

In love and war: The morphometric and phylogenetic basis of ornamentation, and the evolution of male display behavior, in the livebearer genus *Poecilia*

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Exaggerated male traits under sexual selection are often used for both competition and courtship, raising the question of whether ornaments evolved simultaneously for both functions, or if use in one context preceded use in another. Here, we apply a phylogenetic approach to study the evolution of ornamental dorsal fins in male poeciliid fish of the subgenera *Mollienesia* and *Limia*, which exhibit convergent development of an enlarged dorsal fin, and often direct erect-fin displays to male and female conspecifics. Unlike prior categorical assessments of poeciliid adornments, we measure dorsal fin exaggeration with a continuous index of ornamentation. Phylogenetic logistic and generalized least squares regression analyses indicate that high index values are significantly associated with the use of two component postures of courtship and aggressive displays, dorsal fin erection and body curvature, but not with the presence of sexual dichromatism. Male displays initially evolved for male–male aggression in the common ancestor of *Mollienesia* and *Limia*, suggesting that this signal originated for competition, then became co-opted for courtship. These results support the armament-ornament hypothesis for evolution of exaggerated male traits, and are consistent with an evolutionary shift in the predominant mechanisms of sexual selection from intra- to intersexual.

KEY WORDS: Co-opted, displays, ornamentation index, *Poecilia*, sexual selection.

Many organisms exhibit flamboyant and exaggerated morphology and behaviors that appear counterproductive to their survival. In his theory of sexual selection, Darwin (1871) suggested two possible adaptive benefits of these traits. Signals can be used by members of one sex, usually males, in intrasexual aggression or to attract the attention of the opposite sex (Darwin 1871). A huge body of observational and experimental evidence from a wide range of taxa has strongly supported sexual selection as major evolutionary force (Andersson 1994), although most studies of male ornaments have primarily dealt with their use in courtship displays. Yet elaborate sexually selected structures have long been recognized to function in male–male aggressive displays as well

as courtship (Fisher 1930; Noble 1938; Zahavi 1975), raising the question of whether ornaments have evolved simultaneously for both functions, or if use in one context has preceded use in the other.

A prominent hypothesis for the evolutionary origin of dual-utility traits is known as the armament-ornament or preexisting trait hypothesis (Borgia 1979; Berglund et al. 1996). In this hypothesis, elaborate male signals evolve for competitive purposes as “war propaganda” status badges or displays (Borgia 1979), and are subsequently adopted for courtship. Although males may initially use displays to indicate their health to rival males, these displays can become equally useful to females as signals of vitality

and genetic or proximate benefits of potential mates (Borgia 1979; Berglund et al. 1996). Male–male combat should ensure that a displaying male honestly signals his condition through his armaments, as subsequent fights could be considerably costly to fakers (Berglund et al. 1996). Moreover, the ability to perceive display traits should exist in both sexes, so if male traits are valuable signals of fighting ability to other males in a social setting, females are predisposed to recognize these traits and prefer the armaments of victorious males (Berglund et al. 1996). Once co-opted as a signal to females, features used in male displays may be further elaborated to increase motor performance and signal efficiency, which may increase attractiveness of the displays to females (Byers et al. 2010; Tazzyman et al. 2014).

Support for this hypothesis, from comparative studies and ancestral state reconstructions, is mixed. Borgia and Coleman (2000) analyzed behavioral observations and the phylogenetic history of bowerbird calls, and determined that the agonistic “skrraa” call of male bowerbirds was first used in male–male aggression and later adopted for courtship in the genus *Chlamydera*. Likewise, Regalado (2012, 2015) observed social interactions in *Sphaerodactylus* dwarf geckos, and concluded from comparing species repertoires that monochromatic geckos have co-opted an ancestral arched-back threat posture for use in courtship. Yet a reverse case of signal co-option has also been found: Morris et al. (2007) scored behavioral responses of swordtail fish (*Xiphophorus*) to video stimuli, and their phylogenetic analyses showed that dark vertical bars on the bodies of northern swordtails initially served as a display for female mate choice, but gained a new function in contests between males. However, the most parsimonious model of one sex appropriating socially available information used by the other sex predicts that females are more likely to eavesdrop and become choosy over male–male signals, rather than the other way around (Berglund et al. 1996).

Phylogenetic methods, in which traits and behaviors are mapped on to a “family tree,” can potentially reveal the historical patterns of evolution of multiple traits, and hence their order of appearance (Pagel 1999). Not only does the preexisting trait hypothesis predict that male–male aggressive displays will predate displays for courtship (Berglund et al. 1996), but as female choice becomes important in mate selection, this may lead to accelerated evolution of ornament size (Andersson 1994; Emlen 2008). As increases in ornamentation are known to be associated with speciation (Gomes et al. 2016), co-option of signals for courtship may lead to exaggeration of the signaling structure in speciose clades, although little empirical data exist on whether dual-utility traits reach larger sizes than do pure ornaments or pure armaments (Outomuro et al. 2014).

The family Poeciliidae offers a promising system to study the evolution of ornaments because its species vary tremendously in their behavior and display structures. Two well-known exam-

ples are the elongated caudal fins of swordtails (*Xiphophorus*) and the enlarged dorsal fins of sailfin mollies (*Mollienesia*), both of which are associated with male–male aggression and elaborate courtship displays (Noble 1938; Parzefall 1969; Bisazza 1993; Berglund et al. 1996). Poeciliids are small Neotropical freshwater fishes that are predominantly viviparous (Rosen and Bailey 1963) and mate via internal fertilization through a modified male anal fin called a gonopodium (Rosen and Gordon 1953; Rosen and Tucker 1961; Chambers 1987). Differences in poeciliid precopulatory behavior have been linked to significant variation in sexual size dimorphism, relative anal fin length, and the strength of sexual selection (Bisazza 1993; Bisazza et al. 1996; Pollux et al. 2014). In most species, males lack courtship and rely on gonopodial thrusting for copulation (Farr 1989; Bisazza 1993), the sexes are equally dull in coloration, and males are much smaller than females and have relatively long gonopodia (Rosen and Gordon 1953; Rosen and Tucker 1961). On the other hand, males of some species show courtship displays and reduced thrusting rates, or may switch between mating tactics depending on size, dominance, or female receptivity (Farr 1989; Bisazza 1993). These males often are similar in size to females (Bisazza et al. 1996), and have bright coloration, exaggerated fins, and short gonopodia relative to their body lengths (Rosen and Tucker 1961; Chambers 1987). Larger or more colorful males are preferred by females in numerous courting species (Bisazza 1993; Bisazza et al. 1996).

Long gonopodia and covert copulation are both thought to be ancestral traits in poeciliids (Bisazza 1993; Bisazza et al. 1997; Ptacek and Travis 1998). Martin et al. (2010) proposed that the evolution of short gonopodia preceded the evolution of courtship behavior, as courtship elicits cooperation among females and lessens male reliance on covert copulation. Behavioral studies have also found that female choice and male–male competition influence the evolution of mating displays and ornaments in poeciliids (Farr 1989; Bisazza 1993). For example, in males of the green swordtail, *Xiphophorus helleri*, sword length and body size are positively correlated with success in both contests and mate attraction (Benson and Basolo 2006).

To apply the preexisting trait hypothesis to ornament evolution in poeciliids, we focus on species of *Mollienesia* and *Limia*. Mollies are found along the Gulf Coast of North America and throughout Central America, whereas *Limia* are native exclusively to islands of the Greater Antilles (Rosen and Bailey 1963; Hrbek et al. 2007; Weaver 2015). These poeciliids are ideal model organisms because they are closely related (both are subgenera of *Poecilia*), they have independently evolved ornate male display structures, and they use comparable displays for courtship and competition. The close affinity of mollies and *Limia* is well supported, as prior phylogenetic research has robustly combined *Mollienesia*, *Limia*, and the subgenus *Pamphorichthys* in a monophyletic group within the genus *Poecilia* (Rodríguez 1997;

Hamilton 2001; Hrbek et al. 2007; Meredith et al. 2010, 2011; Polux et al. 2014; Weaver 2015; Palacios et al. 2016; Reznick et al. 2017). An exaggerated dorsal fin has convergently evolved in each subgenus: once in the monophyletic sailfin molly clade (Ptacek and Breden 1998); and once in the humpback *Limia*, *Limia nigrofasciata* (Farr 1984). Courtship display behaviors consisting of dorsal fin erection and an S-shaped body posture are used by some species of both subgenera (Farr 1984; Parzefall 1989; Ptacek 1998; Hamilton 2001; Ptacek et al. 2011; Weaver 2015), although shortfin molly males court at lower rates than sailfin molly males (Hamilton 2001; Ptacek 2005). In both subgenera, these display types are also performed in agonistic interactions between males (Parzefall 1969; Balsano et al. 1985; Woodhead and Armstrong 1985; Parzefall 1989; Bierbach et al. 2013; Holz 2015).

The goals of our study are twofold. First, we examine the relationship between ornamentation, morphology, and behavior in mollies and *Limia*. While controlling for phylogeny, we test the prediction that a high ornamentation index (OI) should be positively correlated with sexual dichromatism and multicomponent courtship displays, and negatively correlated with female-biased sexual size dimorphism and relative gonopodium length, because species without ornate fins tend to have long male intromittent organs and larger females. Second, we use ancestral state reconstructions to map the evolution of the OI and display behavior onto a new phylogeny of the two subgenera. Following the pre-existing trait hypothesis, we predict that the use of two display components (dorsal fin erection and sigmoid body postures) in male–male competition should precede co-option of both components for attracting females, as aggression has been observed more often than courtship among species of mollies and *Limia*. These analyses should test the applicability of the preexisting trait hypothesis in a phylogenetic context, to elucidate the origin and evolution of ornaments in the genus *Poecilia*.

Materials and Methods

MORPHOLOGICAL MEASUREMENTS

Body and fin dimensions were obtained from lateral photographs of 14 species of *Limia*, 14 species of *Mollienesia*, five species of *Pamphorichthys*, and four other species in *Poecilia*, which were either collected in the field or borrowed from museum or university laboratory repositories (for information on accession numbers and collection localities, see Tables S1 and S2). Digital images were measured in the program ImageJ (Schneider et al. 2012; Rasband 2014).

The standard length of each fish was measured to the nearest 0.01 mm from the tip of the lower jaw to the base of the caudal peduncle. Relative gonopodium length was calculated as the distance from the base to the distal tip of the male anal fin, divided by standard length. Dorsal fin height was taken as the

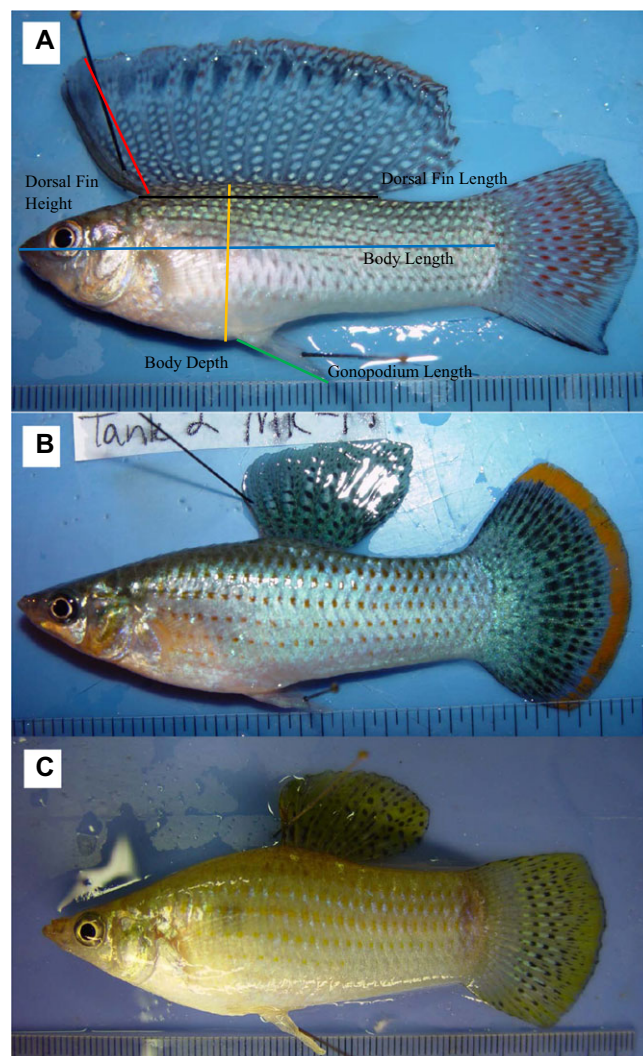


Figure 1. Photographs of representative males of different species of *Mollienesia* that vary in their ornamentation indices. (A) *Poecilia petenensis* (OI = 1.070201), (B) *P. mexicana* (OI = 0.4189795), and (C) *P. orri* (OI = 0.2834045). Reference lines on *P. petenensis* indicate measurements taken of dorsal fin height (red), dorsal fin length (black), body length (blue), body depth (yellow), and gonopodium length (green), in mm. Images courtesy of Margaret Ptacek, Clemson University.

distance from the tip to the base of the longest fin ray, and dorsal fin length was taken as the distance between the anterior and posterior insertion points on the dorsal surface. Finally, body depth was measured from the highest point on the dorsal surface to the lowest point on the abdomen (Fig. 1). Two measurements of sexual size dimorphism were calculated: the natural logarithm of the size ratio between females and males ($SSD = \ln(\text{female size}/\text{male size})$) (Lovich and Gibbons 1992; Smith 1999) and the ratio of the larger to the smaller sex, minus one ($SDI = \text{larger sex}/\text{smaller sex} - 1$) (Lovich and Gibbons 1992). This second index was given a negative value if males are larger and a positive value if

females are larger. Body and fin measurements were natural-log transformed for all statistical analyses (Table S3).

Poeciliid ornamental traits show high variability in size (Meyer et al. 1994; Ptacek 2005), but have historically been treated only as present or absent (Meyer et al. 1994; Martin et al. 2010; Kang et al. 2013; Pollux et al. 2014), or as a few discrete sizes (Bisazza 1993; Basolo 1996). To avoid this information loss, we adopted an alternative approach and treated ornaments as continuous traits. For each species, we quantified an OI with an analysis of covariance (ANCOVA). We regressed the natural log of dorsal fin height against the natural log of standard length, pooling the two sexes. We then calculated the adjusted mean log dorsal fin height for each sex, estimated at the overall mean standard length for that species. We calculated our OI as the difference between the adjusted mean log dorsal fin height of males and of females (Table S4). This created an index that interprets the ratio of male fin height to female fin height, for each species, adjusted for standard lengths of the individual fish (larger individuals have larger fins). This method also accounts for factors that vary among species, like the distribution of standard lengths (body size), any dimorphism in standard length, and different levels of intraspecific allometry between dorsal fin height and standard length (Farr et al. 1986).

This index could overestimate ornamentation in large-bodied species if interspecific allometry (the relationship between average of log dorsal fin height for each species and average log standard length for each species) is strong: then large-bodied species would appear to have greater levels of ornamentation than smaller-bodied species. A test for this effect is the magnitude of the correlation between the OI value and the average log standard length for each species; a large, significant correlation means the OI includes the effect of body size (Mosimann 1975). When all 37 measured species are analyzed, the correlation coefficient between the average log standard length and OI for each species is 0.48 ($P < 0.01$). The significance of this correlation is entirely driven by *Poecilia velifera* and *Poecilia petenensis*; without these species, the correlation coefficient is 0.31, not significant at the $P = 0.05$ level or below. Although the OI is not completely free of a body size effect, it captures the variation among species in ornamentation level, and for most comparisons, especially among similarly sized species, the OI will not confound body size dimorphism with male ornamentation level (comparisons with *P. velifera* and *P. petenensis* obviously excepted).

We used dorsal fin height, rather than length, to quantify trait exaggeration for all species. Dorsal fin length differences reflect species differences in dorsal fin ray counts. Ornate fins are found in species with either narrow fin bases or long fin bases. For example, the ornate fin of *L. nigrofasciata* is formed from protracted dorsal fin rays that emerge from a relatively narrow base, whereas those of the sailfin mollies have long fin bases.

Species below the OI midpoint of 0.5 (ranging from *Limia zonata*, OI = -0.113, to *Poecilia gilli*, OI = 0.433) have traditionally been classified as shortfin species, whereas species above the 0.5 midpoint (ranging from *Poecilia latipinna*, OI = 0.597, to *P. petenensis*, OI = 1.070) have traditionally been classified as sailfin species (Hamilton 2001; Pollux et al. 2014).

BEHAVIORAL AND DICHROMATISM INFORMATION

We derived data on sexual dichromatism and precopulatory and aggressive male behaviors from the published literature and personal correspondence (Table S5). Dichromatism was assessed based on colored images and descriptions from Wischnath (1993), new species descriptions or species revalidations (Rivas and Fink 1970; Menzel and Darnell 1973; Miller 1975; Rivas 1978, 1980; Franz and Burgess 1983; Costa 1991; Miller 1994; Poeser 2003, 2011; Casatti et al. 2006), and comparative behavioral studies (Farr 1984, 1989; Hamilton 2001; Ptacek 2005; Pollux et al. 2014). This character was coded as 0 = monochromatic sexes from a human perspective, or 1 = dichromatic sexes, if color of the dorsal and/or caudal fin differed between males and females over at least 33% of the fin surface.

In poeciliids, competing males display a sigmoid body curvature, or S-posture, in which they swim parallel or antiparallel to each other with their backs arched and their dorsal fins fully spread (Liley 1966; Parzefall 1969). This behavior is characteristic of aggressive male encounters (Parzefall 1969; Bierbach et al. 2013) and courtship displays (Liley 1966; Farr 1989; Ptacek 2005). We coded these displays as present or absent for each sex: dorsal fin erection to males, sigmoid postures to males, dorsal fin erection to females, and sigmoid postures to females. Dorsal fin erection appears in all species with male–male aggression, suggesting an ancestral status.

We obtained behavioral information for *P. gilli* and *Pseudolimia heterandria*, two species not described in the published literature, through personal observation. Study populations were kept in single-species mixed-sex stocks. We observed several focal males within each tank ($N = 3$ for *P. gilli*, $N = 2$ for *P. heterandria*) for periods of 10 min per male, and recorded all displays (dorsal fin erections and sigmoid postures to females and other males) that each focal male performed during this period. These observations served to qualify the existence of certain behaviors (Table S5).

TAXON SAMPLING AND DNA ALIGNMENTS

Taxon sampling included 40 species of *Poecilia* with subgeneric representation as follows: *Limia* (15 species), *Mollienesia* (16 species), *Pamphorichthys* (five species), *Allopoecilia* (one species), *Pseudolimia* (one species), *Psychropoecilia* (one subspecies), and the outgroup subgenus *Poecilia* (one species). Sequences from seven nuclear (ENC1, Glyt, Mhy6, Rag1, Rh,

SH3PX3, and X-src) and four mitochondrial genes (ATPase 8/6, Cytb, COI, and ND2) were obtained from GenBank (Ptacek and Breden 1998; Hamilton 2001; Hrbek et al. 2007; Meredith et al. 2010, 2011; Alda et al. 2013; Pollux et al. 2014) and aligned with MUSCLE (Edgar 2004) in Geneious Version 5.5.4 (Kearse et al. 2012). (For species, genes, and accession numbers, see Table S6). We removed extraneous base pairs from flanking gene segments and redundant base pairs where reading frames overlapped. Protein coding genes were translated in Se-AL Version 2.0a11 (Rambaut 1996). SequenceMatrix 1.7.8 (Vaidya et al. 2011) was used to concatenate the final alignments into a supermatrix (9825 base pairs total), with the four mitochondrial protein-coding genes treated as one gene (3683 bp) with three codon partitions.

PartitionFinder 1.1.1 (Lanfear et al. 2012) was used to find the best partitioning scheme and nucleotide substitution models for the combined dataset, employing linked branch lengths, the greedy search algorithm, and the corrected Akaike Information Criterion. Models combining invariant sites and a Γ distribution of rates across sites were excluded from the search following Yang (2006). Thirteen unique evolutionary models provided the best fit to the sequence data (Table S7). These models were employed in all subsequent phylogenetic analyses except for RAxML where it was necessary to use the more general GTR+ Γ model.

PHYLOGENETIC ANALYSES

Phylogenetic reconstructions were performed on the combined partitioned dataset with maximum likelihood and Bayesian inference (BI). All trees were rooted with the outgroup *Poecilia vivipara* after Pollux et al. (2014), and in all analyses, gaps were treated as missing data. RAxML analyses were performed with Version 8.1.11 (Stamatakis 2006, 2008) on CIPRES (Miller et al. 2010) using rapid bootstrapping (500 pseudoreplicates) and a search for the best maximum likelihood tree all in the same run. We also used GARLI 2.01 (Zwickl 2006) for maximum likelihood analysis, with four search replicates run for 5 million generations each, starting randomized trees generated by a fast stepwise addition algorithm, and 100 attachments evaluated for each taxon. Twenty thousand generations was set as the running limit before topological improvement, and the maximum score improvement required for termination was set to 0.05. Bootstrap support values were obtained with the GARLI Web Service (Bazin et al. 2014) and were summarized onto the best likelihood tree with the DendroPy SumTrees utility (Sukamran and Holder 2010).

BI was performed with MrBayes Version 3.2 (Ronquist et al. 2012) using Metropolis-coupled Markov chain Monte Carlo sampling (MCMCMC) to estimate the posterior probability distributions. Two runs of four Markov chains (three heated and one cold) used random starting trees, default priors, and the GTR+ Γ model of rate variation across sites. Chains were sampled every 50,000

generations and terminated at 14 million generations once average standard deviation of split frequencies reached 0.001. Burn-in was set to 25% (3.5 million generations). Tracer 1.6 (Rambaut et al. 2014) was used to verify that potential scale reduction factors (PSRF) approximated 1, and that effective sample sizes (ESS) exceeded 200. Trees obtained with all three methods were manipulated in FigTree 1.4.0 (Rambaut 2012). We considered nodes highly supported when Bayesian posterior probabilities (BPP) and bootstrap support percentages (BSP) exceeded 0.95 and 95%, respectively, and moderately supported when these values were in the range of 0.75–0.95 (BPP) and 75–95% (BSP).

MOLECULAR DATING ANALYSIS

We implemented a relaxed molecular clock in BEAST Version 1.8.2 (Drummond et al. 2012), that draws branch rates of each lineage independently from a lognormal distribution (Drummond et al. 2006), and used the HKY + Γ model of sequence evolution, as the GTR model can cause convergence issues with a relaxed clock. The BEAST MCMC chain ran for 100 million generations and was sampled every 10,000 generations, with a burn-in of 10%. Given the absence of crown *Poecilia* fossils, we used secondary constraints from prior studies (Hrbek et al. 2007; Alda et al. 2013; Bagley et al. 2015) as follows: 24.39–19.92 million years ago (MYA) for the split of *Mollienesia*, *Limia*, and *Pamphorichthys*; 22.17–18.04 MYA for the split of the Caribbean *Limia* from the South American *Pseudolimia*; 17.6–8.1 MYA for the split of the two sister groups within the shortfin mollies (the *Poecilia sphenops* complex and the *Poecilia mexicana* complex); and 17–14 MYA for the separation of *Limia vittata* and *Limia melanonotata*. Stationarity, convergence, and mixing of the MCMC chain were inspected in Tracer 1.6, to ensure that ESS values of each parameter exceeded 200. TreeAnnotator 1.8.2 was used to summarize the posterior densities of trees from the BEAST run onto a maximum clade credibility tree, and mean node ages were annotated from a sample of post-burn-in trees.

BI OF CORRELATED TRAIT EVOLUTION

To test for correlated evolution between courtship components in *Poecilia*, we used the Discrete module of BayesTraits Version 2.0 (Pagel and Meade 2006, 2014) in a generalized least squares framework based on the protocol of Pollux et al. (2014). All color and behavioral traits (sexual dichromatism, dorsal fin erections to females, sigmoid postures to females, and sigmoid postures to males) are binary variables that we coded as either 0 (absent) or 1 (present) (Table S5). The pruned RAxML maximum likelihood phylogram, with species missing data removed, was used for the BayesTraits Discrete analyses with reversible-jump MCMC to compare dorsal fin erection to females with sigmoid postures to females, under both dependent and independent models of evolution. Two Markov chains were run for 5 million generations,

with sampling every 1000 steps and a burn-in of 50,000. Prior parameter values were set with a hyper-prior that seeded the mean and variance of the gamma prior from a uniform distribution in an interval from 0 to 10 (Pagel and Meade 2014), reducing uncertainty and difficulty of prior choice.

From all BayesTraits results, best-fitting models of behavioral evolution were determined from the logarithm of each Bayes factor, calculated as $2[\log(\text{harmonic mean of the dependent model}) - \log(\text{harmonic mean of the independent model})]$ (Kass and Raftery 1995). Negative log(Bayes factors) support an independent model of evolution, whereas positive values indicate a dependent model of evolution. $\text{Log}(\text{Bayes factors}) > 2$ offer positive evidence for correlation, values > 5 offer strong evidence, and values > 10 offer very strong evidence (Pagel and Meade 2014). A second set of comparisons was performed in Mesquite Version 3.03 (Maddison and Maddison 2015), using Pagel's (1994) correlation method to examine the relationship between dorsal fin erections and sigmoid displays to females. This tests the likelihood of evolutionary models that assume independence versus dependence of binary traits under study. To estimate statistical significance of relationships between behaviors, 10,000 simulation replicates were run in 10 iterations for a more intense likelihood search.

PHYLOGENETIC LEAST SQUARES AND LOGISTIC REGRESSIONS

Phylogenetic regression analyses were performed in MatLab (Mathworks) 7.5.0. The RAXML phylogram was exported in Newick format and converted to a phylogenetic variance-covariance matrix in the APE package of R (Paradis et al. 2004). Morphological and behavioral traits were coded into a tip data file of comparative species values in APE. Phylogenetic generalized least-squares regressions were performed in Regressionv2.m Version 14 March 2011 (Lavin et al. 2008) to compare the OI with morphology. Likewise, phylogenetic logistic regressions to compare the OI with sexual dichromatism and all behavioral traits were performed in PLogReg.m Version 7 September 2012 (Ives and Garland 2010). For both methods, parametric bootstrapping was run with 2000 simulations and the default alpha value of 0.0, to obtain confidence intervals and test for statistical significance of the regression model slope. From the regression parameters and likelihood ratio tests, all *P*-values were obtained with an online calculator (Soper 2016).

ANCESTRAL STATE RECONSTRUCTIONS

Ancestral states for the OI and the two display components were reconstructed on pruned versions of the RAXML phylogram, after eliminating taxa with missing trait data. For continuous traits, the index of ornamentation was plotted onto the tree topology in Mesquite with the Trace Character History command and

maximum parsimony. Second, the BayesTraits Multistate module (Pagel et al. 2004) was used with the most recent common ancestor approach in a reversible-jump MCMC framework, to map the evolutionary history of sigmoid postures and dorsal fin erection to males and females relative to each other ($N = 26$ taxa). All state changes were given equal probability. For each display type, Markov chains were run for 5 million generations, with a burn-in of 50,000 and sampling every 1000 generations to achieve adequate mixing and stationarity. The posterior probabilities for ancestral states at internal nodes were imported into TreeGraph 2 Version 2.9.2 (Stöver and Müller 2010) for visualization.

Results

PHYLOGENETIC AND MOLECULAR DATING ANALYSES

Both the maximum likelihood phylogram obtained with RAXML-VI-HPC on CIPRES for the combined dataset, with 13 best-fitting models (Fig. 2, Fig. S1), and the consensus cladogram obtained with ML and BI methods of RAXML, GARLI, and MrBayes (Fig. S2) provided robust support to all clades within *Mollienesia* and *Limia*, which appeared topologically identical between the three methods. Two major clades were recovered in the mollies—the sailfin molly clade and the shortfin molly clade split into the *P. sphenops* and *P. mexicana* complexes—which are well established by prior phylogenetic studies (Ptacek and Breden 1998; Alda et al. 2013; Bagley et al. 2015; Ho et al. 2016; Palacios et al. 2016). The basal position of the Jamaican *Limia melanogaster*, and the separation of the Cuban *L. vittata* and Cayman Islands *Limia caymanensis* from the Hispaniolan *L. nigrofasciata* and *Limia perugiae* complexes, also fits previous phylogenies of *Limia* (Hamilton 2001; Pollux et al. 2014; Weaver 2015).

The time tree obtained from BEAST (Fig. S3) was nearly identical to the consensus cladogram, indicating that conclusions drawn from either tree will be similar. This time tree had higher nodal support values. The relaxed molecular clock obtained in BEAST for the combined dataset converged on a log marginal likelihood of $-35,797.38$ (with stepping-stone sampling), and had good sampling properties (all ESS > 200). The single topology difference between the trees involved *Allopoecilia caucana*, as it was basal to the sailfin molly clade (BPP = 71%) in the BEAST tree, but was basal to the entire subgenus *Mollienesia* in the RAXML, GARLI, and MrBayes trees, with stronger support values in those trees (BPP and BSP = 100%). Divergence times for major clades (Table S8) were in good agreement with those obtained by Hrbek et al. (2007) and Reznick et al. (2017). This low uncertainty in the phylogenetic relationships among the subgenera of *Poecilia* indicates that results of comparative analyses using either tree should be identical (Hernandez et al. 2013).

Ornamentation Index

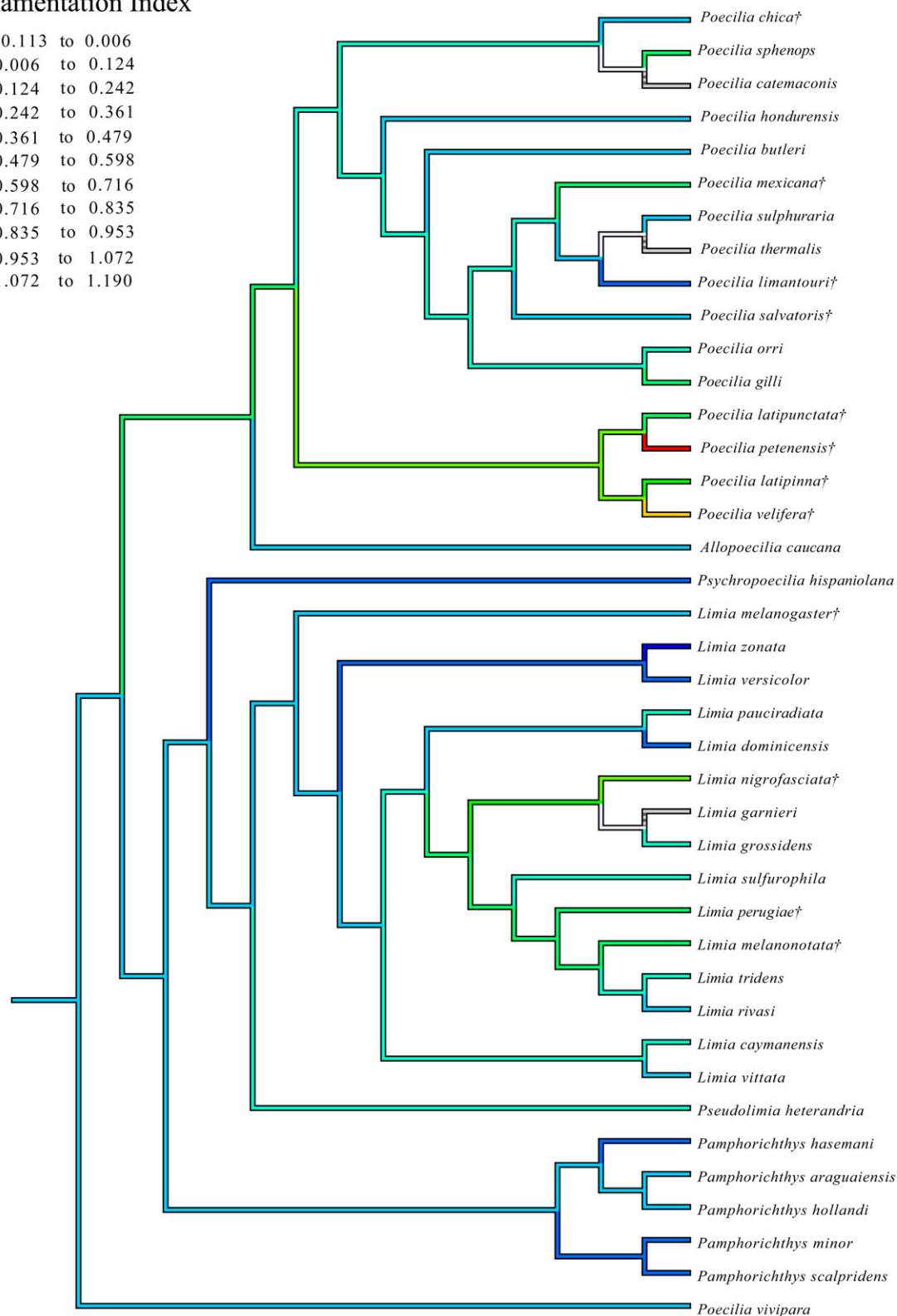
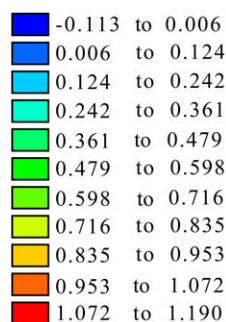


Figure 2. The ornamentation index has become greatly increased in select subgenera of *Poecilia*, as shown on the ultrametric tree created in Mesquite from the RAxML maximum likelihood tree of 40 species of livebearers. Branch colors correspond to a maximum parsimony reconstruction of the ornamentation index, mapped in Mesquite. Warmer colors indicate a taller dorsal fin, which reaches its greatest size in species that have already evolved the male courtship behaviors of dorsal fin displays, sigmoid body curvature, or both (shown with a dagger [†]).

Table 1. Bayesian inference of correlated evolution between fin erection to females and sigmoid curvature to females in *Poecilia*. The dependent model assumes a correlation between the two traits, whereas the independent model sets the correlation to zero. The log(Bayes factor) and *P* indicate a strong correlation and dependent evolution between both behavioral traits.

Program	Number of taxa	Log(harmonic mean), model dependent	Log(harmonic mean), model independent	Log(Bayes factor)	Difference	<i>P</i> -value
BayesTraits (discrete)	26	−36.08	−41.54	10.93		
Mesquite (Pagel 1994)	26	−25.58	−34.38		8.80	<0.001

BI OF CORRELATED BEHAVIORAL TRAIT EVOLUTION

The results of BayesTraits Discrete and Pagel's method showed a significant correlation between the dorsal fin erection and sigmoid postures to females (Table 1). In pairwise comparisons, there was very strong evidence for a correlation between these two component behaviors of courtship (log(Bayes factor) = 10.925, $P < 0.001$), suggesting that they have evolved together.

PHYLOGENETIC LEAST SQUARES AND LOGISTIC REGRESSIONS: ORNAMENTATION, BEHAVIOR, AND DICHROMATISM

Phylogenetic regressions using the RAxML maximum likelihood tree and the BEAST time tree each found similar relationships between the OI and behavior or morphology in *Poecilia*. The Regressionv2.m results (Tables S9 and S10) revealed significant positive correlations between the OI and most morphological variables tested. In species with larger dorsal fins, males surpass females in standard length ($F = 16.36$, $P = 0.001$, Fig. S4A), body depth ($F = 7.90$, $P = 0.001$, Fig. S4C), and dorsal fin length ($F = 32.35$, $P < 0.001$, Fig. S4E). Overall, a positive allometric trend toward greater size was observed for males of highly ornamented species (ln(standard length): $F = 15.98$, $P = 0.001$; ln(body depth): $F = 24.63$, $P < 0.001$; ln(dorsal fin length): $F = 29.79$, $P < 0.001$; Fig. S4G, H, I blue lines), and this was reflected in both Regressionv2.m analyses. Finally, there was a significant negative correlation between relative gonopodium length and the OI, indicating that species with larger dorsal fins have shorter intromittent organs ($F = 36.51$, $P < 0.001$, Fig. 3E).

The PLogReg.m results (Table 2 and Table S11) demonstrate that sexual dichromatism was not significantly associated with a high OI ($b_1 = 0.98$, $P = 0.208$, Fig. 3A), as sex-based differences in coloration are common in mollies and *Limia* regardless of dorsal fin size. There was, however, a significant positive correlation between a high OI and the presence of courtship, in the form of dorsal fin erection to females ($b_1 = 1.05$, $P = 0.008$, Fig. 3B), and sigmoid body postures to females ($b_1 = 1.10$, $P = 0.002$, Fig. 3D). Finally, the use of sigmoid body postures to males was also correlated with a high OI ($b_1 = 1.2$, $P = 0.017$, Fig. 3C), fitting its predicted status as an ancestral behavioral trait.

ANCESTRAL STATE RECONSTRUCTIONS

The ancestral character state reconstruction found sigmoid postures to males appearing at the root of *Poecilia* (Posterior Probability (PP) = 0.62, Fig. 4). The presence of this display was ancestral for both the sailfin and shortfin clades within *Mollienesia* (PP = 0.92) and was further recovered as ancestral for every molly species with behavioral data available (Fig. 4). In *Limia*, on the other hand, the absence of sigmoid postures to males was found to be ancestral (PP = 0.93). Despite an apparent loss in the common ancestor of *Limia*, this behavior reappeared in *L. nigrofasciata* (PP = 0.85), a species that also uses sigmoid postures to females, and in two noncourting species within the *L. perugiae* complex. The two courting species in this clade (*L. perugiae* and *L. melanonotata*) both perform sigmoid postures to females, but not to males. Male–male dorsal fin erections occur in every species of *Poecilia* with behavioral data available, indicating that this display preceded the appearance of dorsal fin erections to females. However, in *Limia*, this display is not linked to the use of sigmoid curvature in male–male agonistic encounters.

Within *Poecilia*, there have been repeated shifts toward an exaggerated dorsal fin, as shown in the evolutionary history of the OI mapped onto the RAxML tree (Fig. 2) and the BEAST time tree (Fig. S5). It is maximized in the sailfin molly clade (excluding *Poecilia latipunctata*), reaching values of 0.6 in *P. latipinna*, 0.87 in *P. velifera*, and 1.1 in *P. petenensis*, species in which male dorsal fins dwarf those of females. Conversely, *P. latipunctata* has an index of 0.41, consistent with the established hypothesis that it has lost the enlarged dorsal fin phenotype (Ptacek and Breden 1998). Among the shortfin mollies, which make up all other members of *Mollienesia* outside the three sailfin species and *P. latipunctata* (Fig. 2), the OI is highest in one courting species (*P. mexicana*, OI = 0.42) and two noncourting species (*P. gilli*, OI = 0.43; and *P. sphenops*, OI = 0.41). An exaggerated dorsal fin has also evolved in *L. nigrofasciata* (OI = 0.66), although other species of *Limia* that have evolved courtship, such as *L. perugiae* (OI = 0.36) and *L. melanonotata* (OI = 0.38), have smaller fins. The lowest value (OI = −0.11) is found in *L. zonata*, in which female dorsal fins are actually larger than male fins. From a predicted ancestral index value of 0.22 in *P. vivipara*, male dorsal fin height

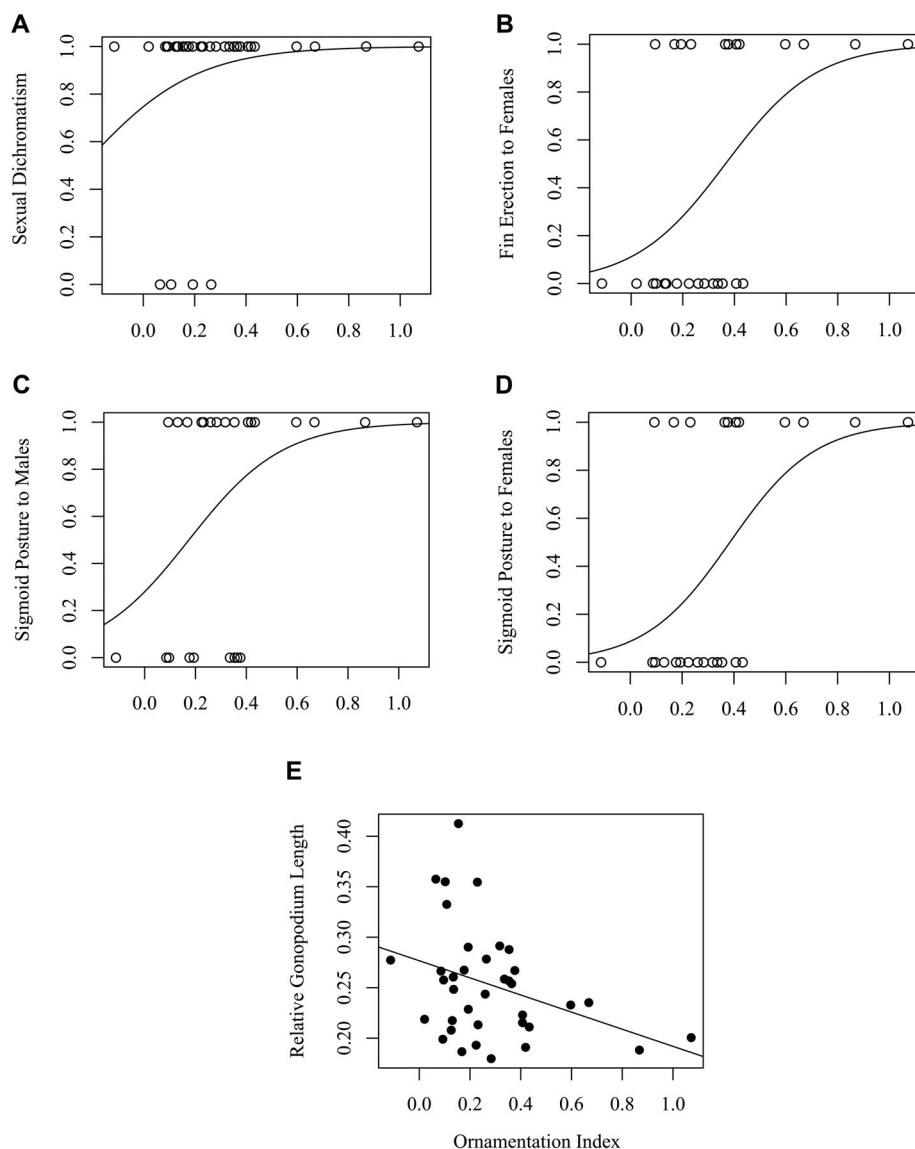


Figure 3. Regression lines show the relationship between the ornamentation index and behavior-related traits. (A) There is no significant correlation with sexual dichromatism ($N = 36$ taxa). (B) There is a significant positive correlation with dorsal fin erection displays to females ($N = 27$), (C) sigmoid body curvature to males ($N = 26$), and (D) sigmoid body curvature to females ($N = 26$). (E) There is a significant negative correlation with relative gonopodium length ($N = 37$).

has undergone drastically different changes in separate poeciliid lineages.

The ancestral character state estimation for the courtship display components (sigmoid postures and dorsal fin erection) revealed, on the whole, a highly synchronous pattern of evolution across the phylogeny of *Poecilia* (Fig. 4). A shift to courtship was observed twice in *Mollienesia* (in the sailfin molly clade, posterior probability = 0.87; and in the *P. mexicana* complex, PP = 0.88), and twice in *Limia* (in *L. nigrofasciata*, PP = 0.79; and in the *L. perugiae* complex, PP = 1.0) (Table S12). Although *L. melanogaster* males court as well (Hamilton 2001), there was insufficient support in favor of a transition at the node at which

this species diverges from the rest of *Limia*. Both behaviors were found to be absent at the root of *Poecilia*, supporting at least four separate origins of courtship within its subgenera.

Discussion

Patterns of morphological and behavioral traits in species of *Mollienesia*, *Limia*, and their close relatives support our predictions that an increase in dorsal fin height (the OI) is correlated with a reduction in gonopodium length and female-biased size dimorphism, an increase in overall body size, and the evolution of courtship. In *Mollienesia*, both components of male–male

Table 2. Parameter estimates for phylogenetic logistic regression with the Firth correction used in PLogReg.m, based on the RAxML phylogeny, to study variation in sexual dichromatism and binary behavioral traits in the genus *Poecilia* as a function of the ornamentation index.

Parameter	Estimate	SE	Bootstrap mean	Bootstrap 95% CI (confidence interval)	Bootstrap <i>P</i> -value
Sexual dichromatism					
<i>a</i>	−4.00		−2.22	(−4.00, 3.99)	0.291
<i>b</i> ₀ (Y-int.)	2.18	0.65	1.70	(0.12, 3.41)	0.026
<i>b</i> ₁ (OI)	0.98	0.84	0.84	(−0.19, 3.00)	0.208
Sigmoid posture to females					
<i>a</i>	−4.00		−3.02	(−4.00, −0.60)	0.519
<i>b</i> ₀ (Y-int.)	−0.64	0.47	−0.61	(−1.57, 0.22)	0.172
<i>b</i> ₁ (OI)	1.10	0.59	1.12	(0.28, 2.80)	0.002
Sigmoid posture to males					
<i>a</i>	−3.97		−2.83	(−4.00, −0.15)	0.420
<i>b</i> ₀ (Y-int.)	0.81	0.49	0.76	(−0.17, 1.86)	0.109
<i>b</i> ₁ (OI)	1.25	0.72	1.26	(0.14, 3.51)	0.017
Dorsal fin erection to females					
<i>a</i>	−4.00		−3.08	(−4.00, −0.76)	0.526
<i>b</i> ₀ (Y-int.)	−0.51	0.44	−0.48	(−1.43, 0.35)	0.252
<i>b</i> ₁ (OI)	1.05	0.55	1.07	(0.23, 2.63)	0.008

aggressive displays (dorsal fin erection and sigmoid body postures) appear co-opted for courtship, while in *Limia* only dorsal fin erection shows evidence of co-option. These results were achieved through the use of a well-resolved molecular phylogeny and a continuous index of ornamentation.

DUAL-UTILITY TRAIT EVOLUTION AND THE OI

By applying a continuous index of ornamentation to dorsal fin height in *Poecilia*, we can include the full range of species-specific sizes exhibited by this trait, regardless of any differences in size or allometry between the sexes. Just as sex-specific analysis of poeciliid body shape has revealed different adaptive landscapes for males and females, which may be diagnosed incorrectly by use of sex-averaged phenotypes (Culumber and Tobler 2017), our results show that a continuous OI provides information that categorical or discrete indices cannot.

Patterns of trait evolution in *Poecilia* are apparent in the distribution of the OI alone (Fig. 5). Most members of *Mollienesis*, *Limia*, and related subgenera have small dorsal fins, and species that do not use aggressive male–male sigmoid postures have an average index of 0.208. However, this average index is higher in species that do not use female-directed sigmoid postures (0.236) or female-directed dorsal fin erection (0.239), which are the two components of courtship displays. This suggests that there may be a threshold size of dorsal fin height that must be reached before poeciliids will evolve aggressive displays between males, and that further increased dorsal fin height is a prerequisite in turn for the evolution of courtship displays to females. The OI is maximized

in three sailfin mollies and the humpback *Limia*, but the values for these species range from 0.6 to 1.1, which spans almost half of the range seen among all species (Fig. 5). A categorical definition of sailfin species as either ornamented or not is insufficient, because it does not factor this variability into account, nor does it consider the possibility that a certain dorsal fin size must be reached before trait exaggeration can occur. Indeed, our quantitative data and the OI revealed that male dorsal fins are smaller in species that use fin displays for a single function (male–male competition) and larger in species that use fin displays for a dual function (both courtship and competition).

EVOLUTIONARY SHIFTS ASSOCIATED WITH INCREASED ORNAMENTATION

A shift toward male-biased sexual dimorphism in association with an increasing OI may result from the effects of sexual selection. Although females are larger than males in most poeciliids (Bisazza et al. 1996), this study offers evidence that in mollies and *Limia* with ornamentation and courtship, males have evolved to surpass females in body size. Small male size may offer an advantage in covert copulation, whereas large male size provides more success in courtship and competition; so sexual size dimorphism has been postulated to covary with male behaviors. The postulated gradient ranges from species with gonopodial thrusting only, in which females are much larger than males, to species with courtship in which males can be larger than females (Bisazza and Pilastro 1997). Both measures of sexual dimorphism used in our study

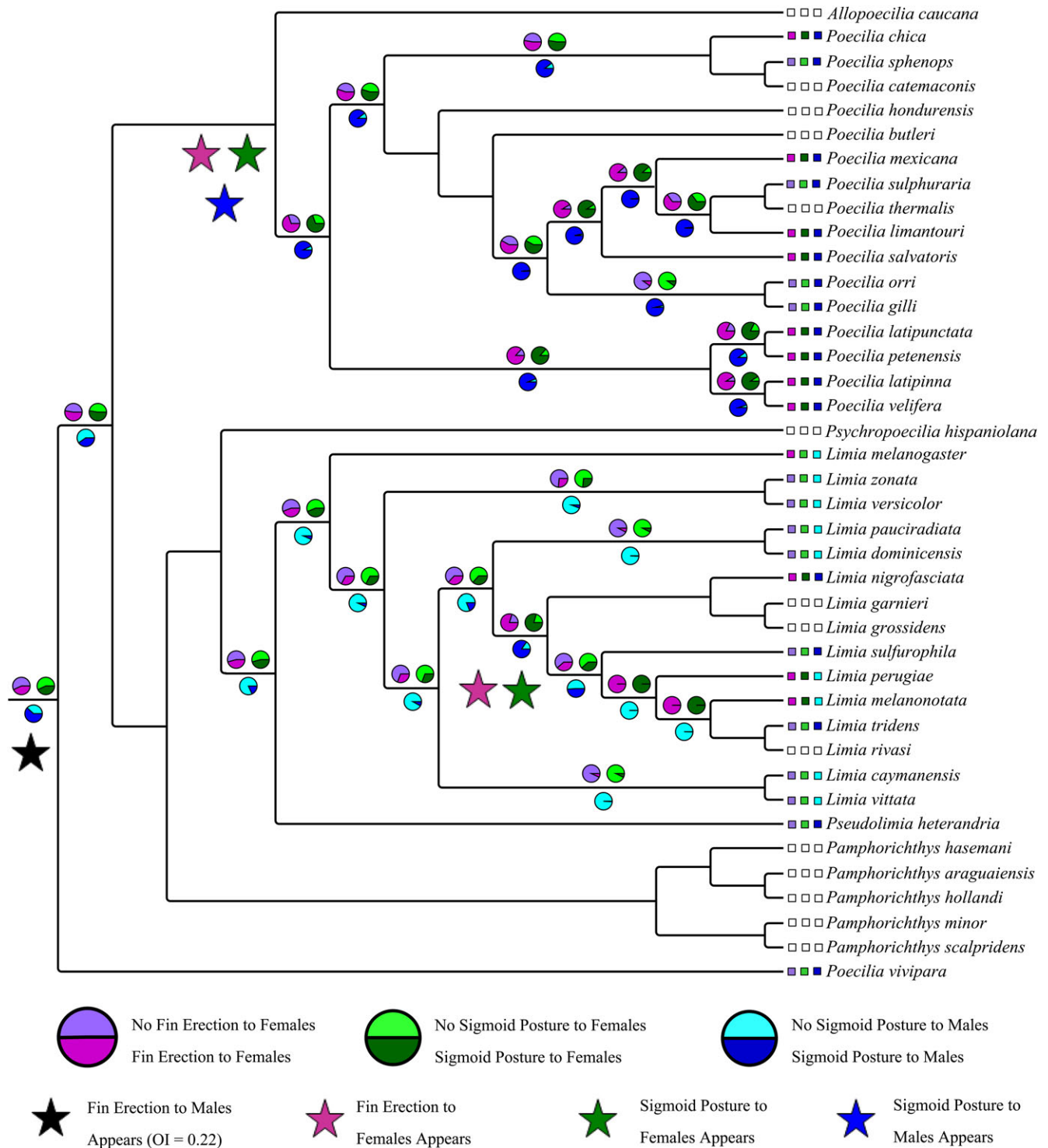


Figure 4. Ancestral character state reconstruction for display behavior in the genus *Poecilia*: dorsal fin erection to females (left above each branch), sigmoid posture to females (right above each branch), and sigmoid posture to males (below each branch). The pie charts plotted on the maximum likelihood cladogram represent posterior probabilities of the occurrence of each state. Boxes at branch tips represent character states for each taxon; white boxes indicate that no behavioral data are available. Stars represent first appearances of each trait. Fin erection to females is classified as absent (lavender) or present (violet). Sigmoid posture to females is classified as absent (light green) or present (dark green). Sigmoid posture to males is classified as absent (light blue) or present (dark blue). Male–male dorsal fin erections (black star) occur in every species with known behaviors (filled-in boxes) and is thus ancestral in *Poecilia*.

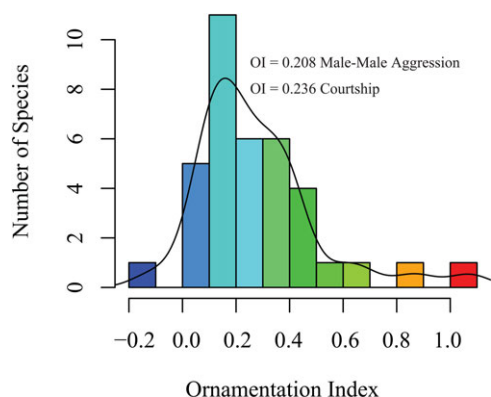


Figure 5. Histogram of the distribution of the ornamentation index relative to the number of species in the genus *Poecilia*. The average ornamentation index of species without aggressive sigmoid postures to males ($OI = 0.208$) is smaller than the average ornamentation index of species without sigmoid postures to females ($OI = 0.236$) or dorsal fin erection displays to females ($OI = 0.239$), which suggests that there are threshold sizes of the ornamentation index that must be reached before a new behavior evolves.

reveal that this contrast occurs within *Poecilia*: females are larger in noncourting species and males are larger in courting species.

Although female poeciliids tend to prefer large males (Bisazza et al. 1996; Bisazza and Pilastro 1997), and these males often exhibit correspondingly large dorsal fins and high courtship rates (Ptacek and Travis 1996, 1997), many poeciliids show extreme polymorphic variation in body size associated with alternative mating strategies. In *Mollienesia* and *Limia*, males that rely on gonopodial thrusting may form a distinct small size class, as in *P. velifera* (Hankison and Ptacek 2007); may be low-ranking, as in *Poecilia limantouri* (Balsano et al. 1985); or may be small and low-ranking, as in *L. nigrofasciata* (Holz 2015). Some species have continuous variation in male size (*L. perugiae*: Farr 1984; Erbelding-Denk et al. 1994; Schröder et al. 1996; *P. latipinna*: Farr et al. 1986; Swanbrow Becker et al. 2012), and *P. latipinna* males also can exhibit phenotypic plasticity. Intermediate-sized males mostly court like large males, but sometimes use gonopodial thrusting like small males (Swanbrow Becker et al. 2012), usually when other males are present (Fraser et al. 2014). This fine-scale variation within poeciliids parallels the patterns of mating tactics between species, further supporting a causal relationship between the evolution of courtship and the emergence of exaggerated ornaments.

As divergence in sexually selected ornamentation has been associated with speciation and may even promote it (Gomes et al. 2016), poeciliid clades that have evolved increased dorsal fin size over time may have correspondingly diverged into new species at higher rates than shortfin clades. *Mollienesia*, in particular, is the most species-rich subgenus of *Poecilia* as well as the one

with the most recent divergences between species (Bagley et al. 2015; Ho et al. 2016; Palacios et al. 2016), so it is unsurprising that courtship displays and dorsal fin enlargement are widespread in mollies. Accordingly, in *Xiphophorus*, the sexually selected elongated caudal fin originated in the ancestor of the genus, prior to its own substantial speciation and the exaggeration of the fin in several swordtail clades (Kang et al. 2013). Within Poeciliidae, *Mollienesia* and *Xiphophorus* show the highest levels of sexual selection and ornamentation (Pollux et al. 2014) and are two of the most speciose clades in the family (Reznick et al. 2017), which indicates that phenotypic evolution of enlarged dorsal or caudal fins may have led to rapid speciation in these livebearers.

CO-OPTION AND CORRELATIONS BETWEEN AGGRESSION AND COURTSHIP

The preexisting trait hypothesis predicts co-option of aggression displays for an additional courtship function (Berglund et al. 1996), but our study shows that this may only have occurred in *Mollienesia*, in which sigmoid postures and dorsal fin erections appear to be ancestral displays that are found in every species that we examined. In *Limia*, only dorsal fin erection is an ancestral display used in male–male competition, and the order of evolution is equivocal for sigmoid curvature. The phylogenetic distribution of male competitive displays is much broader than the distribution of courtship displays, indicating that male–male aggression came first, and that co-option for mate attraction has occurred in a few lineages of *Mollienesia* and *Limia*. Extreme dorsal fin height in sailfin mollies and the humpback *Limia*, which evolved after the appearance of courtship, may have arisen secondarily to enhance the vigor or efficacy of male aggressive and courtship displays (Byers et al. 2010; Tazzyman et al. 2014). This scenario has been proposed for enlarged male dorsal fins in the species *Xiphophorus birchmanni* (Robinson et al. 2011), as fin erection for courtship and competition is common in swordtails as well.

Typically, intersexual and intrasexual selection will reinforce each other by imposing the same form and direction of male trait evolution (Wong and Candolin 2005; Hunt et al. 2009), consistent with the pattern we observed in the genus *Poecilia*. Theoretical models show that mating preferences for competitive traits can indeed evolve and persist over time, and will affect the evolutionary trajectory of dual-utility traits (Stern and Servedio 2017). Despite its potential broad applicability to the evolution of animal displays (Berglund et al. 1996), the preexisting trait hypothesis has seldom been tested in a phylogenetic context prior to this study (Borgia and Coleman 2000; Morris et al. 2007; Regalado et al. 2015). There are many case studies in which both intra-sexual and intersexual selection have been found to act on male traits, such as body size, that are used in competition and are also chosen by females (Hunt et al. 2009). However, these examples have focused on one or a few species at a time, instead of taking

a comparative approach to trait evolution. Dual-utility displays have been found in members of several species-rich groups, such as auks (Jones and Hunter 1999), widowbirds (Andersson et al. 2002), Old World sparrows (Griggio et al. 2007), antbirds (Tobias et al. 2011), darter fish (Zhou and Fuller 2016), and deer (Morina et al. 2018); and phylogenetic analyses of ornamentation in speciose clades (such as poeciliids) can provide a greater picture of the processes by which trait co-option has occurred. Furthermore, the combination of behavioral observations with ancestral state reconstructions should help fill in the gaps for phylogenies in which some species are known to use dual-utility traits, and in which other species may have lost the trait function for courtship, as in *Malurus* fairy wrens (Fan et al. 2018).

If co-option of preexisting traits has occurred in poeciliids, there should be a strong genetic linkage between aggressive and courtship displays. This correlation at the microevolutionary level has been observed in the bluefin killifish, *Lucania goodei*, another species in which males exhibit courtship of females and aggressive displays toward other males and females, and females prefer dominant males, as in poeciliids (McGhee et al. 2007; McGhee and Travis 2010). Courtship rates and rates of aggressive acts toward other males are both significantly heritable in *L. goodei* and appear to have a strong genetic covariance (McGhee and Travis 2013). Multigenerational behavioral studies of poeciliids, similar to those that have been done with *L. goodei*, should reveal genetic covariance and high heritability of courtship and male–male aggression in livebearers, fitting the phylogenetic pattern of coevolution that we have documented in our study. For example, dominance and male–male aggressiveness show strong covariance in the sheepshead swordtail *X. birchmanni* (Wilson et al. 2013), should be correlated with female-directed courtship (Robinson et al. 2011), and should show significant heritability.

It is also possible that courtship in poeciliids has only evolved in clades that exhibit male–male aggressive displays. This may parallel fishes in general, in which territoriality is thought to have preceded and set the stage for the evolution of male care of offspring (Ah-King et al. 2005). Courtship displays have multiple independent origins across the livebearer phylogeny (Pollux et al. 2014), similar to male care in fishes, but the appearances of male–male aggressive displays have not been equally well studied, and their patterns of evolution could indicate whether competition is a prerequisite for courtship. Just as tending offspring is less costly when a male can defend a place to raise his young (Ah-King et al. 2005), attracting females may be easier when a male can draw from a behavioral repertoire that he already uses for competing with other males. Consistent with this idea, large dominant males in many species of poeciliids, which win most aggressive interactions and are preferred by females (Bisazza et al. 1996), spend more time courting females and fending off rivals than smaller and subordinate conspecifics (Erbelding-Denk et al. 1994; Swan-

brow Becker et al. 2012). A minimum level of male–male aggression may be required for courtship to evolve, just as nest and territory acquisition in birds may require a minimal level of aggression (Duckworth et al. 2006). Behavioral studies of poeciliids with unknown competitive behavior could investigate this possibility.

Finally, courtship that is based on an ancestral display used for male–male competition might even have arisen from an “aggressive spillover” (Arnqvist and Henriksson 1997). As aggressiveness in male poeciliids is highly correlated with male sexual activity, regardless of a species’ reliance on gonopodial thrusting or courtship (Bierbach et al. 2013), it is therefore possible that an aggressive spillover has occurred in *Poecilia*. The armament-ornament and aggressive spillover hypotheses may interconnect to explain the existence of exaggerated courtship traits in mollies and *Limia*: preexisting armaments used in aggressive intrasexual contests are required to facilitate a shift to intersexual ornamentation, while aggressive spillover may account for the behavioral transition from sole male–male competition to competition plus female mate choice. Clearly, additional research is needed to determine the genetic covariance of displays for mate attraction and male–male competition in poeciliids, as well as the repeatability of these behaviors and the strength of selection on the ornaments involved.

Conclusions

The results of our comparative study demonstrate that co-option of male displays from contests to courtship can explain the evolution of exaggerated dorsal fins in the livebearer genus *Poecilia*. Our use of a continuous OI reveals that mollies, *Limia*, and their close relatives do not simply fit discrete categories of fin size, as increasingly larger dorsal fins have evolved in species that display for both male–male competition and mate attraction. Increased ornamentation is associated with a shift toward male-biased sexual dimorphism, may account for the species richness of highly ornamented clades, and could have arisen due to strong genetic covariance and heritability of courtship and aggression. Phylogenetic studies of sexually selected traits that are measured as continuous variables should help to elucidate the evolution of extreme ornamental phenotypes and behaviors that function as dual-utility traits in certain animal groups.

AUTHOR CONTRIBUTIONS

D.L.G. and D.N.R. designed the study; J.A.L. and J.T. quantified the ornamentation index; D.L.G. and M.S.S. constructed the molecular phylogeny; M.S.S. taught D.L.G. how to do phylogenetic regression; and D.L.G. measured the morphological traits, performed analyses of the data, and wrote this article. All authors discussed the results and commented on the manuscript.

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DATA ARCHIVING

The doi for our data is <https://doi.org/10.5061/dryad.2h0k50s>.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. The maximum likelihood phylogram for the genus *Poecilia* (ln likelihood = -34650.36) obtained with RAxML-VI-HPC on CIPRES for the combined data set with 13 best-fitting models.

Figure S2. The consensus cladogram of the genus *Poecilia* obtained with RAxML, GARLI, and MrBayes for the combined data set with 13 best-fitting models. Values by each branch correspond to ML bootstrap support percentages obtained with RAxML (first), ML bootstrap support percentages obtained with GARLI (second), and Bayesian posterior probabilities obtained with MrBayes (third), respectively.

Figure S3. Chronogram of the evolution of *Poecilia*, with a timeline in millions of years ago (MYA), based on BEAST analysis of the combined data set with 13 models. Blue bars indicate 95% highest posterior densities (HPDs). Mean estimates of the time to most recent common ancestor (TMRCA) are indicated at the four nodes used to calibrate divergence times. Values by each branch correspond to Bayesian posterior probabilities.

Figure S4. Regression lines show the relationship between the ornamentation index and morphological traits ($N = 37$ taxa). There is a significant negative correlation with (A) the sexual dimorphism index (SDI) for standard length, (B) the size dimorphism index (SSD) for standard length, (C) the SDI for body depth, (D) the SSD for body depth, (E) the SDI for dorsal fin length, and (F) the SSD for dorsal fin length. There is a significant positive correlation with (G) natural-log-transformed standard length, (H) natural-log-transformed body depth, and (I) natural-log-transformed dorsal fin length, for males (blue dots and lines) and females (red dots and lines) of all species.

Figure S5. The ornamentation index has become greatly increased in select subgenera of *Poecilia*, as shown on the ultrametric tree created in Mesquite from the BEAST time tree of 40 species of livebearers. Branch colors correspond to a maximum parsimony reconstruction of the ornamentation index, mapped in Mesquite. Warmer colors indicate a taller dorsal fin, which reaches its greatest size in species that have already evolved the male courtship behaviors of dorsal fin displays, sigmoid body curvature, or both (shown with a dagger †).

Table S1. Collection data for all preserved specimens examined in this study, including the museum where specimens were stored, accession numbers, species, number of males photographed, number of females photographed, preparation type, country of origin, municipality information, locality information, date of collection, and individual collector(s).

Table S2. Collection data for all live and recently preserved specimens whose photographs were measured in this study, including the university campus where stocks were kept, species, number of males photographed, number of females photographed, preparation type (if dead), country of origin, municipality information, locality information, date of collection, and individual collector(s).

Table S3. Species measured for morphological analysis, ornamentation index (OI), number of males measured (NMAL), number of females measured (NFEM), relative gonopodium length (PROPGL), natural-log-transformed male standard length (LNMSL), natural-log-transformed female standard length (LNFSL), natural-log-transformed male body depth (LNMBD), natural-log-transformed female body depth (LNFBD), natural-log-transformed male dorsal fin length (LNMDL), natural-log-transformed female dorsal fin length (LNFDW), size dimorphism index standard length (SDISL), sexual size dimorphism standard length (SSDSL), size dimorphism index body depth (SDIBD), sexual size dimorphism body depth (SSDBD), size dimorphism index dorsal fin length (SDIDL), and sexual size dimorphism dorsal fin length (SSDDL).

Table S4. Species-specific values used to create the Ornamentation Index in R statistical software. For each species, the difference between the adjusted mean of each sex from an ANCOVA analysis [(the estimated least squares mean of ln(Dorsal Fin Length) for each sex, calculated at the overall mean ln(Standard Length), and adjusted for the relationship between ln(Dorsal Fin Length) and ln(Standard Length)] yielded the Index (OI).

Table S5. Species studied with behavioral traits labeled as absent (0), present (1) or unknown (?).

Table S6. Species used in the phylogenetic analysis, gene sequences from GenBank used to construct the phylogeny, and GenBank accession numbers.

Table S7. Molecular evolution models, subsets, partitions, and sites chosen to best fit each codon position of all nuclear and mitochondrial genes, as determined by PartitionFinder.

Table S8. Point estimates (mean node ages) and 95% highest posterior densities (HPD) for the divergence times of major clades, in millions of years, calculated by BEAST for the combined data set with 13 best-fitting models.

Table S9. Statistics for ordinary least squares regressions (OLS) assuming a star phylogeny, phylogenetic least-squares regression (PGLS) assuming a Brownian motion process of trait evolution, and phylogenetic least-squares regression with the Ornstein-Uhlenbeck transformation (RegOU).

Table S10. Statistics for ordinary least squares regressions (OLS) assuming a star phylogeny, phylogenetic least-squares regression (PGLS) assuming a Brownian motion process of trait evolution, and phylogenetic least-squares regression with the Ornstein-Uhlenbeck transformation (RegOU).

Table S11. Parameter estimates for phylogenetic logistic regression with the Firth correction used in PLogReg.m, based on the BEAST phylogeny, to study variation in sexual dichromatism and binary behavioral traits in the genus *Poecilia* as a function of the ornamentation index.

Table S12. BayesTraits posterior probabilities of ancestral state reconstructions for display components to females and males.