



## Patterns of inheritance of mating signals in interspecific hybrids between sailfin and shortfin mollies (Poeciliidae: *Poecilia: Mollienesia*)

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### Abstract

Differences in male morphology and mating behaviors are thought to confer species sexual isolation between sailfin and shortfin species of mollies. This study used interspecific crosses between the sailfin molly, *P. latipinna*, and the shortfin molly, *P. mexicana*, to investigate patterns of inheritance of morphological traits and behavioral rates of three mating behaviors in F1 hybrid males. The two parental species showed clear species differences with respect to the length of the dorsal fin and dorsal fin ray number. First generation hybrid males were intermediate between the two parental species for dorsal fin length and fin ray number, suggesting autosomal control of this trait with little effect of dominance by genes from either parental species. Parental species showed clear species differences in their rates of courtship displays. Unlike the pattern for dorsal fin morphology, F1 hybrid males showed a clear distinction in display rates with respect to the direction of the interspecific cross. Male hybrids whose sires were *P. latipinna* had courtship display rates that were up to three times higher than the rates of displays performed by hybrid males whose fathers were *P. mexicana*. The distribution of phenotypes between the parental species and that of hybrid males sired by that parental species was nearly identical. Such a pattern suggests the influence of Y-linked genes on the inheritance of courtship display rates in mollies.

### Introduction

One of the greatest challenges in evolutionary biology is to identify the forces that promote divergence in reproductive systems and ultimately speciation. Recent studies have provided much insight into how natural selection, genetic drift and sexual selection can result in population divergence and speciation (see recent reviews by Panhuis et al., 2001; Schluter, 2001; Via, 2001). Less attention however, has been given to the kinds of changes that are favored by each of these forces and our knowledge of the genetic changes that occur during speciation is very limited, especially with respect to the types of species studied (Otte & Endler, 1989; Coyne, 1992; Coyne & Orr, 1998; Macdonald & Goldstein, 1999; Orr, 2001). Much of our knowledge of the genetic basis of species differences comes from a few well-studied groups, that is, *Drosophila*, a few

additional insect genera and certain plant taxa, namely *Mimulus* spp. (see Table 1, Orr, 2001).

While both prezygotic and postzygotic isolating barriers may be involved in speciation, recent models suggest that premating isolation, particularly behavioral isolation, may be more important in rapid speciation, especially rapid divergence resulting from sexual selection (Turner & Burrows, 1995; Gavrilets & Boake, 1998; Turelli, Barton & Coyne, 2001). Behavioral divergence of closely related species is a common phenomenon and sexual selection has been implicated in speciation of a variety of taxa (reviewed by Ptacek, 2000; Panhuis et al., 2001). Such studies have concentrated on the contribution of sexual selection to speciation through divergence in mating signals and preferences, yet only a few of these studies have investigated the underlying genetics of mating signal divergence (Tomaru & Oguma, 1994; Shaw, 1996;

Noor & Aquadro, 1998; Boake, Andreadis & Witzel, 2000; Beukeboom & Assem, 2001; Williams, Blouin & Noor, 2001) or divergence in mating preferences (Shaw, 2000).

The study of the genetics of interspecific hybrids between closely related taxa is particularly relevant for understanding the process of species isolation (Beukeboom & Assem, 2001). If sexual traits play a major role in the early stages of speciation, then these traits should show a large degree of divergence between closely related species (Civetta & Singh, 1998). To the extent that high levels of genetic divergence caused this morphological and behavioral divergence, such higher genetic divergence is also expected to translate into a stronger disruption of the phenotypes of these sexual traits in interspecific hybrids due to incompatible gene interactions (Civetta & Singh, 1998; Beukeboom & Assem, 2001). Thus comparisons of the distribution of phenotypic components of a mating signal between parental species and their hybrids provide insights into the constancy of these traits from parental to hybrid generations and provide the first step towards uncovering the underlying genetic control of species differences in the parental taxa.

Mollies (genus *Poecilia*, subgenus *Mollienesia*) are particularly amenable to the study of interspecific divergence in mating signals and its role in speciation for several reasons. First, differences in the two major species complexes of mollies, sailfins and shortfins, are found primarily in males and are strongly associated with divergence in their mating systems. Sailfin species are characterized by a sexual dimorphism in which males possess a greatly enlarged dorsal fin (Regan, 1913; Hubbs, 1933; Parzefall, 1969) that is erected and presented to the female in a courtship display (Parzefall, 1969, 1979; Farr, Travis & Trexler, 1986). Receptive females respond to this display by remaining stationary, folding the median fins, and sometimes twisting the abdomen to accept a copulation (Parzefall, 1969; personal observation). Males of the sailfin species *P. latipinna* show low levels of inter-male aggression (Travis, 1994) and are not known to form permanent dominance hierarchies (Farr, 1989). Thus, reproductive success in sailfin species appears to be more a function of female choice than strong male–male competition.

In marked contrast, shortfin species do not show sexual dimorphism in fin morphology and most species rely primarily on gonopodial thrusting during mating attempts (Parzefall, 1969, 1979, 1989; Brett

& Grosse, 1982; Balsano et al., 1985; Woodhead & Armstrong, 1985; Ptacek, 1998). Gonopodial thrusting is an attempt at insemination without female cooperation, whereby a male orients himself behind a female, brings his gonopodium (modified anal fin that serves as an intromittent organ during internal fertilization) to a forward position and attempts to insert it forcefully into the female's gonopore. Males of shortfin species do not rely on female cooperation for mating and have adopted a completely different mating system than males of sailfin species. Reproductive success is determined primarily by a social structure based on male–male aggression, where dominant males aggressively keep other males from gaining access to females and females are forcibly inseminated by these males through gonopodial thrusting (Miller, 1975; Parzefall, 1969; personal observation). Thus, male–male competition appears to play a much larger role in determining male reproductive success than does female choice in shortfin molly species.

Second, in addition to the strong level of interspecific divergence in mating behaviors and associated dorsal fin morphology, most of the reproductive isolation that exists between sailfin and shortfin species of mollies appears to be primarily due to premating reproductive isolation. Despite few reported instances of interspecific hybrids occurring in natural populations (with the exception of the gynogenetic species *P. formosa*; Turner, 1982), many species of mollies are completely interfertile in laboratory crosses (Hubbs, 1933, 1936; Meyer, Wischnath & Foerster, 1985; Parzefall, 1989). This implies an important role of sexual selection through female mating preferences in promoting and maintaining the interspecific divergence in mating signals between sailfin and shortfin species of mollies (Ptacek, 1998). Thus, speciation between sailfin and shortfin mollies may have occurred through rapid divergence of male mating behavior and/or dorsal fin shape (Ptacek & Breden, 1998). While a large degree of phenotypic divergence is known to exist between males of shortfin and sailfin species (Ptacek, 1998), the underlying genetic control of these phenotypic traits is not known.

Interspecific hybrids can be generated in the laboratory and measured for their degree of trait expression of morphological and behavioral traits associated with mating signals. The phenotypic trait distributions in the F1 hybrid males can be compared to these values for males of the parental species. Such a comparison provides an initial step in understanding the genetic control of species differences between sailfin

and shortfin mollies. In this study, I performed reciprocal laboratory crosses between a sailfin species, *P. latipinna*, and a shortfin species, *P. mexicana*. I compared the distribution of morphological traits and behavioral rates of three mating behaviors between males of the two parental species and F1 hybrid sons from both directions of the interspecific cross. I asked first, what was the degree of phenotypic differentiation in morphology and behavior between the two parental species, *P. latipinna* and *P. mexicana*. Second, I asked whether F1 hybrid sons differed from the parental males in trait distributions of morphology and behaviors. Third, I asked whether the expression of morphological and behavioral traits in F1 hybrid sons was influenced by the parental species of their sires.

## Materials and methods

### *Origin and maintenance of experimental fish*

Dams of *P. latipinna* used in interspecific crosses were obtained from a single population from Live Oak (Wakulla County, Florida). Sires of *P. latipinna* came from two populations, Live Oak ( $N = 2$ ) and nearby Mounds ( $N = 2$ ; Wakulla County, Florida). Stocks of *P. latipinna* from Live Oak were collected in 1990 and maintained first in J. Travis' laboratory at Florida State University (FSU) and then (beginning in 1997) in my laboratory at Idaho State University (ISU). Stocks of *P. latipinna* from Mounds were collected in 1997 and maintained in my laboratory at ISU. Dams and sires of *P. mexicana limantouri* came from two populations, the Río Tigre ( $N = 2$  dams,  $N = 4$  sires; near Tampico, Tamaulipas, Mexico) and the Río Verde ( $N = 2$  dams,  $N = 1$  sire; near Río Verde, San Luis Potosí, Mexico). Fish from the Río Tigre population were collected in 1989 and maintained first in J. Travis' laboratory at FSU and then (beginning in 1998) in my laboratory at ISU. Fish from the Río Verde population were collected in 2000 and maintained in my laboratory at ISU. It was necessary for dams of *P. m. limantouri* to come from multiple populations due to the extinction of the Río Tigre stock population that occurred in 1999. All stock populations of both species were maintained as randomly breeding populations in large tanks (750 l) in the greenhouse facilities at both FSU and ISU.

Reciprocal crosses were made between *P. latipinna* and *P. mexicana* by pairing a single male and a single female of each species and housing them in a 37.5 l

aquarium. Four crosses between a *P. mexicana* dam and a *P. latipinna* sire produced F1 hybrid offspring and five crosses between a *P. latipinna* dam and a *P. mexicana* sire produced F1 hybrid offspring. Sires were removed from the pairing tanks approximately 1 week prior to parturition and were scored for their behavioral profiles and morphological traits. Hybrid offspring were raised initially in groups of four to six individuals in 37.5 l aquaria until nearing maturity. The formation of a pigmented spot dorsal to the anus (gravity spot) signifies maturity in females (Medlen, 1951). Immature males can be distinguished upon initial fusing of the anal fin rays in the developing gonopodium (Constantz, 1989) and were removed from group tanks and housed individually in 37.5 l tanks until reaching maturity. At the time of maturity, an F1 hybrid female from the same direction of the cross, but not a sib, was introduced into the male's tank. The male was housed with this female for at least 2 weeks prior to scoring his behavioral profile in order to provide sexual experience (Parzefall, 1969; Rodd & Sokolowski, 1995).

Stock populations and aquarium fish were kept on a 14:10 light:dark cycle at 25°C, with water at 6 ppt salinity. Fish were fed twice daily with TetraMin commercial flake fish food and filters were changed and approximately one-third of the water replaced weekly for the aquarium fish.

### *Measurements and analysis of male morphology*

To assess morphological differentiation of males between the two parental species and F1 hybrids, I used landmark-based geometrical morphometric analyses (Rohlf & Marcus, 1993; Rohlf, 1996). Twenty-one males from each parental species (*P. latipinna* from Mounds and *P. mexicana* from Río Verde), 65 males from the *P. latipinna* dam  $\times$  *P. mexicana* sire crosses and 42 males from the *P. mexicana* dam  $\times$  *P. latipinna* sire crosses were included in the morphometric analyses. Each male was anesthetized in 0.50% MS-222 (Sigma) and photographed with an Olympus digital camera. Fourteen landmarks were measured from each photograph (Figure 1). I also measured the standard length (straight-line distance from the tip of the snout to the end of the last vertebra; Trautman, 1981) of each male. Males were not photographed until after their behavioral profiles were completed in order to avoid any residual effects of the anesthetic on male behavior. Landmarks were superimposed on photographs using image analysis

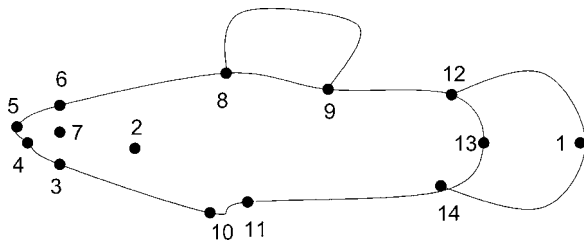


Figure 1. The locations of the 14 landmarks that were used in geometrical morphometric analyses. Landmark 1: insertion point of the ventral-most caudal fin ray into the caudal peduncle; landmark 2: insertion point of the caudal fin ray at the maximum curvature of the caudal peduncle; landmark 3: maximum extent of the caudal fin ray directly opposite to the maximum curvature of the caudal peduncle; landmark 4: insertion point of the dorsal-most caudal fin ray; landmarks 5 and 6: posterior and anterior insertion points of the dorsal fin; landmark 7: edge of the body, dorsal to the center of the eye; landmark 8: tip of the snout; landmark 9: origin of the lower jaw; landmark 10: edge of the body, ventral to the center of the eye; landmark 11: center of the eye; landmark 12: insertion of the pectoral fin; landmarks 13 and 14: anterior and posterior insertion points of the gonopodium.

software (TpsDig program, F.J. Rohlf). Dorsal fin ray counts were made by direct count of anesthetized males.

All landmark configurations were superimposed on a consensus (mean) configuration with generalized orthogonal least-squares Procrustes analysis (Rolf & Slice, 1990; Rohlf & Marcus, 1993). This analysis removes differences in shape due to location, orientation, and size of different individuals. Residuals from the superimposition were used in a relative warp analysis (tpsRelw program, F.J. Rohlf, Bookstein, 1991; Rohlf & Marcus, 1993; Loy et al., 1998; Cavalcanti, Monteiro & Lopes, 1999). Relative warp analysis describes shape change as the deformation of landmarks from a reference configuration, into those of each member in a group of specimens of interest. This analysis finds the thin-plate spline transformations that map the reference configuration of landmarks (mean of each species) onto each specimen. Results of the relative warp analysis produce principal warp scores, called relative warps, which are eigenvectors of a bending energy matrix and are computed using only information from the reference configuration. The principal warp vectors allow one to describe the ways in which it is possible to deform the shape of the reference configuration and include only uniform shape components of the specimens of interest.

The relative warp scores were then used as variables in a multivariate analysis of variance (MANOVA). Univariate ANOVAs were then run to

examine the patterns of variation in each variable separately. Finally, canonical discriminant analysis was used to determine which traits contributed to species and hybrids distinctions. This canonical analysis revealed the linear combinations of the relative warp scores that best distinguished males of the two parental species and their F1 hybrids.

#### Observations and analysis of male behavior

For males of parental species and the F1 hybrids, individual males were kept in isolation for 24 h prior to testing. This protocol appears to motivate males of *P. latipinna* and yields the high rates of behavior patterns typically observed in natural populations (Travis, 1994). Object females used in the behavior trials were mature, visibly gravid females. Object females were housed separately from males, so the test male had no immediate prior experience with the object females. I used visibly gravid females as objects in order to standardize receptivity across females. Female sailfin mollies (*P. latipinna*) are receptive for 1–2 days immediately after producing a brood and signal this receptivity to males (Farr & Travis, 1986; Farr, Travis & Trexler, 1986; Travis & Woodward, 1989; Sumner, Travis & Wilson, 1994), resulting in increased levels of sexual behavior by males towards receptive females. Once females have been inseminated, males lower their rates of sexual behavior, yet still show all three mating behaviors at the rates that their body sizes would predict (Sumner, Travis & Wilson, 1994). By using only gravid females, I could minimize variation in male behavior due to variation in female condition. In addition, gravid females should be unreceptive to males thus male responses to females of different species should be less confounded with patterns of female mating preferences.

I tested males of both parental species only with conspecific object females. I tested each F1 hybrid male with females from both of the species used as dams on two consecutive days. Each male was tested with a female of *P. latipinna* on 1 day and a female of *P. mexicana* on the following day. Half the males were tested first with a *P. latipinna* female and second with a *P. mexicana* female and the other half of the males had the order reversed. Females used as objects were within 10 mm of the male's standard length.

I performed behavioral observations on a single male and a single female in a 37.5 l aquarium. To

minimize disturbance to the fish due to the presence of the observer, I covered three sides of the tank with aluminum foil and the front side with one-way film. I placed the test male in the tank and allowed 15 min for acclimation. I then added the object female to the test tank and allowed an additional 15 min for acclimation. I observed males for 10 min and scored three measures of sexual behavior with a laptop event recorder: the number of courtship displays, the number of gonopodial thrusts and the number of gonopodial nibbles. A total of 26 males of *P. latipinna* (Mounds), 12 males of *P. mexicana* (Río Verde), 66 males from the *P. latipinna* dam  $\times$  *P. mexicana* sire crosses and 42 males from the *P. mexicana* dam  $\times$  *P. latipinna* sire crosses were assayed for male behavioral profiles.

I transformed male standard length and the rates for each behavior to natural logs (ln) to obtain linearity in the behavior rate-body size relationships and to make the estimates of average body size independent of the variance in body size. In some cases a male did not show one of the three behaviors during the observation period. To eliminate zeros prior to ln-transformation, I added 0.05 to all behaviors from each observation. This value is small relative to the scale of the measurement, and examination of the regression residuals from several candidates indicated that this value produced the distribution of residuals that best conformed to the assumptions of the general linear model.

To examine the effect of body size on the rate of each behavior, I performed least squares regression using the ln-transformed value of standard length and behavior rate. To test for differences between parental species and F1 hybrid crosses in behavior rates independently of body-size variation, I performed an analysis of covariance (ANCOVA). I extracted the effects of body size first as a covariate and then tested for parental species or type of hybrid cross effects. I used Fisher's protected LSD method as a *post hoc* comparison of means between the two parental species and two types of hybrid crosses for the rate of each behavior where the ANCOVA showed a significant main effect of male type. I performed similar ANCOVAs (with body size as a covariate) to test for the effects of parental species of sire and species of the object female on the variation in the rates of the three behaviors for the F1 hybrid males from both directions of the interspecific cross. Again I used Fisher's protected LSD method as a *post hoc* comparison of

means when ANCOVAs revealed a significant main effect.

## Results

### *Morphological differences between parental species and F1 hybrids*

The landmark-based geometrical morphometric analysis yielded 24 relative warp factors. The first three relative warps explained 75.29% of the variance among the males. Nine of the 24 relative warp factors were significant based on univariate ANOVAs ( $F_{3,145}$  value range = 3.48–450.02,  $P_s \leq 0.019$ ). A scatterplot of the second relative warp (RW2) against the first relative warp (RW1) for the 149 individuals shows evidence of clear shape differences between the two parental species (Figure 2). The F1 hybrids showed complete overlap with respect to the direction of the cross and both classes of hybrids were intermediate in shape to the parentals, especially with respect to the axis of RW1.

The first relative warp, which explains 44.36% of the variation among males, represents a longitudinal compression of the insertion points of the dorsal fin (landmarks 5 and 6, Figure 3). The first relative warp is least compressed in males of *P. latipinna* (resulting in a longer dorsal fin) and maximally compressed in males of *P. mexicana* (resulting in a shorter dorsal fin). Hybrid males show an intermediate degree of compression of the first relative warp regardless of the direction of the cross. The second relative warp, which accounts for 18.32% of the variation among males, represents the insertion point of the gonopodium (landmarks 13 and 14). Hybrids of both directions of the cross have an insertion point of the gonopodium that is slightly more anterior than either parental species, especially than that of *P. latipinna* (Figure 2).

The overall MANOVA revealed a highly significant species effect (Wilk's lambda = 0.006,  $F_{72,365} = 23.24$ ,  $P < 0.001$ ). The discriminant analysis used two canonical factors to correctly classify all 21 males (100%) of each of the two parental species. Among the hybrid males, 39 of the 42 hybrids with *P. latipinna* sires (93%) were correctly classified; the three misclassified individuals were grouped with hybrid males with *P. mexicana* sires. Among the hybrid males with *P. mexicana* sires, 55 of 65 (85%) were correctly classified and 10 were misclassified as hybrid males with

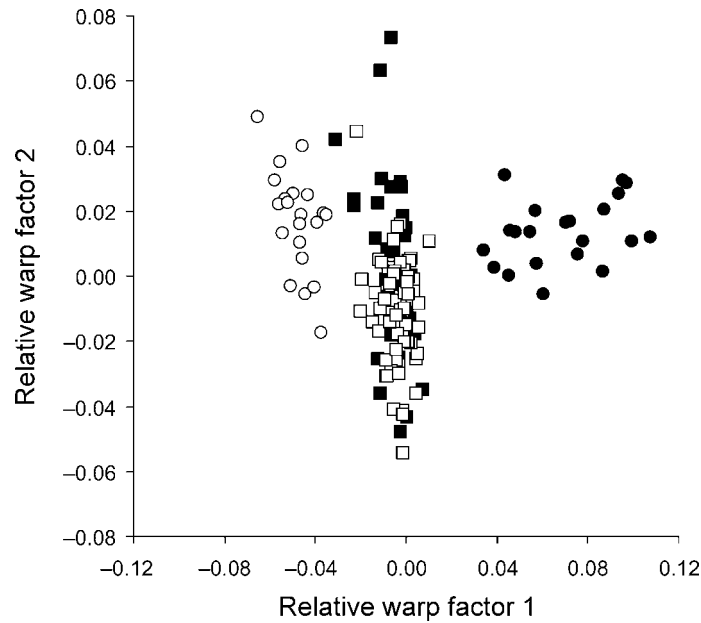


Figure 2. Scatterplot of the projections of 14 landmarks from the 149 aligned individual fish onto the first two relative-warp (RW1, RW2) axes. Solid circles: *P. latipinna*, open circles: *P. mexicana*, solid squares: males from the *P. mexicana* dam  $\times$  *P. latipinna* sire crosses, open squares: males from the *P. latipinna* dam  $\times$  *P. mexicana* sire crosses.

*P. latipinna* sires. Canonical factor 1 of the discriminant analysis was most highly correlated with RW1 ( $r = 0.968$ ,  $P < 0.001$ ) which described the length of the dorsal fin based upon the degree of compression of its insertion points. Males from the parental species *P. latipinna* had the longest dorsal fins and males from the parental species *P. mexicana*, the shortest. The F1 hybrid males were intermediate with respect to this canonical factor and did not differ based upon the parental species of the sire of the cross (Figure 4). Canonical factor 2 was most highly correlated with RW2 ( $r = 0.558$ ,  $P < 0.001$ ) which described the insertion point of the gonopodium. The insertion of the gonopodium was more anterior in both types of F1 hybrid males than in either parental species (Figure 4). Canonical factor 2 was also correlated with RW6 ( $r = -0.406$ ,  $P < 0.001$ ), but this relative warp factor only explained 2.91% of the variation among males in overall shape.

Males differed between the two parental species with respect to the number of fin rays in the dorsal fin. Dorsal fins of males of *P. latipinna* ranged from 15 to 17 fin rays while dorsal fins of males of *P. mexicana* varied between 8 and 10 dorsal fin rays. Dorsal fins of males of F1 hybrids were intermediate in fin ray number; hybrid males with *P. latipinna* sires ranged from 11 to 13 dorsal fin rays while hybrid males with

*P. mexicana* sires ranged from 10 to 13 dorsal fin rays. Thus neither dorsal fin ray number nor dorsal fin shape appear to have a strong paternally controlled genetic component.

#### *Behavioral differences between parental species and F1 hybrids*

Males of *P. latipinna* performed characteristic courtship displays, by raising the dorsal fin, orienting their bodies in front of females, and fanning the fin towards the female accompanied by a sigmoid curving of their body. Males of *P. mexicana* swam alongside females with their dorsal fin erect and pelvic fins extended away from their body. While they did not fan the dorsal fin, many males would shimmy their bodies and bend them towards the female in a manner similar to the sigmoid behavior of courting male guppies (Liley, 1966; Farr, 1975). Hybrid males from both directions of the cross, performed courtship displays. Their display behavior was more similar to that of *P. latipinna* males, where F1 hybrid males would erect the dorsal fin and many males would fan their dorsal fin and curve their body in a sigmoid fashion towards the anterior end of females during the display.

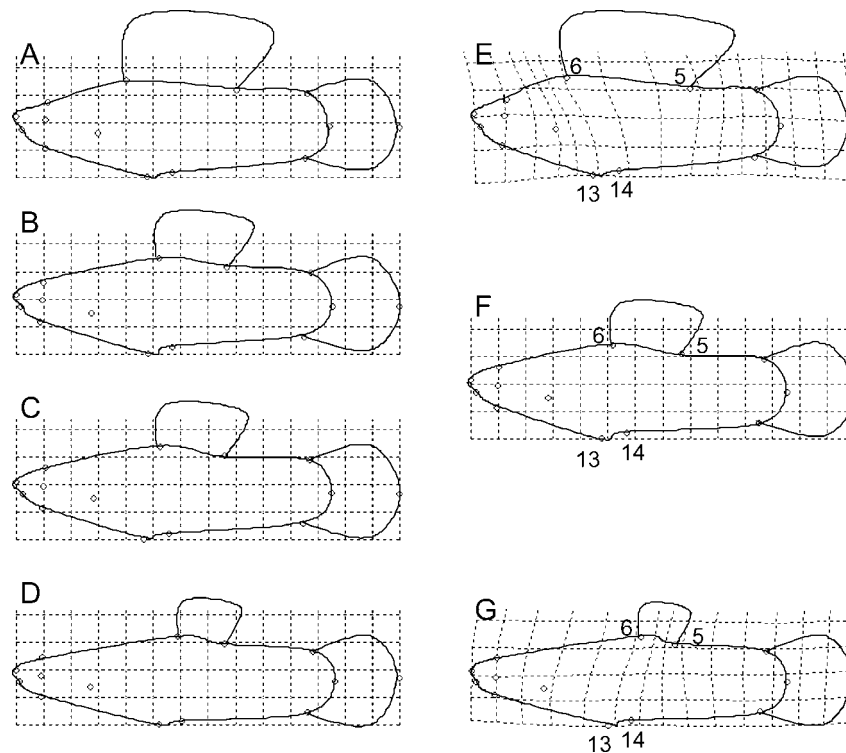


Figure 3. Shape changes implied by variation along the first relative warp axis (RW1). Uniform shape changes are shown as deformations in the thin-plate spline transformations from the consensus (mean) configuration of the 149 individuals included in the analysis. The consensus (mean) configuration for each type of male phenotype is shown for reference (A)–(D). (A) Consensus configuration of the 21 *P. latipinna* males, (B) consensus configuration of the 42 males from the *P. mexicana* dam  $\times$  *P. latipinna* sire crosses, (C) consensus configuration of the 65 males from the *P. latipinna* dam  $\times$  *P. mexicana* sire crosses, (D) consensus configuration of the 21 *P. mexicana* males. Influence of relative warp factor 1 (RW1) on shape changes from the mean are as follows: (E) shape change for a positive deviation from the mean, (F) consensus configuration of all 149 males, (G) shape change for a negative deviation from the mean. Note the similarity in shape between F1 hybrid males from either direction of the crosses (B and C) to that of the consensus configuration for all males (F). The landmarks (5, 6, 13, 14) that contribute the most to the shape changes are identified (see Figure 1).

Descriptive statistics for each parental species and the two classes of F1 hybrids for standard length and the rates of each of the three sexual behaviors are presented in Table 1. The values for slopes of the regressions of  $\ln$  behavior rate on  $\ln$  standard length for each behavior are also presented. There was no significant relationship between behavioral rate and male size for either parental species for any behavior except gonopodial thrusting. There was a significant negative relationship between rates of gonopodial thrusting and male standard length for *P. latipinna*. Male body size did influence the rates of gonopodial thrusts and gonoporal nibbles but not courtship displays in both types of hybrid males. In all cases where there was a significant relationship, the relationship was positive. Thus larger F1 hybrid males showed higher rates of thrusting and nibbling, but rates of courtship display were not influenced by male size.

Males of the F1 hybrids did not differ significantly in their behavioral response to females presented on the first day of testing or the second day of testing for any of the three behaviors ( $F_{1,214}$  value range = 0.567–3.045,  $P_s > 0.082$ ). Results of ANCOVAs (with body size as the covariate) to test for the effects of parental species of the sire and species of the object female on the rates of each of the three behaviors for the F1 hybrid males are shown in Table 2. Hybrid males from both directions of the cross, performed similar rates of all three behaviors in response to both species of females with which they were tested (Table 1). In addition, there was no significant effect of female species for any of the three behavior rates based upon the ANCOVA (Table 2). Thus there was no evidence that parental species of dam influenced the behavior rates of the F1 hybrid sons.

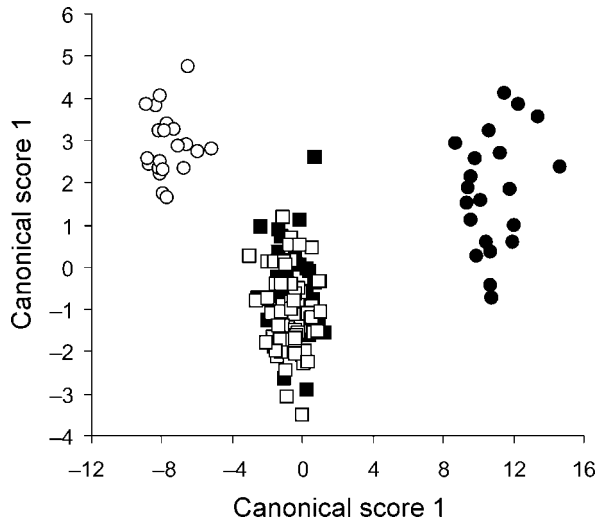


Figure 4. Species and F1 hybrids scores on the first two canonical axes from a canonical discriminant analysis of 24 relative warp factors from two parental species of mollies and F1 hybrids from both types of reciprocal cross. The relative warp factors with the highest correlations with each canonical axis are discussed in the text. Solid circles: *P. latipinna*, open circles: *P. mexicana*, solid squares: males from the *P. mexicana* dam  $\times$  *P. latipinna* sire crosses, open squares: males from the *P. latipinna* dam  $\times$  *P. mexicana* sire crosses.

The parental species of the sire did have a highly significant effect on the rates of courtship displays (Table 2). Hybrid males with *P. latipinna* sires performed on average, three times higher rates of courtship displays than did hybrid males with *P. mexicana* sires (Table 1). Rates of gonopodial thrusting and gonoporal nibbling were similar for the two types of hybrid males and not influenced significantly by the parental species of the sire (Table 2).

Since the ANCOVA found no significant effect of species of object female, the remaining analyses and results used only the behavioral rates of F1 hybrid males with *P. latipinna* sires in response to *P. latipinna* object females and F1 hybrid males with *P. mexicana* sires in response to *P. mexicana* object females. In all cases, behavioral rates reported for the two parental species are in response to conspecific females.

Males from the two parental species showed little overlap in courtship display rates and hybrid males strongly resembled their sires (Figure 5). In contrast, males from the two parental species showed considerable overlap in rates of gonopodial thrusts and rates of gonoporal nibbling (Figure 5). In similar fashion, hybrids from both directions of the cross, overlapped with each other and with the parental species for rates

of these two behaviors (Figure 5). An ANCOVA (with body size as a covariate) to test for the effect of male type (parental species or hybrid type) on each behavior rate revealed a highly significant effect of male type on rates of all three behaviors (Table 3). The results of Fisher's LSD mean separation method showed that F1 hybrids with *P. latipinna* sires did not differ significantly ( $P = 0.131$ ) from *P. latipinna* males for rates of courtship displays. There was also, no significant difference ( $P = 0.365$ ) between rates of courtship displays for F1 hybrid males with *P. mexicana* sires and males of *P. mexicana*. Multiple comparisons between members of these two groups were highly significant ( $P$ s range = 0.001–0.0001). Fisher's LSD also showed significant differences among male types for mean rates of gonopodial thrusting. For this behavior, both hybrid classes showed significantly lower rates of thrusting than did males of *P. latipinna* ( $P = 0.018$  with F1–*P. latipinna* and  $P = 0.003$  with F1–*P. mexicana*). The remaining comparisons were not significant ( $P$ s range = 0.210–0.635). Mean rates of gonoporal nibbling also showed significant effects of male type based on Fisher's LSD method. Males of *P. latipinna* performed significantly fewer nibbles than males of *P. mexicana* ( $P = 0.029$ ) or males of either hybrid type ( $P = 0.001$  with F1–*P. latipinna* and  $P = 0.0001$  with F1–*P. mexicana*). No other comparisons were significant ( $P$ s range = 0.762–0.935).

## Discussion

### *Mating signal divergence in sailfin and shortfin mollies*

Results of this study confirm the existence of a high degree of phenotypic divergence in morphological and behavioral traits associated with mating signals in the two parental species, the sailfin molly *P. latipinna* and the shortfin molly *P. mexicana*. The clearest morphological distinction between the two parental species is in the length of the dorsal fin (RW1, Figure 2) with males of the sailfin species having a dorsal fin that is more than twice as long and consisting of twice as many fin rays. Males of the two species overlap considerably in other morphological shape variables. These results are consistent with a previous study (Ptacek, 1998) that examined morphological shape distinctions between males of *P. latipinna* and males of two shortfin species, *P. mexicana*, and *P. orri*. This study also found length of the dorsal fin to be the most

Table 1. Means of male standard length and rates of three mating behaviors for parental species and two types of F1 hybrids<sup>a</sup>

Male species	Female species	N	Standard length (mm)			Displays		Thrusts		Nibbles	
			Mean	SE	Range	Mean	Slope	Mean	Slope	Mean	Slope
<i>P. latipinna</i>	<i>P. latipinna</i>	26	32.15	1.79	22–50	26.11	0.101	22.38	−3.283*	11.04	2.442
<i>P. mexicana</i>	<i>P. mexicana</i>	12	40.75	2.75	35–47	5.50	1.878	19.42	3.493	29.58	4.687
F1– <i>P. lat.</i>	<i>P. latipinna</i>	42	34.98	1.11	24–48	17.50	0.334	21.74	5.970*	30.48	3.474*
F1– <i>P. lat.</i>	<i>P. mexicana</i>	42	34.98	1.11	24–48	15.19	1.581	24.69	3.278	33.83	1.829
F1– <i>P. mex.</i>	<i>P. latipinna</i>	66	33.26	0.78	25–45	5.39	−0.686	24.11	6.693*	36.48	5.558*
F1– <i>P. mex.</i>	<i>P. mexicana</i>	66	33.26	0.78	25–45	6.14	−0.362	12.58	5.897*	24.79	4.171*

<sup>a</sup> Values for slopes of the regressions of ln behavior rate on ln standard length for each behavior for each type of male are included.

\* Indicates slopes significantly different from zero at  $P < 0.05$ .

Table 2. ANCOVAs for the effects of body size, species of sire, and species of object female on rates of three mating behaviors in the two types of F1 hybrids

Source	df	Displays			Thrusts			Nibbles		
		SS	F	P	SS	F	P	SS	F	P
Body size	1	0.127	0.062	0.803	249.089	38.441	0.0001	122.074	36.744	0.0001
Sire species	1	91.970	45.225	0.0001	1.617	0.250	0.618	0.001	0.000	0.984
Female species	1	1.037	0.510	0.476	4.051	0.625	0.430	0.363	0.109	0.741
Sire species × female species	1	1.273	0.626	0.430	0.169	0.026	0.872	0.297	0.900	0.765
Error	211	2.034			1367.218			701.003		

Table 3. ANCOVAs for the effects of body size and male type on rates of three mating behaviors in parental species and two types of F1 hybrids

Source	df	Displays			Thrusts			Nibbles		
		SS	F	P	SS	F	P	SS	F	P
Body size	1	0.003	0.002	0.966	57.644	10.890	0.001	70.207	21.133	0.0001
Male type	3	100.347	21.337	0.0001	53.054	3.341	0.021	50.647	5.077	0.002
Error	141	221.041			746.349			468.867		

important trait in distinguishing sailfin males from males of either shortfin species. Indeed, height of the dorsal fin did not distinguish sailfins from shortfins (see Figure 7 in Ptacek, 1998).

Clearly the species distinctions in dorsal fin shape contribute to the interspecific divergence observed in courtship display rates. While males of *P. mexicana* do perform a modified type of courtship display (Ptacek, 1998; this study), males of *P. latipinna* rely on courtship displays as a predominant mating tactic and perform them on average at five times higher rates than do males of *P. mexicana* (Table 1, Figure 5). Females of *P. mexicana* have been observed to cooperate during copulation (Balsano et al., 1985), however the degree

to which males rely on female cooperation to enhance mating success in this species is unknown.

Males of the two parental species showed considerable overlap in rates of the two mating behaviors not associated with female cooperation, gonopodial thrusting and gonopodial nibbling (Figure 5). Gonopodial thrusting is an attempt at forced insemination without female cooperation and males of all sizes of both species perform this behavior. This study found a significant negative relationship between rates of gonopodial thrusting and male body size for males of *P. latipinna*. Such a negative relationship has been reported in prior studies for this population of *P. latipinna* males and for several other populations as

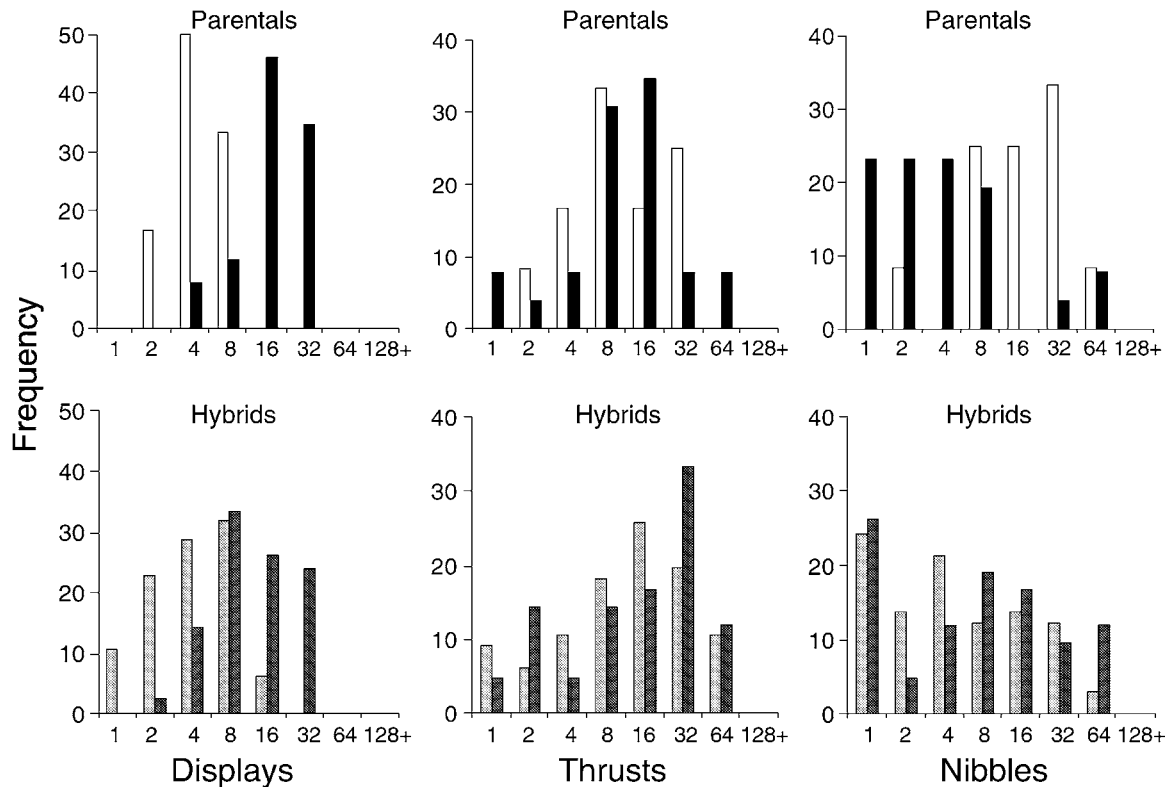


Figure 5. Distribution of courtship display rates, gonopodial thrust rates, and gonoporal nibbling rates for males from two parental species of mollies (top panels) and F1 hybrids from both types of reciprocal cross (bottom panels). Black solid bars: *P. latipinna*, white solid bars: *P. mexicana*, black stippled bars: males from the *P. mexicana* dam  $\times$  *P. latipinna* sire crosses, white stippled bars: males from the *P. latipinna* dam  $\times$  *P. mexicana* sire crosses.

well (Farr, Travis & Trexler, 1986; Ptacek & Travis, 1996). In most populations, small males of *P. latipinna* rely more heavily on gonopodial thrusting while large males perform higher rates of courtship displays (Farr, Travis & Trexler, 1986; Ptacek & Travis, 1996). Such differences in size-associated rates of gonopodial thrusting and courtship display are likely mediated through strong female preferences for large males (Ptacek & Travis, 1997; Gabor, 1999). No such size-association was observed for males of *P. mexicana*, a species where males of all sizes rely on gonopodial thrusting as a primary mating strategy (Balsano et al., 1985; Woodhead & Armstrong, 1985; Parzefall, 1989; Ptacek, 1998).

Gonoporal nibbling is a behavior in which males make nasal or oral contact with the female's gonopore, sometimes nipping at the female. Its complete function is unclear, but it appears to aid a male in determining a female's reproductive condition (Farr & Travis, 1986; Travis & Woodward, 1989). Males of *P. mexicana* in this study performed gonoporal nibbles

at nearly three times the rate of males of *P. latipinna* (Table 1, Figure 5). While the exact reason for this difference is unknown, the increased reliance of males of *P. latipinna* on courtship as an indicator of female receptivity may play a role in contributing to the species differences observed in gonoporal nibbling rates. Alternatively, there may be differences in the degree to which females of these two species signal their receptivity to males (Parzefall, 1973; Sumner, Travis & Wilson, 1994).

#### *Patterns of inheritance of male morphological and behavioral traits in F1 hybrids*

The first generation hybrid males resulting from the interspecific crosses between *P. latipinna* and *P. mexicana* were clearly intermediate in morphology to the parental species (Figure 4). There was little evidence for distinctions based upon morphological shape between the two types of F1 hybrid males, those with *P. latipinna* sires versus those with *P. mexicana* sires.

This result suggests no strong effect of autosomal dominance by a parental gene from either parental species or an influence of parental species of the sire through Y-linkage on the inheritance of morphological shape. Male F1 hybrids had dorsal fins of intermediate-length and an intermediate number of dorsal fin rays with respect to the two parental species. This pattern of trait distribution in F1 hybrids is expected under single locus, codominance or that of polygenic control. Unfortunately my breeding design did not extend beyond the F1 generation so I am unable to distinguish between these two genetic hypotheses for the inheritance of dorsal fin length and fin ray number.

Another study of interspecific hybridization between a sailfin and shortfin species of molly also found a similar pattern of phenotypic distribution for dorsal fin ray number in F1 hybrids (Parzefall, 1989). Parzefall (1989) performed interspecific crosses between a sailfin species, *P. velifera* and the shortfin species, *P. mexicana*. Males of these F1 hybrids had an intermediate number of fin rays (12–15) as compared to the parental species (10–11 for *P. mexicana* and 17–21 for *P. velifera*). Variability in the F2 generation increased and the backcrosses to each parental species were clearly separated. Based upon these observations and results from a scaling test (Broadhurst & Jinks, 1961; Mathers & Jinks, 1971), Parzefall (1989) concluded that the number of rays in the dorsal fin is controlled by an additive polygenic system. Results of my study show a qualitatively similar result with respect to the pattern of inheritance of dorsal fin ray number in F1 hybrids.

The pattern of inheritance of courtship display rate was distinctly different than that seen for dorsal fin morphology with F1 hybrid males showing a clear distinction with respect to the direction of the interspecific cross. Male hybrids whose sires were *P. latipinna* had courtship display rates that were up to three times higher than the rates of displays performed by hybrid males whose fathers were *P. mexicana* (Table 1). Even more striking was the degree of resemblance of a hybrid male's courtship display rate to that of the parental species of his sire. The distribution of phenotypes between a parental species and that of the hybrid males sired by that parental species was nearly identical (Figure 5). Such a pattern suggests that Y-linkage influences the inheritance of courtship display rates in mollies.

The developmental pathways that lead from a specific genetic condition to a particular behavior pattern

are poorly known. Many behaviors are polygenically inherited (Franck, 1974; Noakes, 1986; Parzefall, 1989; Zimmerer & Kallman, 1989; Shaw, 1996), and it is unlikely that a single gene controls courtship display behavior in mollies. However, the strong resemblance of courtship display rates in F1 hybrids to their parental species' sires and the significant sire effect on variation in display rates between the two types of hybrid crosses strongly argues that a Y-linked gene or genes may interact with autosomal genes that control the expression of courtship display behavior.

The existence of Y-linked genes, which control the expression of sexually-selected male characters, is not uncommon in poeciliids (reviewed by Lindholm & Breden, in press). A pattern of Y-linkage is known to influence the inheritance of male size at maturity in several poeciliids (swordtails: *Xiphophorus* sp., Kallman, 1984, 1989; sailfin mollies: *P. latipinna*, Travis, 1994). In several species of *Xiphophorus*, size at maturity is controlled by a Y-linked multiple-allelic series at the *P* (pituitary) locus, which controls the onset of sexual maturity (Kallman, 1984, 1989). Males with small body-size *P* alleles mature much sooner (weeks to months) than males with large body-size *P* alleles. An influence of linked, additional Y-chromosome gene loci, with alleles at the *P* locus has been suggested for the inheritance of alternative mating behaviors, including courtship displays, in *X. nigrensis* (Zimmerer & Kallman, 1989). The frequency of various components of the male courtship behavior of the Trinidad guppy, *P. reticulata* is also inherited as a Y-linked trait and variation among crosses in display rates has been attributed to autosomal factors that modify the primary influence of Y-linked genes (Farr, 1983). Parzefall (1989) found a similar influence of the species of the sire on the inheritance of courtship behaviors in hybrids and backcrosses between the sailfin molly *P. velifera* and the shortfin molly *P. mexicana*. Hybrid males from the F1 generation strongly resembled the parental species of their sires in the number of sexual displays performed. Thus mollies appear to show a similar pattern of Y-linked influence on the inheritance of courtship display behavior as that reported for other poeciliids.

Interestingly, I did not observe a strong influence of the size of a sire on the size at maturity of his F1 sons. Sons ranged in size at maturity from 22 to 50 mm while their sires ranged from 35 to 55 mm. Size at maturity in *P. latipinna* is known to be strongly Y-linked (Travis, 1994). However, the lack of a strong paternal effect on size at maturity in this study may

be due to the fact that the sons were interspecific hybrids. Hybrids from several types of interspecific crosses between species of *Xiphophorus* have shown significant changes in maturation schedules, including both delayed maturation and precocious maturity, compared to the parental species (reviewed by Bao & Kallman, 1982; Kallman, 1989).

While I did not observe evidence for delayed maturity in my hybrids (all juveniles from each cross matured in less than 1 year), I did observe a high degree of precocious maturation. As many as half of the male hybrid offspring within a family matured at sizes less than 28 mm despite their sires' sizes, which ranged from 35 to 55 mm. These males matured in as little as 3 weeks, while their male sibs that matured at around 45 mm, took up to 4 months to reach maturity. Hybrid males that showed precocious maturation occurred with equal frequency in families from both directions of the cross. Parzefall (1989) also reported the occurrence of such 'dwarf' males among hybrid males from his interspecific crosses between *P. velifera* and *P. mexicana*. Thus placing the Y-linked *P* locus in a heterospecific autosomal background may influence its control of the timing of male maturation.

Despite the occurrence of these precocious male hybrids, their small size did not appear to influence their rates of courtship displays. Small males displayed at similar rates to their larger brothers within a family and there was no significant effect of male body size on display rates for hybrid males from either direction of the interspecific cross. Thus the Y-linked influence on the inheritance of courtship display rate appeared to be independent of Y-linked expression of male size at maturity in these F1 hybrid male offspring.

## Conclusions

A growing body of evidence suggests that sexual selection by female choice can have a direct genetic effect on alleles determining the expression of male traits (see Brooks, this volume). Behavioral divergence of closely related species is common and studies are beginning to show that behavioral differences that confer species isolation have a genetic basis. The divergence of sailfin mollies from shortfin ancestors is a clear case where behavioral isolation mediated by strong female mating preferences has played an important role in potentially rapid speciation. The pattern of inheritance of courtship display behavior revealed

in the F1 hybrid males used in this study, suggests that a change in Y-linked alleles may have contributed to the divergence of sailfin mollies through behavioral premating isolation. Indeed an association between Y-linked *P* alleles for large male size and the expression of courtship display behavior has been described for a related poeciliid, *X. nigrensis* (Ryan & Causey, 1989; Zimmerer & Kallman, 1989). Strong female choice for large, courting males has altered the distribution of *P* alleles in certain populations of this species (Ryan, Hews & Wagner, 1990). Such a *P* allele mutation, that changed the size-courtship display rate association within a molly population, would have rapidly increased in frequency through strong female preferences for large, courting males (Ptacek & Travis, 1997; Ptacek 1998; Gabor, 1999). Future studies should focus on determining the degree of genetic correlation that exists between alleles at the *P* locus and autosomal genes, which influence the expression of courtship display behavior. Understanding the genetic basis of traits that confer premating isolation in mollies will provide important insights into how sexual selection can result in speciation and expand our knowledge of the genetics of species differences to include vertebrate systems.

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