes (Cyprinodontiformes: Poeciliidae). *Copeia*. 1961:102–212.
- Ross PJ, Crews D. 1977. Influence of the seminal plug on mating behaviour in the garter snake. *Nature*. 267:344–345.
- Ryan MJ, Causey BJ. 1989. Alternative mating behavior in the swordtails *Xiphophorus nigrensis* and *Xiphophorus pygmaeus* (Pisces: Poeciliidae). *Behav Ecol Sociobiol*. 24:341–348.
- Servedio MR, Price TD, Lande R. 2013. Evolution of displays within the pair bond. *Proc R Soc B*. 280:1–7.
- Shapiro AM, Porter AH. 1989. The lock-and-key hypothesis: evolutionary and biosystematics interpretation of insect genitalia. *Annu Rev Entomol*. 34: 231–245.
- Simmons LW. 2001. Sperm competition and its evolutionary consequences in the insects. Princeton (NJ): Princeton University Press.
- Thornhill R. 1983. Cryptic female choice and its implications in the scorpionfly *Harpobittacus nigriceps*. *Am Nat*. 6:765–788.
- Trivers RL. 1972. Parental investment and sexual selection. Chicago (IL): Aladine.
- Wachtmeister C, Enquist M. 2000. The evolution of courtship rituals in monogamous species. *Behav Ecol*. 4:405–410.
- Wang SMT, Ramsey ME, Cummings ME. 2014. Plasticity of the mate choice mind: courtship evokes choice-like brain responses in females from a coercive mating system. *Genes Brain Behav*. 13:365–375.
- Warburton BC, Hubbs BC, Hagen DW. 1957. Reproductive behavior of *Gambusia heterochir*. *Copeia*. 1957:299–300.
- Zimmerer EJ, Kallman KD. 1989. Genetic basis for alternative reproductive tactics in the pygmy swordtail *Xiphophorus nigrensis*. *Evolution*. 43:1298–1307.