Mating Signal Divergence, Sexual Selection, and Species Recognition in Mollies (Poeciliidae: *Poecilia*: *Mollienesia*)

Margaret B. Ptacek

Department of Biological Sciences, Clemson University,
Clemson, South Carolina, USA

Mating Signal Divergence, Sexual Selection, and Species Recognition in Mollies (Poeciliidae: *Poecilia*: *Mollienesia*)

Margaret B. Ptacek

Abstract

Mating preferences for particular features of mating signals can have a pronounced effect on the divergence of these traits both at the intra- and interspecific level. Divergent female mating preferences have led to reproductive isolation and speciation. This implies that traits that are the targets of sexual selection also serve as reliable species recognition signals. Mollies (Poeciliidae: *Poecilia*: *Mollienesia*) provide an excellent model system in which to examine the role of female mating preferences in shaping both intra- and interspecific divergence in male mating signals because of the enormous diversity that exists both within and between species of mollies in male mating behaviors and associated morphological traits. Sailfin mollies differ from shortfin mollies both behaviorally and morphologically. Sailfin species show a sexual dimorphism in which males have a greatly enlarged dorsal fin erected and presented to the female in a courtship display behavior. Most shortfin species show neither sexual dimorphism in fin morphology nor perform courtship display behaviors. Phylogenetic analyses show that sailfin mollies form a monophyletic group diverging from a shortfin ancestor. Changes in size and shape of the dorsal fin and a switch to courtship display behavior occurred during speciation of sailfin mollies. Females of a single sailfin species, *P. latipinna* prefer males from native populations to those from foreign populations whose males show divergent values of dorsal fin shape and courtship display rates. Studies of female mating preferences have compared patterns of female choice using heterospecific males and F1 hybrid males that show similar, intermediate dorsal fin morphology yet vary considerably in courtship display rates due to Y-linked effects on this behavior. Results suggest that females rely on differences in dorsal fin shape and courtship display rate when distinguishing between conspecific, heterospecific and hybrid males. Females from shortfin species also prefer sailfin males to heterospecific, shortfin males suggesting that the preference for the "sailfin male phenotype" may be a pre-existing bias. Thus female mating preferences in mollies are based upon the same components of the mating signal both within and between species, which argues for an important role of sexual selection in promoting the initial speciation of sailfin mollies from shortfin ancestors.

Resumen

En las señales de apareo, las preferencias por características particulares pueden tener un efecto pronunciado en las divergencias de estos rasgos, tanto en nivel interespecífico como intraespecífico. La divergencia de las preferencias sexuales de las hembras, ha llevado a su especiación y aislamiento. Esto implica que los rasgos blanco de la selección sexual también son señales confiables en el reconocimiento de las especies. Los mollies (Poeciliidae: *Poecilia: Mollienesia*) son un excelente modelo en el estudio del papel de las preferencias sexuales de hembras en la formación de divergencias intraespecíficas e interespecíficas sobre las señales de apareo en el macho, debido a la enorme diversidad que existe dentro y entre las especies de mollies en cuanto al comportamiento del macho en el apareo y sus rasgos morfológicos asociados. Los mollies "aleta de velero" (*sailfin*) difieren de los mollies "aleta corta" (especies *shortfin*) en comportamiento y morfología. Las especies sailfin se caracterizan por un dimorfismo sexual en el cual los machos tienen una aleta dorsal muy alargada, erguida y presentada ante la hembra durante el cortejo. La mayoría de las especies *shortfin* no presentan dimorfismo sexual en las aletas ni realizan exhibiciones durante el cortejo. Análisis filogenéticos muestran que los mollies *sailfin* forman un grupo monofilético que se bifurca de su ancestro, el mollie *shortfin*. Cambios en el tamaño y forma de la aleta dorsal, así como en los comportamientos de exhibición durante el cortejo, ocurrieron durante la especiación de los mollies *sailfin*. Las hembras de una única especie de mollies *sailfin*, *P. latipinna*, prefieren machos de poblaciones nativas, en relación a machos de poblaciones extranjeras, los cuales presentan valores divergentes en la forma de la aleta dorsal y ritmos en la exhibición durante el cortejo. Estudios relacionados con preferencias sexuales de hembras han comparado patrones de selección usando machos heteroespecíficos y machos híbridos F1, los cuales presentan morfología similar en la aleta dorsal intermedia pero varían considerablemente en los ritmos de exhibición en el cortejo debido a efectos "*Y-linked*" en este comportamiento. Los resultados sugieren que las hembras se basan en las diferencias en la morfología de la aleta dorsal y en los ritmos de exhibición y cortejo para distinguir entre machos híbridos, conespecíficos y heteroespecíficos. Las hembras de las especies *shortfin* también prefieren los machos de las especies *sailfin* entre los machos heteroespecíficos. Esto sugiere que la preferencia para el "fenotipo del macho *sailfin*" puede ser una predisposición preexistente. Por lo tanto, las preferencias sexuales de las hembras mollies están basadas en los mismos componentes que las señales de apareo, dentro y entre las especies, lo cual sugiere la importancia que la selección sexual juega en promover la especiación inicial de los mollies *sailfin* de sus ancestros, los mollies *shortfin*.

• *Viviparous Fishes*
Harry J. Grier and Mari Carmen Uribe, book editors.
New Life Publications, Homestead, Florida, 2005. p 73-89.

Introduction

Evolutionary biologists have long recognized the importance of sexual selection in shaping the evolution of mating signals within species (Andersson, 1994). Yet the role that sexual selection plays in promoting divergence among species in mating signals and its potential importance in the speciation process has only recently begun to be explored (Ptacek, 2000; Panhuis *et al.,* 2001). A number of authors have argued that sexual selection and species recognition represent a continuum in animal communication (Verrell, 1988; Ryan and Rand, 1993; Endler and Houde, 1995; Boake *et al.,* 1997; Littlejohn, 1999; Boake, 2000), which implies that the same forces of evolution that influence signal properties used in species recognition also influence features of the signal that make the signaler a more attractive mate.

These signal features are not mutually exclusive and thus, mating preferences for certain features of a mating signal can simultaneously promote divergence at both the intra- and interspecific levels (Ptacek, 2000). For example, divergence in mate recognition signals among conspecific populations can lead ultimately to reproductive isolation and speciation (Lande, 1981, 1982; Kirkpatrick, 1982; Thornhill and Alcock, 1983; West-Eberhard, 1983, 1984; Kaneshiro and Boake, 1987; Lande and Kirkpatrick, 1988; Kirkpatrick and Ryan, 1991; Schluter and Price, 1993; Iwasa and Pomiankowski, 1995; Payne and Krakauer, 1997; Verrell, 1999; Uy and Borgia, 2000).

Mating signals are often complex traits where different components of the signal provide information to the receiver concerning species identity, gender, readiness to mate, individual identity and even mate quality (*e.g.,* Crapon de Caprona and Ryan, 1990; Barlow, 1992; Rand *et al.,* 1992; Gerhardt, 1994; McLennan and Ryan, 1997, 1999). Determining the message and meaning of a mating signal and how mating preferences have shaped various components of the signal is the first step in making a link between mating preferences and species recognition. In order to demonstrate that female mating preferences can shape interspecific divergence in mating signals, studies must ask whether the same male traits that are preferred by females within a species also function to distinguish conspecific from heterospecific males. This implies that male traits that confer high mating success also confer a high degree of sexual incompatibility. Only a few studies to date have directly tested this hypothesis (Wiernasz, 1989; Wiernasz and Kingsolver, 1992; Boake *et al.,* 1997).

Poeciliid fishes in general, and mollies (subgenus *Mollienesia*) in particular, have provided ideal model systems in which to examine the role of sexual selection in generating the enormous divergence observed in male mating signals at both the intra- and interspecific levels. Mollies have fascinated biologists for decades because of their diversity in habitat, body size, male secondary sex characters and mating behaviors. Mollies are an ideal group in which to examine the role of sexual selection in generating interspecific patterns of phenotypic diversity; differences in the two major species complexes (sailfin and shortfin species) are found primarily in males and are strongly associated with divergence in their mating systems. Males from the two species complexes differ dramatically both morphologically and behaviorally. Sailfin species are characterized by a sexual dimorphism in which males have 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 *et al.,* 1986). Most shortfin species show neither sexual dimorphism in fin morphology nor perform courtship display behaviors (Parzefall, 1969, 1979; Brett and Grosse, 1982; Balsano *et al.,* 1985; Woodhead and Armstrong, 1985; Ptacek, 1998). Thus, divergence in morphology and behavior associated with mating system differences between these two species complexes implies an important role for sexual selection in promoting premating reproductive isolation.

In this review I explore mating signal divergence in mollies at both the intra- and interspecific levels and the role that female mating preferences have played in shaping this divergence. I draw examples mostly from my own studies on population variation in male morphology and mating behaviors and patterns of female mating preferences for these traits in the sailfin molly, *P. latipinna*, a species native to the coastal marshes of the southeastern United States and northeastern Mexico. I further examine whether patterns of female mating preferences within a species mirror those between sailfin and shortfin species. Lastly, I present evidence from interspecific hybrid studies that explores the relative importance of male dorsal fin morphology and courtship display behavior as the targets of sexual selection and species recognition in mollies.

Characteristics of *Mollienesia*

The *Mollienesia* group (*sensu* Rauchenberger, 1989) consists of at least 15 described species

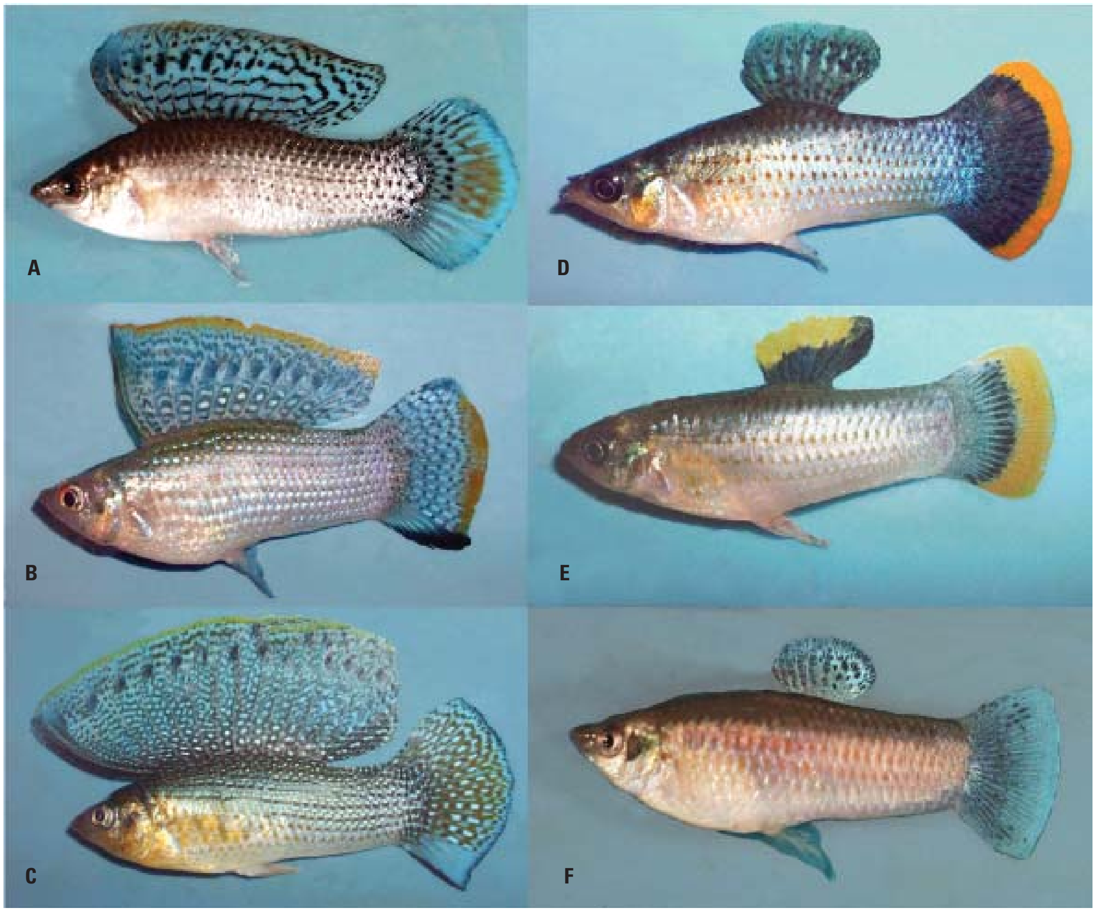


Figure 1.
Photographs shown are representative examples of members of *Mollienesia*. Photographs in the left panels show species in the *P. latipinna* species complex: A) *P. latipinna*, B) *P. petenensis*, C) *P. velifera*. Photographs in the right panels show species in the *P. sphenops* species complex: D) *P. mexicana mexicana*, E) *P. mexicana limantouri*, F) *P. sphenops*

distributed throughout southern North America and Central America (see distribution map in Ptacek and Breden, 1998). There are two readily distinguishable species complexes: the sailfin mollies of the *P. latipinna* complex and the shortfin mollies of the *P. sphenops* complex (Fig. 1; Hubbs, 1933). The sailfin group is confined to the Atlantic slope ranging from the coastal southern US, south through Mexico and into northern Guatemala and Belize. The four, sailfin species are mostly allopatric with respect to one another, although sympatric populations of *P. velifera* and *P. petenensis* have been observed in north central Campeche, Mexico (E. Harrison and M. Ptacek, unpubl. data). Each sailfin species is sympatric with one or more shortfin species throughout much of their distribution. The shortfin group, which occupies both Atlantic and Pacific slopes from the Rio Grande through Central America, consists of at least 11 taxonomically described species.

The difference in size of the dorsal fin between sailfin and shortfin species is dramatic; dorsal fin area differs by 2-50 fold between the groups (Fig. 1, unpubl. data). The two species complexes also have striking differences in mating systems (Table 1; Farr, 1989). All sailfin species perform a courtship display in which the dorsal fin of the male is erected and presented to the female (Parzefall, 1969, 1979; Luckner, 1979; Farr *et al.,* 1986; Niemeitz *et al.,* 2002) in an attempt to elicit female cooperation in mating. Courtship behaviors have not been observed for most shortfin species; instead males attempt to forcefully insert the tip of the gonopodium into the female's gonopore in a behavior called gonopodial thrusting. Many shortfin species rely on a dominance hierarchy system wherein large, dominant males aggressively prevent smaller males from approaching females (Miller, 1975; Parzefall, 1979).

Many pairs of the *Mollienesia* are interfertile in the laboratory, but few examples of hybrids are found in nature (Hubbs, 1933, 1936; Meyer *et al.,* 1985). The only known naturally occurring hybrid species is the all-female gynogenetic species *P. formosa*, which has arisen through a hybridization of *P. mexicana* with *P. latipinna* (Avise *et al.,* 1991; Schartl *et al.,* 1995). Despite the occurrence of many sympatric populations of shortfin and sailfin species throughout the distribution of *Mollienesia*, no other naturally occurring hybrid populations resulting from crosses between sailfin and shortfin species have been reported. This suggests that premating isolating mechanisms are important in preventing interspecific hybridization in sympatric populations of sailfins and shortfins. Indeed male mollies of both *P. latipinna* and *P. mexicana* strongly discriminate against both heterospecific females and females of *P. formosa* where the three species co-occur (Schlupp *et al.,* 1991; Ryan *et al.,* 1996). Females from an allopatric population of *P. latipinna* strongly discriminated against males of both *P. mexicana* and *P. orri* in laboratory dichotomous choice tests (Ptacek, 1998). These patterns of mating preferences suggest that divergence in mate recognition systems may have played an important role during speciation of mollies.

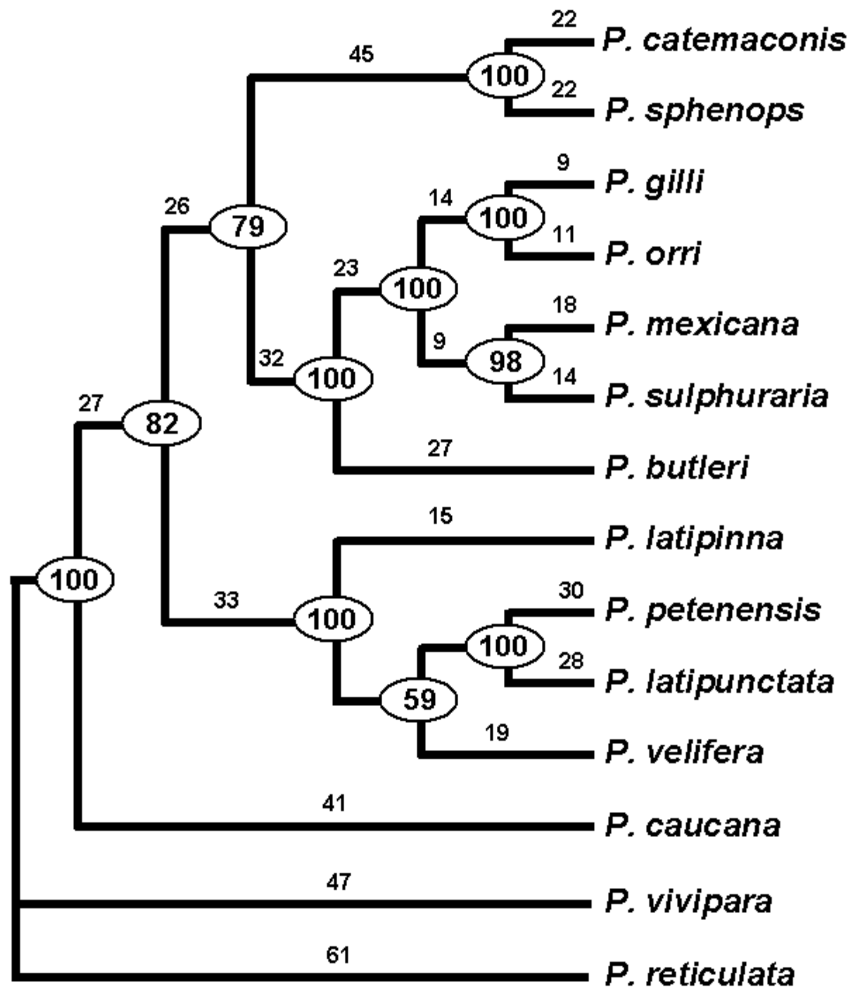


Figure 2.
Single most parsimonious tree based upon analysis of the combined D-loop/ND2 data set for the 14 taxa examined. Tree length = 686; Consistency index = 0.531; Homoplasy index = 0.469; Retention index = 0.593. Bootstrap support from 1000 iterations is given in the circles at the nodes. Numbers on branches are branch lengths measured as number of nucleotide substitutions. (From Ptacek and Breden, 1998)

Phylogenetic Relationships among Members of *Mollienesia*

A molecular phylogeny for 11 of the 15 species of *Mollienesia* has been done, based upon nucle-

Table 1.
Secondary sexual differentiation among species* in the subgenus *Mollienesia*

Species	Sexual Dimorphism in: Color	Sexual Dimorphism in: Dorsal Fin Size	Presence of Courtship Displays	References
P. sphenops complex:				
P. butleri	No	No	Unknown	Farr 1989
P. catemaconis	Yes	No	Unknown	Miller 1975
P. chica	Yes	No	No	Miller 1975; Brett & Grosse 1982
P. gilli	No	No	Unknown	Farr 1989
P. maylandai	No	No	Unknown	Farr 1989
P. mexicana	Yes	No	Yes, but at low rates	Woodhead & Armstrong 1985; Balsano *et al.* 1985; Ptacek 1998
P. orri	Yes	No	No	Farr 1989; Ptacek 1998
P. sphenops	No	No	No	Parzefall 1969, 1979; Farr 1989
P. sulphuraria	No	No	Unknown	Farr 1989
P. teresea	No	No	Unknown	Greenfield 1990
P. latipinna complex:				
P. latipinna	Yes	Yes	Yes	Luckner 1979; Farr *et al.* 1986, Ptacek & Travis 1996
P. latipunctata	No	No	Yes	Niemeitz *et al.* 2002
P. petenensis	Yes	Yes	Yes	Parzefall 1969; Farr 1989
P. velifera	Yes	Yes	Yes	Parzefall 1989; Farr 1989

**P. formosa* is not included because it is an all-female gynogenetic species (Turner, 1982).

otide sequence data from the mitochondrial genome, 1047 base pairs (bp) of the entire *ND2* gene and 500 bp of the noncoding control region (Ptacek and Breden, 1998). The Trinidad guppy, *P. reticulata*, was used as the outgroup taxon. The single most parsimonious tree is presented (Fig. 2) showing support for monophyletic groups from 1000 bootstrap iterations. Neighbor-joining and maximum likelihood analyses recovered similar topologies (Ptacek and Breden, 1998).

The phylogeny is consistent with monophyly of *Mollienesia* with respect to the position of two South American mollies, *P. caucana* and *P. vivipara*, outside of the *Mollienesia* clade. *Mollienesia* consists of two well-supported clades. The first of these contains seven of the eight previously classified shortfin species (Rauchenberger, 1989). The second clade of *Mollienesia* contains the three, sailfin species of the *P. latipinna* species complex and one member of the *P. sphenops*, shortfin species complex, *P. latipunctata* (Rauchenberger, 1989).

While the inclusion of *P. latipunctata* as part of a monophyletic sailfin clade is surprising, it has now been supported by two separate phylogenetic studies based on both nuclear DNA (Schartl *et al.,* 1995) and mtDNA (Ptacek and Breden, 1998). From a morphological standpoint, *P. latipunctata* is clearly a shortfin, however, recent behavioral observations have shown that males of *P. latipunctata* perform courtship displays much like those of other sailfin species (Niemeitz *et al.,* 2002; personal observation). This observation argues for the importance of behavioral as well as morphological trait divergence during speciation of sailfin mollies.

Sexual Selection and Phenotypic Divergence in Mating Signals among Populations of the Sailfin Molly *P. latipinna*

The sailfin molly, *P. latipinna*, is remarkable with respect to the extensive variation that exists among males in body size and several size-associated traits, most notably courtship display rates and dorsal fin shape (Farr *et al.,* 1986; Travis and Trexler, 1987; Travis, 1989; Ptacek and Travis, 1996). Variation among populations in these male traits is most likely the result of the interplay between natural selection and sexual selection that may or may not select for the same overall male size within a population (Trexler *et al.,* 1990, 1992, 1994; Travis, 1994; Ptacek and Travis, 1997).

Males from different populations vary over threefold in size-specific rates of courtship displays and gonopodial thrusts (Ptacek and Travis, 1996). Similar variation exists in morphological traits used in the courtship display, *i.e.,* "sexual characters," such as relative length and height of the dorsal fin and relative length of the gonopodium. For example, males of the same size from different populations can vary nearly twofold in dorsal fin length and height (range of means = 8.7-14.2 mm for length, 2.8-9.9 mm for height). Morphological traits that are not involved with courtship displays, *i.e.,* "morphometric characters," such as caudal fin height and mid-body depth, do not show nearly this level of variation among populations (range of means = 6.4-9.6 mm for caudal fin height, 9.0-12.4 mm for mid-body depth). In particular, in populations where males are on average small in size at maturity, size of the dorsal fin is larger than expected based upon the allometric relationship with male size (Travis and Ptacek, unpubl. data; Fig. 3). The lack of an association between increased male size at maturity and relative dorsal fin size suggests that sexual selection through female choice for enhanced apparent body size appears to drive the evolution of dorsal fin morphology, to some degree, independently of body size. A similar pattern of female mating preferences for increased apparent size has been found for females of *P. latipinna* using models of male *P. latipinna* that varied in dorsal fin size and body size (MacLaren *et al.,* 2004) and for females of *Xiphophorus helleri* (Rosenthal and Evans, 1998).

In addition, while a strong positive association exists between male body size and courtship display rates within many populations (Farr *et al.,* 1986; Ptacek and Travis, 1996), little relationship exists among populations (Fig. 3). Again, this suggests that female mating preferences can drive divergence among populations in courtship display rates to some extent independently of either male size or dorsal fin size.

For sexual selection to have generated these phenotypic distinctions among sailfin molly populations, females in different populations must exhibit divergent preferences. Evidence exists for just such a pattern of divergence among populations in female preferences (Ptacek and Travis, 1997). Females of *P. latipinna* prefer males from their own population when those males are matched for body size with males from another population; however, they do not do so against all alternatives (Ptacek and Travis, 1997). In most populations, females were best able to discriminate between native and foreign males that did not overlap in either courtship display rate or dorsal fin morphology (Fig. 3) and less able to discriminate when either of these traits showed considerable overlap (Ptacek and Travis, 1997).

These results argue that females use population-specific values of dorsal fin shape or rates of courtship displays to distinguish males from native populations. Thus intraspecific, interpopulation variation in female choice is based on the same phenotypic trait distinctions that separate the sailfin and shortfin species (Ptacek, 1998). The observed pattern of population differentiation within *P. latipinna* argues that divergence among conspecific populations in male traits associated with mate recognition could have led to the observed divergence of mating signals between sailfin and shortfin mollies.

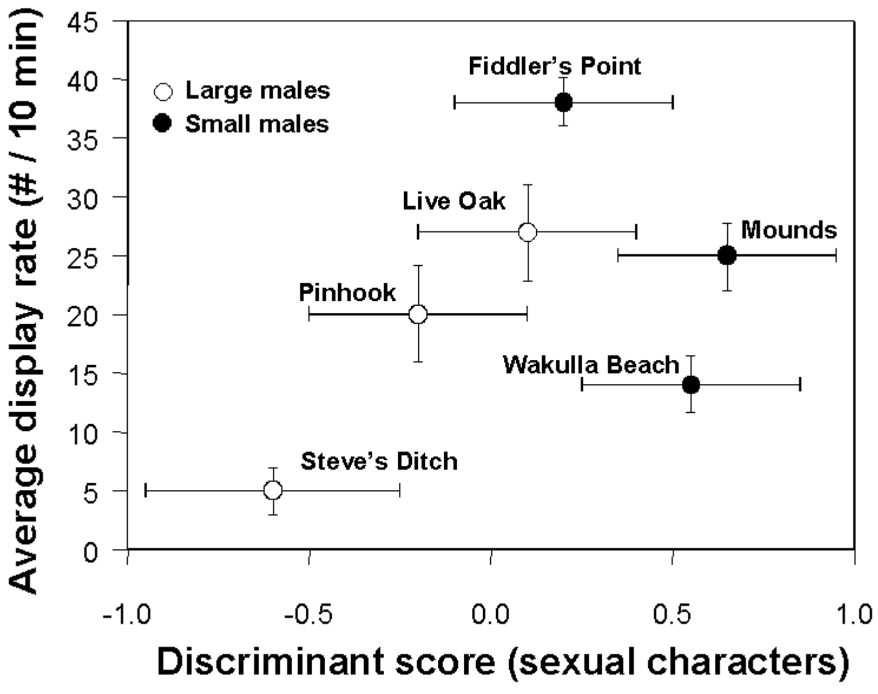


Figure 3.
Relationship between average courtship display rate (number/10 min. ± SE) and discriminant function score (±95% confidence intervals) for "sexual" morphological characters for males from six populations of *P. latipinna.* See text for definition of "sexual" morphological characters

The Role of Sexual Selection in Interspecific Mating Signal Divergence in Mollies

Several lines of evidence implicate an important role for sexual selection in the divergence between shortfin and sailfin groups. First and foremost is the difference in relative fin size (fin size adjusted for body size) and the marked distinction in mating behaviors; sailfin mollies show courtship behaviors and some level of female choice. Males of most species of sailfin mollies are easily distinguishable from shortfin males on the basis of differences in the length of the dorsal fin (Ptacek, 1998, 2002). Indeed the number of fin rays in the dorsal fin is the diagnostic character used to distinguish members of the sailfin molly complex from those of the shortfin molly complex (Miller, 1983). Males of most shortfin species have 6-11 dorsal fin rays, while males of *P. latipinna* range from 12-16, males of *P. petenensis* range from 12-16 and males of *P. velifera* range from 15-21 dorsal fin rays. Dorsal fin length rather than height appears to be the morphological trait that best separates sailfin species from shortfin species (Ptacek, 1998), with *P. latipunctata* being the sole exception. Males of *P. latipunctata* from a single population in Tamaulipas, Mexico, have 10-11 dorsal fin rays, despite considerable variation in male size at maturity (range from 23 to 45 mm SL) in this population. Thus the dorsal fin morphology of *P. latipunctata* is clearly that of a shortfin species.

Males of all four species of sailfin mollies however, perform courtship displays to females in an attempt to elicit female cooperation in mating (Table 1; Farr, 1989; Parzefall, 1989; Niemeitz *et al.,* 2002). Both the form of the display and the rate of courtship displays performed vary between sailfin and shortfin species. Males of sailfin species orient their bodies in front of females and raise the dorsal fin, spreading the fin rays and fanning the dorsal fin towards the female. Males often bend their bodies towards the female in a sigmoid fashion characteristic of guppies and other poeciliids (Liley, 1966; Farr, 1975, 1989). Displays can last from a few seconds to up to a minute in duration. Males of *P. velifera* have longer duration displays than those of *P. latipinna* and *P. petenensis* and males often swim in a circle around the female almost enclosing the female in the arc formed by the erect dorsal fin. Males of *P. petenensis* perform courtship displays that are more similar to those of *P. latipinna*, having a shorter duration and often directed along the side of the female. Males of *P. latipunctata* swim in semi-circles around the front of the female while spreading and fanning the dorsal fin (Niemeitz *et al.,* 2002). Such displays generally end with the male approaching the female's gonopore from the side and performing gonopodial thrusts (personal observation). Interestingly, courtship behavior in *P. latipunctata* appears to be strongly influenced by male-male competition with larger, more dominant males becoming extremely dark in coloration and performing higher rates of courtship displays than normally-colored, subordinate males. The darker coloration and higher rates of courtship displays performed by large, dominant males of *P. latipunctata* has been observed both in the field (Niemeitz *et al.,* 2002) and in the laboratory (personal observation). The rates of courtship displays performed by courting males of all sailfin species can be quite high, with males of *P. latipinna* having been observed to perform as many as 8-10 displays per minute, although the average is 2-4 displays per minute (Ptacek and Travis, 1996; personal observation).

In marked contrast, males of most species of shortfin mollies have not been observed to perform courtship displays (Table 1; Farr, 1989). A form of courtship display has been described for *P. mexicana*. Males of *P. mexicana* swim alongside females with their dorsal fin erect and their pelvic fins extended away from the body. Males will shimmy their bodies and bend them towards females in a sigmoid fashion. However, the rates at which they perform these courtship behaviors are nearly five times lower than the rates of courtship displays performed by males of *P. latipinna* (Ptacek, 1998, 2002). While females of *P. mexicana* have been observed to cooperate with males during copulation attempts (Balsano *et al.,* 1985), the degree to which males of *P. mexicana* rely on courtship to enhance reproductive success is unknown.

In order for sexual selection to have played an important role in divergence of sailfin mollies from shortfin mollies, females should exhibit strong preferences for the sailfin male phenotype over the shortfin male phenotype. Female mollies prefer large, courting males and this is particularly true for females of the sailfin molly, *P. latipinna* (Schlupp *et al.,* 1994; Ptacek and Travis, 1997; Gabor, 1999). Interspecific patterns of female choice have also been examined for three species of mollies: the sailfin species *P. latipinna* and two shortfin species, *P. orri* and

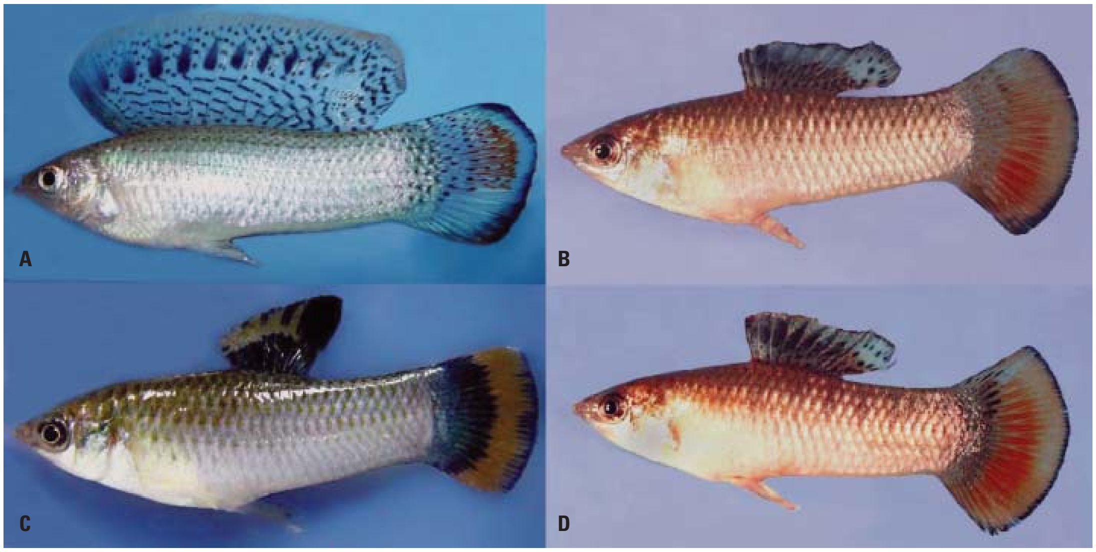


Figure 4.
Photographs showing parental species used as sires in the reciprocal crosses and their F1 hybrid sons: A) *P. latipinna*; B) F1 hybrid son with a *P. latipinna* sire; C) *P. mexicana*; D) F1 hybrid son with a *P. mexicana* sire

P. mexicana (Ptacek, 1998). Females of the sailfin species *P. latipinna* consistently preferred conspecific males across all treatment combinations containing conspecifics. This result implies that female choice by sailfin species helps promote premating isolation between sailfin and shortfin species in sympatry. Females of the two shortfin species had lower levels of female preference and spent less time interacting with males. However, females of both shortfin species did show preferences for the sailfin *P. latipinna* males when comparing them to heterospecific shortfin males, but exhibited no preference for either male type when comparing the sailfin males with conspecific males. This suggests that shortfin females recognize and are attracted to the sailfin phenotype, but that species-specific cues of conspecific males may inhibit this attraction.

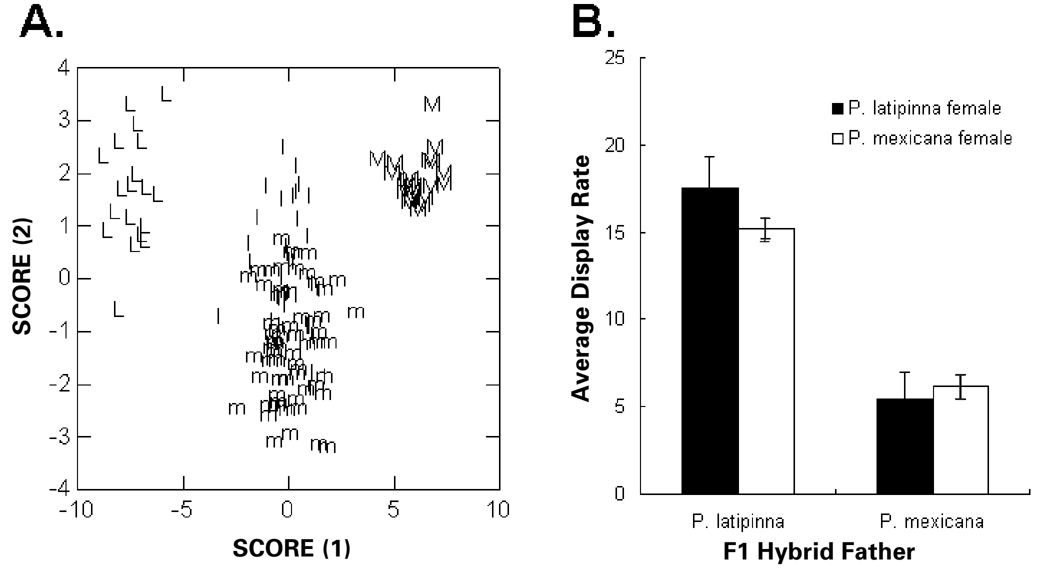
The lack of a preference for sailfin males by shortfin females when comparing them with conspecific males may explain the absence of interspecific hybrids in natural populations where sailfin and shortfin species co-occur. Clear avoidance of heterospecific females by sailfin males from sympatric populations (Ryan *et al.,* 1996; Schlupp *et al.,* 1991) coupled with the lack of a strong female preference for the sailfin male phenotype in the presence of conspecific, shortfin males may aid in decreasing the chances of interspecific hybridization. The potential for a pre-existing bias (Basolo, 1990; Ryan, 1990) for the sailfin phenotype in females of shortfin species however, would suggest that when the initial traits leading to premating isolation (courtship display behavior and/or enlarged dorsal fins) first evolved in molly populations, the females' sensory system was already biased towards favoring these traits in males, hence hastening their establishment and promoting divergence of sailfins. This implies an important role for sexual selection in generating and maintaining the observed divergence in mate recognition systems in mollies.

Targets of Sexual Selection and Species Recognition in Mollies

Theoretical models have demonstrated that sexual selection can lead to speciation when premating reproductive isolation develops in parallel with divergence of sexually-selected traits (Lande, 1982; Thornhill and Alcock, 1983; West-Eberhard, 1983, 1984; Kaneshiro and Boake, 1987; Lande and Kirkpatrick, 1988; Iwasa and Pomiankowski, 1995; Payne and Krakauer, 1997). Such a model of speciation requires that the phenotypically varying male traits that are the targets of sexual selection also serve as reliable species recognition signals. A definitive test of which traits are essential for species recognition can only be conducted by varying traits systematically, one at a time, holding all other traits constant. Such a task is particularly challenging when the mating signal is a multivariate visual cue that varies in both morphological and behavioral components.

I have been using a novel approach to examine female mating preferences for different components of the mating signal in the sailfin molly *P. latipinna*. Male phenotype in mollies can be manipulated by producing interspecific hybrids

Figure 5.
A. Species scores on the first two canonical axes from a discriminant analysis of 14 morphological shape variables from males of *P. latipinna* (L), *P. mexicana* (M) and F1 hybrids with *P. latipinna* sires (l) and F1 hybrid with *P. mexicana* sires (m).
B. Average number of courtship displays (number/10 min. ±SE) performed by F1 hybrid males during the 10-min observation period in response to *P. latipinna* females and *P. mexicana* females. (From Ptacek, 2002)



between sailfin and shortfin species that vary in their expression of certain behavioral and morphological trait values (Ptacek, 2002). First generation male hybrid offspring have been reared from reciprocal crosses between the sailfin species *P. latipinna* and the shortfin species *P. mexicana* (Fig. 4). Four crosses between a *P. mexicana* dam and a *P. latipinna* sire yielded 42 F1 hybrid sons and five crosses between a *P. latipinna* dam and a *P. mexicana* sire yielded 66 F1 hybrid sons. Comparisons of phenotypic trait distributions for 14 morphological shape characters and rates of three mating behaviors (courtship displays, gonopodial thrusts and gonoporal nibbles) between hybrid males from the two directions of the interspecific cross and males of the two parental species suggest that courtship display behavior can evolve independently of morphology in F1 hybrid males (Ptacek, 2002). Canonical discriminant analysis shows that F1 hybrid males are intermediate with respect to the two parental species along the first canonical axis, which describes relative dorsal fin length, regardless of the direction of the cross (Fig. 5A). Males of the two parental species and the F1 hybrids overlap considerably along the second canonical axis, which describes the insertion point of the gonopodium; the insertion of the gonopodium is more anterior for both types of F1 hybrid males than for males of either parental species (Fig. 5A).

Unlike dorsal fin morphology, behavioral variation among the F1 hybrid males differs markedly depending upon the direction of the interspecific cross (*P. latipinna* sire or *P. mexicana* sire) for rates of courtship displays. The difference between the two types of crosses in their rate of courtship displays is striking (Fig. 5B). Hybrid males whose sires were *P. latipinna* performed courtship displays at rates up to three times higher than did those of hybrid males whose sires were *P. mexicana*. Even more striking is the similarity between the phenotypic trait distributions of males from the parental species and F1 hybrid males sired by each parental species (Ptacek, 2002). Hybrid males performed courtship display rates that were nearly identical to the rates performed by the parental species of their sires. Such a pattern suggests that Y-linkage influences the inheritance of courtship display rates in mollies. Indeed, another study of interspecific hybridization in mollies involving crosses between the sailfin *P. velifera* and the shortfin *P. mexicana* also reported a strong resemblance between the number of sexual displays performed by an F1 hybrid male and that of his sire (Parzefall, 1989). Sex-linked effects on courtship display rates have also been suggested as a basis for population-specific rates in guppies (Houde, 1997). The other mating behaviors, gonopodial thrusting and gonoporal nibbling, show considerable overlap for rates of these behaviors both between the two parental species and for F1 hybrid males from both directions of the interspecific cross (Ptacek, 2002). Thus courtship display rates alone, can be adjusted independently of dorsal fin morphology by manipulating the direction of the interspecific cross.

Table 2.
Summary of the time (mean +/- standard error) spent in apathy and difference in time spent with each male for Mounds and Live Oak females for six paired comparisons of males matched for size (Plat = *P. latipinna*, Pmex = *P. mexicana*, F1lat = F1 hybrid male with *P. latipinna* sire, F1mex = F1 hybrid male with *P. mexicana* sire). Mean differences were statistically analyzed using a paired t-test.

Population	Treatment	Male Species		Apathy	Difference	Paired t-test		
		Male 1	Male 2	Mean ± SE	Mean ± SE	df	t-value	P-value
Mounds	1	Plat	Pmex	211.8±41.6	178.1±88.2	11	2.019	0.069
	2	F1mex	Plat	234.2±34.3	-2.7±75.5	11	-0.035	0.972
	3	Pmex	F1mex	235.7±45.2	216.5±86.1	11	2.515	0.029
	4	Plat	F1lat	308.3±34.6	84.0±56.4	11	1.489	0.165
	5	F1lat	Pmex	228.6±45.1	217.6±84.9	11	2.562	0.026
	6	F1lat	F1mex	281.8±34.8	-29.3±64.8	11	-0.451	0.660
Live Oak	1	Plat	Pmex	227.0±16.2	235.8±17.6	11	13.426	0.001
	2	F1mex	Plat	248.5±13.2	83.7±51.3	11	1.629	0.132
	3	Pmex	F1mex	287.6±12.8	71.6±45.8	11	1.564	0.146
	4	Plat	F1lat	233.3±13.7	87.8±48.7	11	1.800	0.099
	5	F1lat	Pmex	250.0±22.1	141.2±48.6	11	2.902	0.014
	6	F1lat	F1mex	313.3±19.9	-0.7±40.5	11	0.016	0.987

These F1 hybrid males were then used in a series of dichotomous female choice tests (see Ptacek and Travis, 1997; Ptacek, 1998 for a description of the choice test design) designed to test whether courtship display behavior or size of the dorsal fin is a more important cue when females of the sailfin species, *P. latipinna* distinguish between conspecific, heterospecific and F1 hybrid males. Two different populations of females were tested, Mounds and Live Oak, Wakulla County, Florida, USA. These two populations were chosen because prior tests of female mating preferences had shown that females from each of these populations preferred males from their own populations over males from foreign populations, in at least some male population combinations (Ptacek and Travis, 1997). Females were controlled for receptivity by testing only virgin females (Live Oak) or a mix of virgin females and experienced females within 24 hours post-parturition (Mounds). Six male treatment combinations were used, which represented all pair-wise combinations of the four types of males: *P. latipinna*, *P. mexicana*, F1 hybrid with *P. latipinna* sire, and F1 hybrid with *P. mexicana* sire. Males used in each treatment combination were matched for size. Two different groups of females from Mounds (N = 12 per group) were tested. Each group was tested with three of the six different male treatment combinations (two 3 x 3 latin square designs). Live Oak females (N = 12) were each tested with all six male treatment combinations, three combinations each day for two consecutive days (6 x 6 latin square design). No effects of trial order (Mounds, $F_{2,69}$ = 0.403, P = 0.670; Live Oak, $F_{2,69}$ = 0.302, P = 0.740) were observed for females from either population. For Live Oak females, there were no effects of day of the trials, first or second, on the preference scores of females ($F_{1,70}$ = 0.343, P = 0.560). Apathy levels (proportion of the trial spent not interacting with males, Table 2) were fairly high for females from both of these populations, which may be due to their both being from laboratory-reared stocks rather than recently wild-caught females.

Results of choice tests with Mounds females suggest that females did not distinguish between the two types of F1 hybrid males nor between F1 hybrid males and conspecific males (Fig. 6). The strongest preferences shown by these females were in trials comparing *P. latipinna* or the two types of F1 hybrid males with males of *P. mexicana*. The time spent with both types of F1 hybrid males was significantly greater than that spent with males of *P. mexicana* (Table 2). Females from the Mounds population, on average, spent more time with *P. latipinna* males

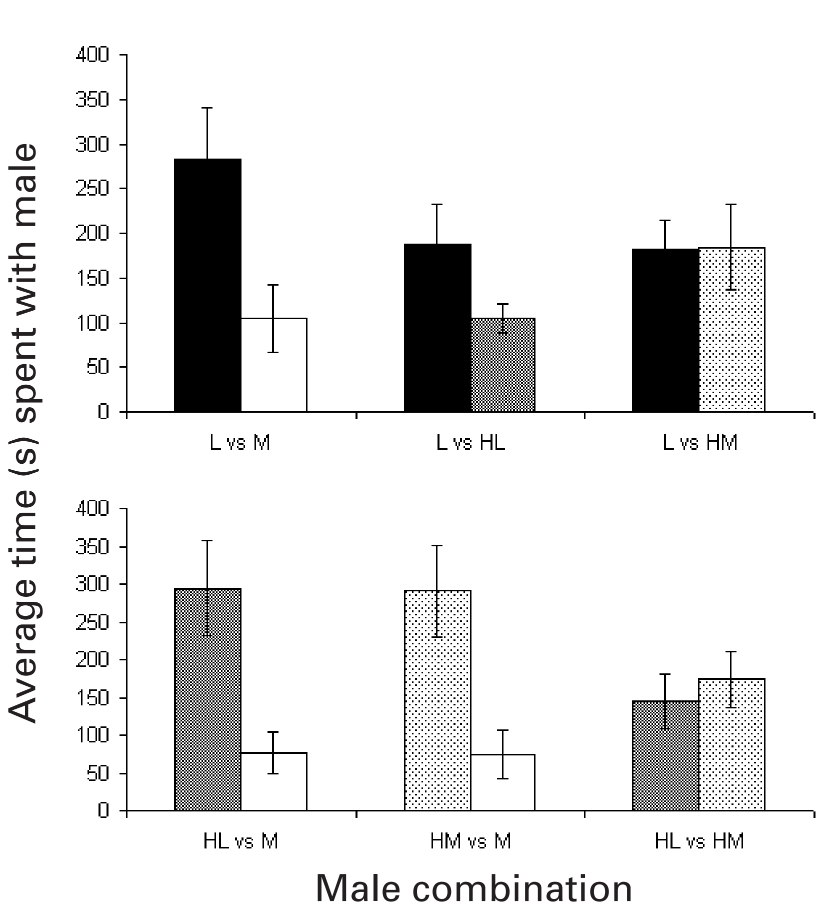


Figure 6.
Response of Mounds females to size-matched males presented in six different male treatment combinations. The average ±SE time (in seconds) that females spent with each male for each combination during the 10-min observation period is plotted. Male treatment combinations are as follows: L = *P. latipinna*, M = *P. mexicana*, HL = F1 hybrid with *P. latipinna* sire, HM = F1 hybrid with *P. mexicana* sire

than males of *P. mexicana*, but this was not significant (Table 2), probably due to one female that spent almost the entire trial with the *P. mexicana* male. These results suggest that Mounds females find F1 hybrid males as attractive as conspecific males and that courtship display rate differences between the two types of hybrid males are not used by females from this population to distinguish between them. Also the results suggest a strong avoidance of males of *P. mexicana* with females clearly preferring both *P. latipinna* males and F1 hybrid males of both types in trials comparing them with the shortfin males. The strength of these preferences (Difference, Table 2) in the treatment combinations containing *P. mexicana* was very similar, suggesting that differences in dorsal fin shape may be the target of female choice in this population of *P. latipinna*. These results are consistent with the pattern of female choice for Mounds females at the intraspecific level as well. Difference in dorsal fin shape appears to be the most important cue used by females from Mounds when distinguishing males from the alternative populations of Pinhook and Live Oak. Considerable overlap exists between males from Mounds and males from these two alternative populations in courtship display rates, but not in dorsal fin shape (Ptacek and Travis, 1997; Fig. 3).

Live Oak females responded in a similar manner as Mounds females, but these females showed stronger preferences for conspecific males than for F1 hybrid males from either direction of the interspecific cross (Fig. 7). There was a trend, although not significant, for females to prefer conspecific males over either type of F1 hybrid male (Fig. 7) and apathy level was considerably higher for females in the male combination that compared both types of hybrid males (Table 2). Females from Live Oak spent significantly more time with conspecific males and F1 hybrid males with *P. latipinna* sires than with males of *P. mexicana* but there was no significant preference in the combination with an F1 hybrid male with a *P. mexicana* sire and *P. mexicana* (Table 2). This result suggests that behavioral differences in courtship display rates may more strongly influence patterns of female mating preferences in this population of *P. latipinna*, but only when a clear distinction in dorsal fin size exists between the males being compared. Similar results were found for Live Oak females in their intraspecific mating preferences where females from this population clearly preferred native males to males from Pinhook, but not to males from Mounds (Ptacek and Travis, 1997). Males from Live Oak and Pinhook differ most in courtship display rates, while considerable overlap exists in courtship display rates between males from Live Oak and males from Mounds (Fig. 3).

Results of these female choice trials suggest that females of *P. latipinna* show the strongest preferences for males with larger dorsal fins when the relative difference between males in fin size is greatest (*P. latipinna* or F1 hybrid males compared to *P. mexicana* males). A similar result has been found in *P. latipinna* when testing females with male models, constructed from photographs of male *P. latipinna*, that varied in dorsal fin size, body size and overall lateral projection area (MacLaren *et al.,* 2004). In these trials, females of *P. latipinna* could only distinguish between males with different-sized dorsal fins when the disparity between the two model males in this characteristic was relatively large (difference in lateral projection area ≥ 1.7

cm^2). Such results suggest that dorsal fin size differences between sailfin and shortfin species may serve as an important species recognition signal.

Conclusions and Avenues of Future Research

Because of their enormous diversity in male mating behaviors and secondary sexual morphological traits at both the intra- and interspecific levels, members of the subgenus *Mollienesia* provide an ideal model system in which to examine the influence of sexual selection in shaping mating signal evolution both within and between species. The major axis of interspecific differentiation in mollies lies between the sailfin and shortfin species complexes where clear distinctions exist in levels of courtship display behavior and dorsal fin shape. However, differentiation among populations within a single sailfin species, *P. latipinna*, parallels divergence at the interspecific level. This argues strongly for an important role of female mating preferences in shaping both intra- and interspecific divergence in mating signals in mollies. Indeed, females of *P. latipinna* appear to use varying rates of the courtship display and shape differences in the dorsal fin of males to distinguish among conspecific males from different populations and between conspecific and heterospecific males.

Our understanding of the phylogenetic relationships within *Mollienesia* has important implications for speciation in the group. First, all extant sailfin species arose from a single speciation event, diverging from a shortfin ancestor. Changes in both male mating behavior and dorsal fin morphology occurred during speciation of sailfin mollies. One member of the sailfin clade, *P. latipunctata*, lacks the enlarged dorsal fin characteristic of males of the other three species, but retains the courtship display behavior that is a hallmark of the group. A second important implication of our knowledge of the phylogenetic relationships of *Mollienesia* is that future research now has a framework from which to study the evolution of male signaling traits and female preferences for these traits. In this manner, we can reconstruct ancestral signal properties and mating preferences. Using a comparative method approach to studying the origin and direction of change in male traits and female preferences for these traits will allow future work to focus on testing models of sexual selection and to examine the degree to which female mating preferences and male signaling traits may be evolving in concert.

One important aspect of speciation in mollies that has been somewhat overlooked is that of the role of natural selection in promoting and maintaining population divergence among sailfin species. We know that natural selection places important constraints on male body size distributions in populations of *P. latipinna*. For example, large males are favored in colder habitats because of larger fat reserves that increase over-winter survival (Trexler *et al.,* 1992). In contrast, wading birds preferentially prey on large males favoring smaller male size distributions in open saltmarsh populations that are exposed to higher levels of wading bird predation (Trexler *et al.,* 1994). Studies are just beginning to explore the relationships between environmental factors, predator regimes and other types of interspecific interactions, all of which, may promote phenotypic divergence

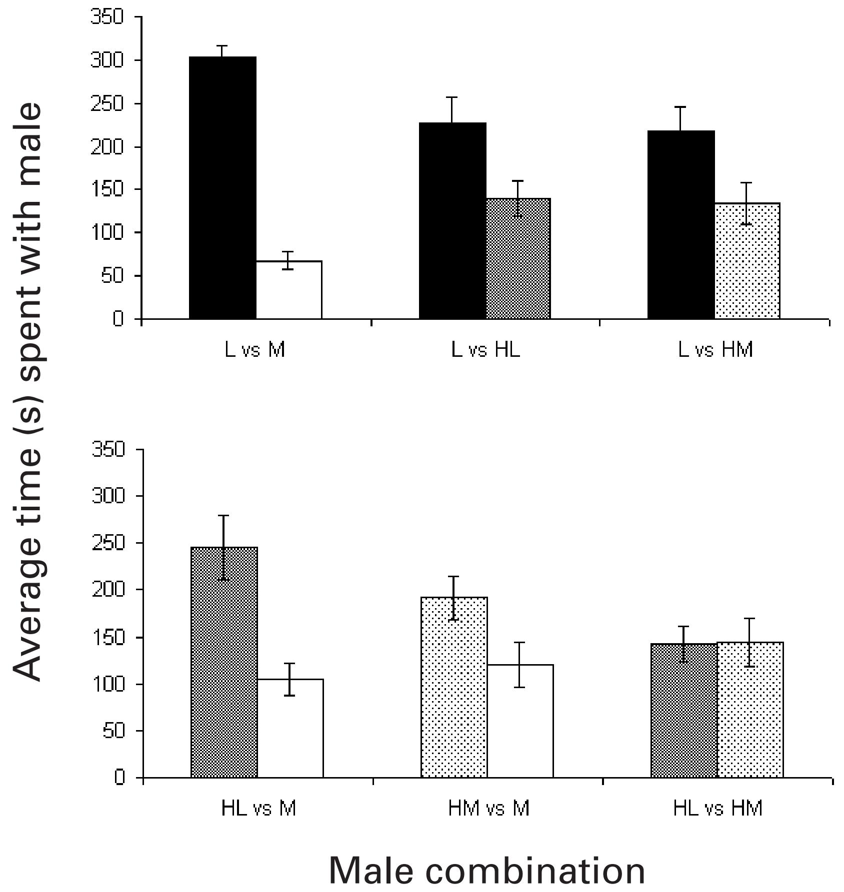


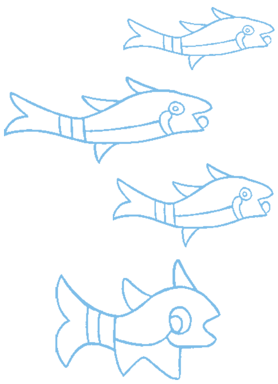
Figure 7.
Response of Live Oak females to size-matched males presented in six different male treatment combinations. The average ±SE time (in seconds) that females spent with each male for each combination during the 10-min observation period is plotted. Male treatment combinations are as follows:
L = *P. latipinna*, M = *P. mexicana*,
HL = F1 hybrid with *P. latipinna* sire,
HM = F1 hybrid with *P. mexicana* sire

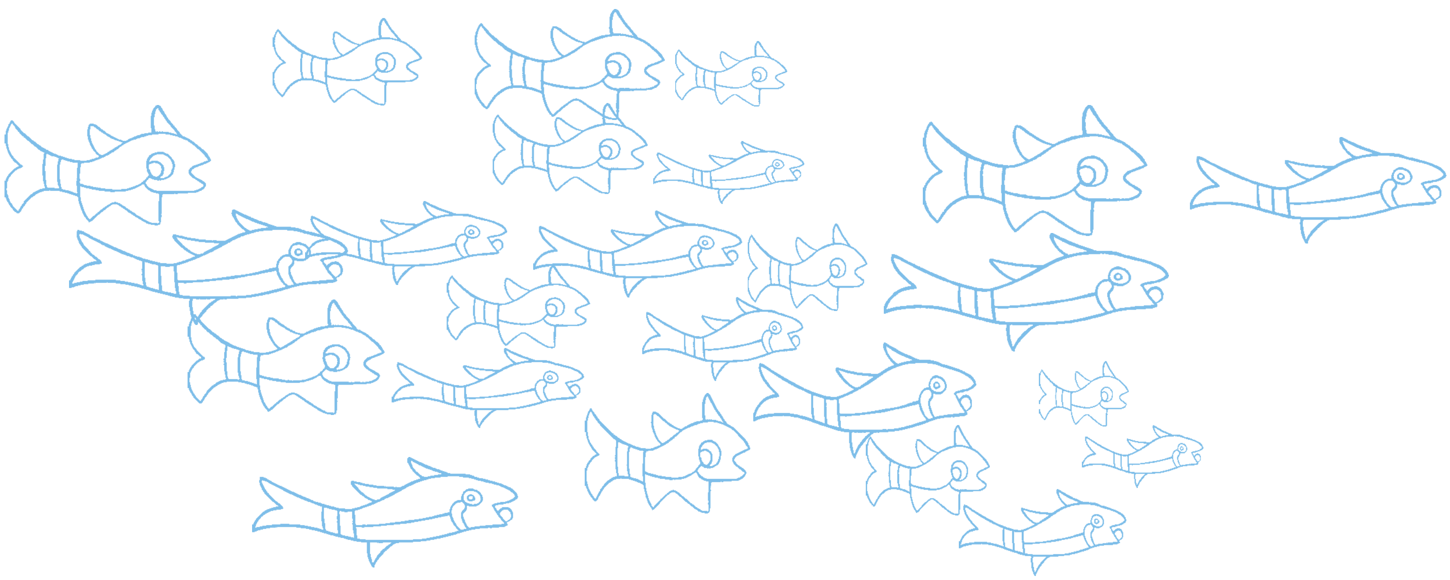
among populations as well as differences in female mating preferences. Female mating preferences in mollies may be reinforcing initial divergence in male phenotypic traits favored by natural selection. Such divergence in color patterns and courtship display rates that is promoted both by natural selection through differential predation pressures and variation among populations in female mating preferences is known for guppies (Endler, 1983; Endler and Houde, 1995) and sailfin mollies may have evolved under similar circumstances.

Comparative studies are needed that include all of the species of sailfin mollies, with emphasis placed on geographic variation among conspecific populations in both signal properties and mating preferences and how these factors covary with changes in environmental factors. Understanding how natural selection, sexual selection and genetic drift interact to shape population divergence within several sailfin species will provide insight into the potential importance of each of these evolutionary forces during speciation of sailfin mollies.

Lastly, future efforts should capitalize upon the ease with which interspecific crosses can be made in the laboratory between shortfin and sailfin species. The amenity of mollies to laboratory rearing and breeding design studies makes them the ideal vertebrate system in which to examine the genetic basis of traits that confer premating reproductive isolation.

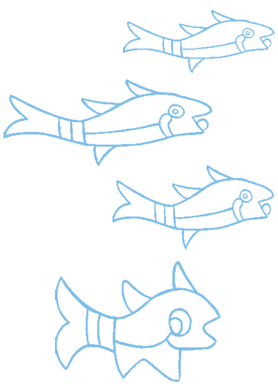
I strongly encourage future studies to build upon our current knowledge of mating signal divergence and patterns of female mating preferences in mollies. Mollies provide a wonderful opportunity for us to begin to understand the importance of sexual selection in mating signal divergence and speciation. I hope that future work will ultimately allow us to uncover the processes of speciation in this fascinating group of poeciliid fishes.





Acknowledgments

Special thanks to J. Travis and J. Trexler for introducing me to this fascinating group of poeciliids, commonly known as mollies. Their suggestions and support have guided my research and greatly improved our understanding of diversification and speciation in mollies. Helpful discussions with F. Breden, M. Childress, H. C. Gerhardt and F. H. Rodd over the past 15 years have improved my thinking on sexual selection and its role in speciation. I am indebted to a number of exceptional technicians, M. Kittell, A. Magnani, N. Martin, T. McKay, H. Ray, S. Strzalkowska, A. Tomasso and C. Wilson, for their husbandry skills and years of careful data collection. Thanks to M. Childress for help in analysis of experiments and preparation of figures and to S. Brock for preparation of the color plates. Thanks to J. L. Ramírez for help in collecting mollies in Mexico and translating documents and the abstract into Spanish. I am grateful to J. Wourms for suggesting that I be included in this symposium and to H. Grier and M. C. Uribe for inviting me to participate. I also wish to thank the Mexican government for their support of my research efforts in Mexico through the granting of collecting permits No. 10.04.01.613.03 and No. 01.01.02.613.03. My research has been generously supported by the National Science Foundation through a NSF-Idaho EPSCoR Program grant (EPS-9720634) and an Animal Behavior Program grant (IBN-0091735).



References

Andersson M. 1994. Sexual Selection. Princeton University Press, Princeton, New Jersey.

Avise JC, Trexler JC, Travis J, Nelson WS. 1991. *Poecilia mexicana* is the recent female parent of the unisexual fish *P. formosa*. Evolution 45:1530-1533.

Balsano JS, Randle EJ, Rausch EM, Monaco PJ 1985. Reproductive behavior and maintenance of all-female *Poecilia*. Environ Biol Fish 12:251-263.

Barlow GW. 1992. Is mating different in a monogamous species? The midas cichlid as a case study. Am Zool 32:91-99.

Basolo AL. 1990. Female preference predates the evolution of the sword in swordtail fish. Science 250:808-810.

Boake CRB. 2000. Flying apart: Mating behavior and speciation. BioScience 50:501-508.

Boake CRB, DeAngelis MP, Andreadis DK. 1997. Is sexual selection and species recognition a continuum? Mating behavior of the stalk-eyed fly *Drosophila heteroneura*. Proc Natl Acad Sci (USA) 94:12442-12445.

Brett BLH, Grosse DJ. 1982. A reproductive pheromone in the Mexican poeciliid fish *Poecilia chica*. Copeia 1982:219-223.

Crapon de Caprona M-D, Ryan MJ. 1990. Conspecific mate recognition in swordtails, *Xiphophorus nigrensis* and *X. pygmaeus* (Poeciliidae): olfactory and visual cues. Anim Behav 39:290-296.

Endler JA. 1983. Natural and sexual selection on color patterns in poeciliid fishes. Environ Biol Fishes 9:173-190.

Endler JA, Houde AE. 1995. Geographic variation in female preferences for male traits in *Poecilia reticulata*. Evolution 49:456-468.

Farr JA. 1975. The role of predation in the evolution of social behavior in natural populations of the guppy, *Poecilia reticulata* (Pisces: Poeciliidae). Evolution 29:151-158.

Farr JA. 1989. Sexual selection and secondary sexual differentiation in poeciliids: Determinants of male mating success and evolution of female choice. In: Meffe GK, Snelson FF, Jr, editors. Ecology and Evolution of Livebearing Fishes (Poeciliidae), Prentice Hall, Englewood Cliffs, New Jersey. p 91-123.

Farr JA, Travis J, Trexler JC. 1986. Behavioral allometry and interdemic variation in sexual behaviour of the sailfin molly, *Poecilia latipinna* (Pisces: Poeciliidae). Anim Behav 34:497-509.

Gabor C. 1999. Association patterns of sailfin mollies (*Poecilia latipinna*): alternative hypotheses. Behav Ecol SocioBiol 46:333-340.

Gerhardt HC. 1994. The evolution of vocalization in frogs and toads. Annu Rev Ecol Syst 25:293-324.

Greenfield DW. 1990. *Poecilia teresae*, a new species of poeciliid fish from Belize, Central America. Copeia 1990:449-454.

Houde AE. 1997. Sex, Color, and Mate Choice in Guppies. Princeton University Press, Princeton, New Jersey.

Hubbs CL. 1933. Species and hybrids of *Mollienesia*. Aquarium 1:263-268, 277.

Hubbs CL. 1936. Fishes of the Yucatan peninsula. In: Pearse AS, Creaser EP, Hall FG, editors. The Cenotes of Yucatan, a Sociological and Hydrological Survey Carnegie Inst Washington Publ 457. p 157-287.

Iwasa YA, Pomiankowski A. 1995. Continual change in mate preferences. Nature 377:420-422.

Kaneshiro KY, Boake CRB. 1987. Sexual selection and speciation: Issues raised by Hawaiian *Drosophila*. Trend Ecol Evol 2:207-212.

Kirkpatrick M. 1982. Sexual selection and the evolution of female choice. Evolution 36:1-12.

Kirkpatrick M, Ryan MJ. 1991. The evolution of mating preferences and the paradox of the lek. Nature 350:33-38.

Lande R. 1981. Models of speciation by sexual selection on polygenic traits. Proc Nat Acad Sci (USA) 78: 3721-3725.

Lande R. 1982. Rapid origin of sexual isolation and character divergence in a cline. Evolution 36:213-223.

Lande R, Kirkpatrick M. 1988. Ecological speciation by sexual selection. J Theor Biol 133:85-98.

Liley NR. 1966. Ethological isolating mechanisms in four sympatric species of poeciliid fishes. Behaviour (Suppl) 13:1-197.

Littlejohn MJ. 1999. The evolution and breakdown of homogamy. In: Foster SA, Endler JA, editors. Geographic Variation in Behavior Oxford University Press, Oxford. p 209-233.

Luckner CL. 1979. Morphological and behavioral polymorphisms in *Poecilia latipinna* males (Pisces: Poeciliidae). Ph.D. Dissertation, Louisiana State University, Baton Rouge, Louisiana, USA.

MacLaren RD, Rowland WJ, Morgan N. 2004. Female preferences for sailfin and body size in the sailfin molly, *Poecilia latipinna.* Ethology 110:363-379.

McLennan DA, Ryan MJ. 1997. Responses to conspecific and heterospecific olfactory cues in the swordtail *Xiphophorus cortezi*. Anim Behav 54:1077-1088.

McLennan DA, Ryan MJ. 1999. Interspecific recognition and discrimination based upon olfactory cues in northern swordtails. Evolution 53:880-888.

Meyer MK, Wischnath L, Foerster W. 1985. Lebendgebarende Zierfische Arten der Welt. Mergus-Verlag, Melle, West Germany.

Miller RR. 1975. Five new species of Mexican poeciliid fishes of the genera *Poecilia*, *Gambusia*, and *Poeciliopsis*. Occasional Papers of the Museum of Zoology, Univ. of Michigan 672:1-44.

Miller RR. 1983. Checklist and key to the mollies of Mexico (Pisces: Poeciliidae: *Poecilia,* subgenus *Mollienesia*). Copeia 1983:817-822.

Niemeitz A, Kreutzfeldt R, Schartl M, Parzefall J, Schlupp I. 2002. Male mating behaviour of a molly, *Poecilia latipunctata*: a third host for the sperm-dependent Amazon molly, *Poecilia formosa*. Acta Ethologica 5:45-49.

Panhuis TM, Butlin R, Zuk M, Tregenza T. 2001. Sexual selection and speciation. Trends Ecol Evol 16:364-371.

Parzefall J. 1969. Zur vergleichenden Ethologie verschiedener *Mollienesia*-Arten ein-schliesslich einer Hohlenform von *M. sphenops*. Behaviour 33:1-37.

Parzefall J. 1979. Zur genetik und biologischen bedeutung des aggressionsueshaltens von *Poecilia sphenops* (Pisces, Poeciliidae). Z Tierpsychol 50:399-422.

Parzefall J. 1989. Sexual and aggressive behaviour in species hybrids of *Poecilia mexicana* and *Poecilia velifera* (Pisces, Poeciliidae). Ethology 82:101-115.

Payne RJH, Krakauer DC. 1997. Sexual selection, space, and speciation. Evolution 51:1-9.

Ptacek MB. 1998. Interspecific mate choice in sailfin and shortfin species of mollies. Anim Behav 56: 1145-1154.

Ptacek MB. 2000. The role of mating preferences in shaping interspecific divergence in mating signals in vertebrates. Behav Processes 51:111-134.

Ptacek MB. 2002. Patterns of inheritance of mating signals in interspecific hybrids between sailfin and shortfin mollies (Poeciliidae: *Poecilia*: *Mollienesia*). Genetica 116:329-342.

Ptacek MB, Breden F. 1998. Phylogenetic relationships of the mollies (Poeciliidae: *Poecilia*: *Mollienesia*) based on mitochondrial DNA sequences. J Fish Biol (Supplement A) 53:64-81.

Ptacek MB, Travis J. 1996. Interpopulation variation in male mating behaviours in the sailfin molly, *Poecilia latipinna*. Anim Behav 52:59-71.

Ptacek MB, Travis J. 1997. Mate choice in the sailfin molly, *Poecilia latipinna*. Evolution 51:1217-1231.

Rand AS, Ryan MJ, Wilczynski, W. 1992. Signal redundancy and receiver permissiveness in acoustic mate recognition by the Tungara frog, *Physalaemus pustulosus*. Am Zool 32:81-90.

Rauchenberger M. 1989. Annotated species list of the subfamily Poeciliinae. In: Meffe GK, Snelson FF Jr, editors. Ecology and Evolution of Livebearing Fishes (Poeciliidae), Prentice Hall, Englewood Cliffs, New Jersey. p 359-368.

Regan CT. 1913. A revision of the cyprinodont fishes of the subfamily Poeciliinae. Proc Zool Soc Lond 11: 977-1018.

Rosenthal GG, Evans CS. 1998. Female preference for swords in *Xiphophorus helleri* reflects a bias for large apparent size. Proc Natl Acad Sci (USA) 95:4431-4436.

Ryan MJ. 1990. Sexual selection, sensory systems, and sensory exploitation. Oxf Surv Evol Biol 7:157-195.

Ryan MJ, Dries LA, Batra P, Hillis DM. 1996. Male mate preferences in a gynogenetic species complex of Amazon mollies. Anim Behav 52:1225-1236.

Ryan MJ, Rand AS. 1993. Species recognition and sexual selection as a unitary problem in animal communication. Evolution 47:647-657.

Schartl M, Wilde B, Schlupp I, Parzefall J. 1995. Evolutionary origin of a parthenoform, the Amazon molly *Poecilia formosa*, on the basis of a molecular genealogy. Evolution 49:827-835.

Schlupp I, Marler C, Ryan MJ. 1994. Benefit to male sailfin mollies of mating with heterospecific females. Science 236:373-374.

Schlupp I, Parzefall J, Schartl M. 1991. Male mate choice in mixed bisexual/unisexual breeding complexes of *Poecilia* (Teleostei: Poeciliidae). Ethology 88:215-222.

Schluter D, Price T. 1993. Honesty, perception, and population divergence in sexually selected traits. Proc R Soc Lond B. 253:117-122.

Thornhill R, Alcock J. 1983. The Evolution of Insect Mating Systems. Harvard Univ. Press, Cambridge, Massachusetts.

Travis J. 1989. Ecological genetics of life-history traits in poeciliid fishes. In: Meffe GK, Snelson FF Jr., editors. Ecology and Evolution of Livebearing Fishes (Poeciliidae). Prentice Hall, Englewood Cliffs, New Jersey. p 185-200.

Travis J. 1994. Size-dependent behavioral variation and its genetic control within and among populations. In: Boake CRB, editor. Quantitative Genetic Approaches to Animal Behavior. University of Chicago Press, Chicago. p 165-187.

Travis J, Trexler JC. 1987. Regional variation in habitat requirements of the sailfin molly, with special reference to the Florida Keys. Nongame Wildl Tech Rept No. 3, Florida Game and Freshwater Fish Commission, Tallahassee, Florida, USA.

Trexler JC, Tempe R, Travis J. 1994. Size-selective predation of sailfin mollies by two species of heron. Oikos 69:250-258.

Trexler JC, Travis J, McManus M. 1992. Effects of habitat and body size on mortality rates of *Poecilia latipinna*. Ecology 73:2224-2236.

Trexler JC, Travis J, Trexler M. 1990. Phenotypic plasticity in the sailfin molly, *Poecilia latipinna* (Pisces: Poeciliidae). II. Laboratory experiment. Evolution 44:157-167.

Turner, BJ. 1982. The evolutionary genetics of a unisexual fish, *Poecilia formosa*. In: Barigozzi C., editor. Mechanisms of Speciation. Alan R Liss, New York. p 265-305.

Uy JA, Borgia G. 2000. Sexual selection drives rapid divergence in bowerbird display traits. Evolution 54:273-278.

Verrell PA. 1988. Stabilizing selection, sexual selection, and speciation: a view of specific-mate recognition systems. Syst Zool 37:209-215.

Verrell PA. 1999. Geographic variation in sexual behavior. In: Foster SA, Endler JA, editors. Geographic Variation in Behavior. Oxford University Press, Oxford. p 262-279.

West-Eberhard MJ. 1983. Sexual selection, social competition, and speciation. Quart Rev Biol 58:155-183.

West-Eberhard MJ. 1984. Sexual selection, competitive communication, and species-specific signals in insects. In: Lewis T., editor. Insect Communication. Academic Press, New York. p 283-324.

Wiernasz DC. 1989. Female choice and sexual selection on male wing melanin pattern in *Pieris occidentalis* (Lepidoptera). Evolution 43:1672-1682.

Wiernasz DC, Kingsolver JG. 1992. Wing melanin pattern mediates species recognition in *Pieris occidentalis*. Anim Behav 43:89-94.

Woodhead AD, Armstrong N. 1985. Aspects of mating behaviour of male mollies (*Poecilia* spp.). J Fish Biol 27:593-601.