

SPECIES AND MATE-QUALITY RECOGNITION
IN *POECILLIA LATIPINNA*

THESIS

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ABSTRACT

Examination of the species recognition process may shed light on an evolutionary paradox: the persistence of Amazon mollies (*Poecilia formosa*) that are unisexual gynogens. Amazon mollies require sperm from the closely related sailfin molly, *P. latipinna*, to initiate embryogenesis, however, inheritance is strictly maternal. In the following chapters, I address both proximate and ultimate questions pertaining to species recognition by male sailfin mollies in this unisexual-bisexual species complex.

In Chapter II, I examine a mechanism for the persistence of Amazon mollies via conflict between species and mate-quality recognition cues. Previous work has found that male sailfin mollies in sympatry exhibit a significantly greater mating preference for female sailfin mollies over Amazon mollies compared with males in allopatry. In addition, male sailfin mollies prefer to mate with larger conspecific females and these females are more fecund and represent higher quality mates. Therefore, when choosing mates, male sailfin mollies from populations sympatric with Amazon mollies may rely on traits indicating species identity (genetic compatibility) rather than those indicating mate-quality (increased fitness benefits). Conversely, allopatric males may rely more on traits indicating mate-quality. I hypothesized that Amazon mollies may be taking advantage of body size as a mate-quality cue. To test this, I paired males with a larger Amazon molly and a smaller female sailfin molly to determine whether there is a conflict in species and mate-quality recognition. I tested males from three allopatric and five sympatric populations. In each trial I scored the number of mating attempts that males directed

to conspecific and heterospecific females. I found that males in most sympatric and allopatric populations no longer demonstrate the preference for conspecifics found in previous work when females were matched for standard length. In addition, I found a significant difference in mating preference between allopatric and sympatric populations with males from allopatry showing a greater heterospecific mate preference. These results indicate a conflict between species and mate-quality cues in sympatry, which may contribute to the persistence of gynogenetic Amazon mollies.

Chapter III examined morphological cues that may be used for species recognition. Understanding the cues used for species recognition is important in closely related sympatric species where there is a high risk of mating with heterospecifics. Multiple cues may be used or there may be more emphasis on specific individual traits. Herein, I examine possible cues used by male sailfin mollies (*Poecilia latipinna*) to distinguish between conspecific females and sympatric Amazon mollies (*P. formosa*). Digital photos were used to create models to test male *P. latipinna* preference for model female *P. latipinna* and *P. formosa* with a full suite of traits and altered *P. latipinna* and *P. formosa* models. I found that *P. latipinna* males significantly preferred models of either species over no stimulus, demonstrating that models elicit a male response. Second, I found that males significantly preferred model *P. latipinna* females over *P. formosa*. Third, I tested male preference for altered models in the following combinations; (1) *P. formosa* vs. *P. formosa* with a female *P. latipinna* fin (2) *P. formosa* vs. *P. formosa* with a female *P. latipinna* lateral spot pattern (3) *P. formosa* vs. *P. latipinna* with a *P. formosa* fin and their spotless lateral pattern. Males did not significantly prefer models with any

isolated traits over the unaltered *P. formosa* models. Thus, males may be using traits other than the ones isolated for species recognition or males may be using a suite of multiple traits to recognize conspecific females.

CHAPTER I

INTRODUCTION

Sexual selection is selection due to differences in reproductive success caused by competition over mates (Darwin 1871). The mechanisms of differential reproductive success are intersexual selection (mate choice) and intrasexual selection (competition between individuals of the same sex) (Darwin 1871). Mate recognition is a process included in mate choice, and it may consist of avoiding heterospecifics, identifying gender and assessing relatedness, genetic quality or parental resources. First I will cover the background to sexual selection theory and then discuss recognition systems and their relevance to sexual selection.

Female choice

Mate choice is a pattern of behavior that results in members of one sex being more likely to mate with certain members of the opposite sex (Halliday 1983). Females are usually the choosier sex due to the high-energy cost of producing eggs as compared to the relatively inexpensive production of sperm by males (Trivers 1972). Females are also the more discriminating sex as the operational sex ratio, or number of receptive males to receptive females in a population, is usually biased towards males (Emlen & Oring 1977). A female may choose mates for direct benefits received in resource based mating systems. When no obvious resources are offered, females may choose mates based on indicator mechanisms, where conspicuous costly male traits become targets of female choice. Females prefer male secondary sexual characters because such traits may indicate high heritable viability which may increase the fitness of a females' offspring. Non-indicator mechanisms may also be the basis of female choice when they choose male traits for reasons that may be unrelated to mate choice and/or the traits' indication of heritable viability. Non-

indicator mechanisms are important as they may explain the origin of the indicator mechanisms. There are numerous hypotheses proposed for each of these mechanisms.

Indicator mechanisms. The “good genes” hypothesis states that females who mate with males that exhibit a high quality appearance or courtship display benefit by acquiring viability promoting genes for their offspring. These offspring then have a greater ability to survive, compete, and reproduce (reviewed by Andersson 1994). Evidence for this hypothesis is provided by Welch et al. (1998) based on studies of the gray tree frog (*Hyla versicolor*). Female gray tree frogs prefer males who produce long calls. In this study, offspring of males with long calls had better performance, in terms of survival and growth rates, during larval and juvenile stages than did offspring of males who produced short calls. Therefore, call duration can serve as a reliable indicator of genetic quality in this species.

The “Handicap principle”, proposed by Zahavi (1975, 1977), suggests that females prefer males with extreme phenotypes that indicate high genetic quality. The handicap indicates viability as males must be able to build or maintain the cost imposed by the phenotype. Both the phenotype and the high genetic quality are then passed on to the offspring. A large amount of empirical evidence exists for this hypothesis (Andersson 1994).

Non-indicator mechanisms. “Runaway Selection” as proposed by Fisher (1930) suggests that females prefer males that are sexually attractive due to exaggerated male traits despite the trait not conveying information directly about mate quality. Male offspring then inherit the trait and female offspring inherit the preference for the trait. Evidence for runaway selection has been shown in mathematical models (O’Donald

1962; Lande 1980; Kirkpatrick 1982; Seger 1985) but experimental evidence is limited (Andersson 1994).

The “pre-existing bias” hypothesis (Endler and McLellan 1988; Endler 1992) examines how the environment and sensory biology of the species allow for separate evolution of male traits and female preferences. For example, a male trait may arise independently of a female preference but may be favored by a previously established female bias that may not have evolved via sexual selection (Basolo 1990; Witte & Curio 1999).

“Sensory exploitation” hypothesis is another extension of the sensory drive hypothesis (Endler and McLellan 1988), as postulated by Ryan (1990). This hypothesis suggests that females prefer male traits that elicit the greatest stimulation from the sensory system (Ryan 1997, 1998). Therefore the signal value of the display is more important than its indicative value. There are many examples of studies supporting this hypothesis (Ryan et al. 1990; Ryan 1997; Shaw 1995; Ryan 1998).

Male mate choice

Although female choice is usually seen, male choice can be an important factor in some species. Often in male choice, males prefer to mate with larger females due to an increased fecundity benefits (reviewed in Andersson 1994), or when sperm is limiting (Aspbury & Gabor 2004). Another case where male choice is important is in mating systems where unisexual females are reliant on sperm from males of a bisexual species to reproduce. In this situation, males are sexually parasitized by heterospecific females and sperm is limiting, so males are expected to both choose high quality mates and recognize conspecific mates over heterospecific females.

Male-Male Competition

The second component of sexual selection, intrasexual selection arises when members of one sex, usually males, compete for mates in a variety of ways (Darwin 1871). Males engage in fights or contests over direct access to females or over resources that will attract females (Cox & Le Boeuf 1977; Heinze et al. 1998).

Alternatively, males attempt to prevent other males from gaining access to a potential or actual mate, mate guarding, which improves a males' chances of mating success both before and after insemination (Alcock 1994; Polak et al. 2001). Additionally, a post-copulatory form of male-male competition has been the focus of many recent studies. Sperm competition occurs within a single female between the sperm from two or more males for fertilization of the ova (Parker 1970).

Recognition systems

In addition to considering the evolutionary theory behind mate choice, we must also give consideration to *how* mate choice occurs. An individual must recognize an appropriate mate by assessing, discriminating, and ultimately accepting a mate. Mate recognition may consist of avoiding heterospecifics (species recognition), identifying gender (sex recognition) and assessing relatedness (kin recognition), genetic quality (mate-quality recognition) or parental resources (mate-resource recognition). On a mechanistic level, the mate recognition process consists of three steps (Sherman et al. 1987); (1) production of cues by an individual, (2) sensory detection of cues and assessment of the cues, and (3) discriminating action based on the cues.

Due to selection, individuals' recognition systems should be free of error. However, cues produced by appropriate and inappropriate mates often overlap (Pfennig 1998) and inappropriate mates may benefit from mimicking cues of desirable mates (Reeve 1997). As a result, individuals make both acceptance errors, in which they accept inappropriate mates, and rejection errors, where they reject appropriate mates. One system where acceptance errors are common is in the unisexual-bisexual complex of mollies. Unisexual Amazon mollies (*Poecilia formosa*) rely on sperm from closely related bisexual sailfin mollies (*P. latipinna*). Acceptance errors by male sailfin mollies are one factor that may be important to the persistence of Amazon mollies for over 100,000 years (Avise et al. 1991; Scharl et al. 1995).

In order to avoid recognition errors, individuals often assess multiple cues for mate choice. These multiple cues may be assessed concurrently, may differ in level of importance to mating decisions, or may interact with each other (reviewed in Candolin 2003). This may be especially important to species that mate with closely related heterospecifics as assessing multiple cues can be adaptive and increase fitness by reducing mate choice errors or costs of choice itself.

Several hypotheses have been proposed to support the adaptive preferences for multiple cues. The "multiple message" hypothesis proposes that different cues give information about different mate qualities (Johnstone 1997). These may be assessed concurrently, indicating the overall quality of the potential mate, or individuals may differ in their use of cues. If this is the case, each cue indicates quality in a different way to different individuals (Wedekind 1992). The "back-up

signals” hypothesis, in contrast to the multiple message hypothesis, proposes that multiple cues are used to reflect the same quality in an individual (Johnstone 1997). Since each signal allows for some error, evaluating multiple cues simultaneously gives a more accurate assessment of mate-quality. The “species recognition” hypothesis proposes that to avoid hybridization, additional cues used exclusively for species recognition or mate-quality recognition will evolve (Pfennig 2003). Though species recognition and sexual selection are often thought of as a continuum (Boake et al. 1997), the cues used for each may differ in species that are sympatric with closely related species. Cues for species and mate-quality recognition may also conflict when high-quality conspecifics resemble heterospecifics (Pfennig 1998, 2000).

Study system

Poeciliidae

The family Poeciliidae contains 200 species in 20 genera of livebearing fishes. They are found in tropical and subtropical latitudes of the New World. In many of these, females typically have a 30 d ovarian cycle, and are usually more receptive to males for 1-2 d after dropping a brood (Liley 1966). Females store sperm for several months (Baerends et al. 1955) and many females mate multiply. Insemination takes place after a male inserts his gonopodium (modified anal fin used to transfer sperm packets) into the females’ gonopore.

Amazon mollies

The Amazon molly (*Poecilia formosa*) is native to streams and coastal lagoons ranging from southern Texas to Vera Cruz, MX and is introduced in areas of

Central Texas. Amazon mollies are a result of a hybridization event between *P. latipinna* and *P. mexicana*, with the maternal contribution coming from *P. mexicana* (Awise et al. 1991; Scharl et al. 1995). It is a unisexual species that reproduces through gynogenesis. Though genetic transmission is clonal (Balsano 1989), sperm from *P. latipinna* (sailfin molly) or *P. mexicana* (Atlantic molly) is needed to start embryogenesis (Hubbs & Hubbs 1946; Kallman 1962; Darnell et al. 1967). This results in a system where the Amazon molly is essentially a sexual parasite of both *P. mexicana* and *P. latipinna*, as it must mate with one of the two host species to reproduce. Niemeitz et al. (2002) found that male *P. latipunctata*, the Tamsei molly, may also serve as hosts for Amazon mollies both in the lab and the field. The range of the Tamsei molly is very restricted, and falls within the range of another Amazon molly host, *P. mexicana*. Thus, the parental hosts are the main hosts of Amazon mollies.

The successful persistence of *P. formosa* for over 100,000 years (Awise et al. 1991), may be due to five factors. (1) Amazon mollies that successfully acquire mates produce twice the number of female offspring compared to female sailfin mollies, translating into a higher rate of reproduction. (2) Female sailfin mollies copy the mate choice of Amazons, making it advantageous for male sailfin mollies to mate with Amazon mollies (Schlupp et al. 1994). (3) Amazon mollies are more aggressive than female sailfin mollies when interacting with male sailfin mollies (Foran & Ryan 1994). (4) Amazons share genes with sailfin and Atlantic mollies and may be falsely recognized as conspecifics by males of these species (Dries 2003). Finally, (5) Amazon mollies might be exploiting a conflict in species and mate-quality

recognition cues used by male sailfins to select mates. I plan to investigate this last possible mechanism of the maintenance of Amazon mollies.

Sailfin mollies

The sailfin molly, *P. latipinna*, is found along the Gulf coast from North Carolina to the Yucatan Peninsula. It is also found inland in Florida, Louisiana, and Texas in ponds, streams, drainage ditches and saltwater marshes. In this sexually dimorphic species, males have secondary sexual characteristics consisting of a large dorsal fin and enhanced coloration compared to gray females with no enlarged fin. The dorsal fin is erected and presented to the female with a sigmoid curving of the body in courtship displays (Travis & Woodward 1989). This is accompanied by nibbling on the female's gonopore and gonopodial thrusting at the female. Males may also mate without courtship by gonopodial thrusting. This is an attempt at insemination without female cooperation in which the male orients himself behind a female and attempts to insert his gonopodium into the female's gonopore.

Males exhibit genetic polymorphism for body size that is associated with differences in secondary sexual characters and behavior patterns (Farr et al. 1986; Travis & Woodward 1989). Male size is correlated with age at maturity with variation in size at maturity being based on a series of Y-linked alleles (Travis 1994). Larger males take up to 60d to mature whereas smaller males mature within 30d. Larger males court more often than smaller males, who rely solely upon gonopodial thrusting for mating. All mature males are continuously sexually active (Travis & Woodward 1989). Large males aggressively display to other smaller males and limit their access to females (Travis & Woodward 1989; Travis et al. 1990). Aspbury & Gabor (2004)

found that female availability positively affects the amount of sperm male sailfin mollies have ready to transfer to females. Additionally, this response was strongest when large females were available to males. Females gain no known material benefits from males but females prefer to associate with larger males (Ptacek & Travis 1997; Ptacek 1998; Gabor 1999). Males also prefer to associate with larger females (Travis & Trexler 1987; Ptacek & Travis 1997; Gabor 1999) and female size may be an important mate-quality cue in sailfin mollies.

It is not known why this unisexual-bisexual mating complex persists as both male and female sailfin mollies can distinguish between conspecifics and heterospecifics (Gabor & Ryan 2001), even though they live together in mixed shoals (Schlupp & Ryan 1996). It has been postulated that mate-choice copying may help explain this paradox of heterospecific mating. Mate-choice copying is non-independent mate choice in which the probability of choosing a given mate increases if other individuals have previously chosen that mate (Pruett-Jones 1992). As mentioned previously, female sailfin mollies copy the mate choice of Amazon mollies in the laboratory (Schlupp et al. 1994). Male sailfin mollies have also been found to copy the mate choice of other males in the laboratory (Schlupp & Ryan 1997), and both male and female sailfin mollies show mate-choice copying in the wild as well (Witte & Ryan 2002). It must be noted, however, that the studies of mate-choice copying in mollies equated association time with mate choice. Gabor (1999) found that association preference based only on time may not reflect mate choice, thus the interpretation of the mate-choice copying results are not clear. Furthermore, it is unclear from the study of Schlupp et al. (1994) what the costs of mismating are for a

male and if the benefit of increased attractiveness of males outweighs the cost of mating with a heterospecific.

Objectives

I examined two aspects of species recognition that are related to the persistence of Amazon mollies. First, I examined whether male mate preference for larger females could facilitate the maintenance of Amazon mollies. Size as a mate-quality cue may cause conflict when presented concurrently with species recognition cues. Second, I used model fish to examine the relative importance of individual visual cues used by male *P. latipinna* for species recognition. Examining individual cues is the first step towards a complete picture of species recognition under more complex scenarios.

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CHAPTER II

ASEXUALS LOOKING FOR SEX: CONFLICT BETWEEN SPECIES RECOGNITION AND MATE-QUALITY RECOGNITION CUES IN *POECILIA LATIPINNA*

The process of mate choice requires two potentially overlapping processes: species recognition, in which conspecific breeding individuals of the opposite sex are identified and intersexual selection in which high-quality mates are selected (Ryan & Rand 1993; but see Abt & Reyer 1993). Traits indicating high-quality mates are often exaggerated and costly to produce whereas species recognition traits are usually mean values for a population or species (Pfennig 1998). The processes of species recognition and mate-quality recognition may result in a conflict when heterospecifics resemble high-quality conspecifics (Pfennig 1998). In response to this conflict, selection should favor mate preferences that minimize costs associated with heterospecific mating or mating with low-quality conspecifics. One outcome may be that individuals forgo one type of recognition for the other (Pfennig 1998). For example, spadefoot toads (*Spea multiplicata*) are distributed across both sympatric and allopatric populations. Pfennig (2000) found that male *S. multiplicata* that enhance female fertilization success are characterized by an extreme call resembling that of heterospecific males (*S. bombifrons* and *S. couchii*). Female *S. multiplicata* from populations that are allopatric with *S. bombifrons* and *S. couchii* prefer this extreme conspecific call, and this leads to higher fertilization success. Females from populations sympatric with heterospecifics, however, prefer calls of males that are less extreme and are closest to the mean for their population. Therefore, female *S. multiplicata* in sympatric populations on average, exhibit reduced fertilization success and thus compromise potential mate-quality benefits to ensure conspecific matings (Pfennig 2000).

Although female choice is most often examined, male choice can be important in some species. One such case involves mating systems where unisexual females of one species are reliant on sperm from males of a bisexual species to reproduce. In this situation, males incur the dual costs of sperm production/ expenditure and time/ energy expended courting heterospecific females. Males would benefit from both recognizing conspecific mates and choosing high-quality mates.

A well-studied system where species recognition and mate-quality recognition may conflict is the unisexual-bisexual complex of mollies. If shown to be present, this conflict may help explain the maintenance of gynogenetic species. The Amazon molly, *Poecilia formosa*, is a unisexual species that reproduces through gynogenesis and although genetic transmission is clonal (Hubbs & Hubbs 1932; Balsano et al. 1989), sperm from male sailfin mollies, *P. latipinna*, or male Atlantic mollies, *P. mexicana*, is needed to initiate embryogenesis (Hubbs & Hubbs 1946; Kallman 1962; Darnell et al. 1967; Schlupp et al. 2002). From an evolutionary perspective, mating with heterospecifics has no apparent benefits to males (but see Schlupp et al. 1994). Males that mate with Amazon mollies may gain no direct reproductive benefits and, therefore, this behavior should be selected against.

Heterospecific mating can be costly for males with limited resources. When mating, males spend time finding and courting a mate, risk greater exposure to predators, and expend energy through sperm production (reviewed in Andersson 1994). Recent evidence indicates that sperm production costs may be higher than previously thought. Aspbury & Gabor (2004) found that the presence of females positively affects the amount of sperm male sailfin mollies have available to transfer.

Additionally, this response was strongest when large females were available to males, indicating that males may be conserving energy resources in the absence of female stimuli and that there may be constraints on sperm production in males. Thus, it may also be energetically important for males to avoid using sperm for heterospecific matings.

Amazon mollies, however, have successfully persisted for 100,000 years (Avisé et al. 1991; Scharl et al. 1995; Dries 2003). Their persistence may be due to five non-mutually exclusive hypotheses: (1) Amazon mollies have higher reproduction rates; mated Amazon mollies produce twice the number of female offspring as do female sailfin mollies. (2) Female sailfin mollies copy the mate choice of Amazons, making it less costly for male sailfin mollies to mate with Amazon mollies (Schlupp et al. 1994). (3) Amazon mollies are more aggressive than female sailfin mollies when interacting with male sailfin mollies (Foran & Ryan 1994). (4) Amazons share genes with sailfin and Atlantic mollies and may be falsely recognized as conspecifics by males of these species (Dries 2003). Finally, (5) Amazon mollies might be exploiting a conflict in species and mate-quality recognition cues used by male sailfins to select mates. Herein, I investigate the hypothesis that a conflict between species and mate-quality recognition is a possible contributing mechanism of the maintenance of Amazon mollies.

Male sailfin mollies can discriminate between conspecific and heterospecific females, with sympatric males showing a stronger preference for conspecific females than allopatric males (Hubbs 1964; Ryan et al. 1996; Gabor & Ryan 2001). Both sympatric and allopatric males also prefer to associate with larger female sailfin

mollies (Ptacek 1998; Gabor 1999) and larger females are more fecund (Travis et al. 1990; Trexler et al. 1997). Large Amazon mollies may be taking advantage of large body size as a mate-quality cue. Further, males from allopatry have no recent history with Amazon mollies, therefore, one would expect that this behavior has not been selected against by the costs of heterospecific matings, whereas there would have been such selection in sympatry. Consequently, individuals from populations allopatric to Amazon mollies may rely more on traits indicating mate-quality rather than those indicating species identity while males from populations that are sympatric with Amazon mollies may rely more on traits indicating species identity rather than those indicating mate-quality.

I examined whether male mate preference for larger females facilitates the maintenance of Amazon mollies. Size as a mate-quality cue may cause conflict when presented concurrently with species recognition cues. I tested males from allopatric and sympatric populations with a large Amazon molly and a smaller female sailfin molly. I predicted that males would no longer clearly prefer conspecifics as they had in previous studies (Gabor & Ryan 2001). In addition, males from allopatric populations may be more likely to mate with larger Amazon mollies based on the exaggerated mate-quality cue than males from sympatric populations.

MATERIALS AND METHODS

Natural History

Mollies are livebearing fishes. Females typically have a 30 d ovarian cycle, and are usually more receptive to males for 1-2 d after parturition (Liley 1966).

Insemination takes place after a male inserts his gonopodium (modified anal fin used to transfer sperm) into the females' gonopore.

The Amazon molly is native to streams and coastal lagoons ranging from southern Texas to Vera Cruz, MX and is introduced in areas of Central Texas from Brownsville, TX (Brown 1953). Amazon mollies are a result of a hybridization event between sailfin and Atlantic mollies (Hubbs & Hubbs 1932; Hubbs 1964; Avise et al. 1991) with the maternal contribution coming from Atlantic mollies (Avise et al. 1991; Schartl et al. 1995). In the field, Amazon mollies are frequently larger than female sailfin mollies, yet female sailfin mollies have significantly larger standard length (SL) than Amazon mollies ($N_{\text{female sailfin}} = 64$ (mean \pm SD = 38.3 ± 4.6); $N_{\text{Amazon}} = 139$ (36.5 ± 6.5); unpaired t -test $t = -2.0$, $p = 0.047$). However, Amazon mollies show significantly more variation in size (Kolmogorov-Smirnov $C^2_{(2)} = 18.2$; $p = 0.0002$). In other words, although female sailfin mollies are on average larger, Amazon mollies grow larger than female sailfin mollies. From these field measurements, the largest female sailfin had a SL of 47mm while the largest Amazon molly I measured had a SL of 57.7 mm.

Sailfin mollies are found along the North American Gulf coast from North Carolina, USA to the Yucatan Peninsula. They are also found inland in Florida, Louisiana, and Texas in ponds, streams, drainage ditches and saltwater marshes. Males are sexually dimorphic with secondary sexual characteristics consisting of a large dorsal fin and enhanced coloration compared to gray females with no enlarged fin. The dorsal fin is erected and presented to the female with a sigmoid curving of the body in courtship displays (Travis & Woodward 1989). This is accompanied by

nibbling on the female's gonopore and gonopodial thrusting at the female. Males may also mate without courtship by sneaky gonopodial thrusting. This is an attempt at insemination without female cooperation in which the male orients himself behind a female and attempts to insert his gonopodium into the female's gonopore. Females gain no known material benefits from males.

Collection and Maintenance

All fishes were collected from natural populations by seine and dip-net. The following five populations of sailfin mollies are sympatric with Amazon mollies (date collected): Venus, Mexico (May 2002); Alfred Bonfil, Mexico (May 2002); Vincente Guerrero, Mexico (March 2003); Comal, Texas (May 2002); and Martindale, Texas (May 2002). Allopatric populations of sailfin mollies were collected from two sites: Spring Lake (Aquarena Springs), Texas (May 2002); and Lafayette, Louisiana (March 2003); Destin, Florida (1997). All population were first generation fish except for the allopatric Destin, FL population, which was lab reared. The Amazon mollies used in test of allopatric populations were lab reared from populations collected at Rio Tigre, MX (1989; 1998). All Mexican populations are from the Tamaulipas region of Mexico and are naturally sympatric. The Martindale, TX and Comal, TX sites represent introduced sympatry, with fish coming from populations in Florida and Louisiana in the 1930's (Brown 1953). Spring Lake, TX is an allopatric population, introduced in 1944, with fish originating from populations in Florida (Brown 1953).

Fishes were transported by car to Texas State University at San Marcos, TX, where they were maintained in a laboratory in 38 l aquaria (54 cm x 29 cm x 33 cm)

and 53 l aquaria (76 cm x 32 cm x 32 cm). Fishes were maintained at a constant temperature (22-25 C) and on a 14 L:10 D cycle with UV florescent lighting that simulates daylight (40 W Coralife Day-Max Aquarium daylight, 40 W Coralife Actinic Blue, 40 W Coralife 10,000k high intensity purified super daylight, and 40 W General Electric). Fishes were fed Spirulina and Freshwater flake food (Ocean Star International Inc.) twice a day and supplemented with live and freeze-dried brine shrimp. Populations were housed separately and within each population I separated sex and species for at least 30 d before testing. The isolation of females from males for at least 30 d increases the chance that the females will be at a similar stage in the brood cycle. As females have a 30 d ovarian cycle, most females will have dropped any broods that they may have been carrying (Farr & Travis 1986; Snelson et al. 1986). Only mature males, identified by the fusion of the anal fin into the gonopodium, were used in trials.

Species Recognition vs. Mate Quality Recognition Mating Trials

Mating trials were conducted in a 38 l aquarium (54 x 29 x 33 cm) that contained tan gravel and 24 cm of aerated and filtered water. A 15 W Sun Glo full spectrum fluorescent light (General Electric) was placed directly on top of the aquarium. Three sides were covered with black plastic to prevent test fishes from being distracted by the environment around the tank. The front of the tank was covered with one-way film to minimize disturbance of the fishes during observation. All fishes were fed prior to testing. I conducted trials from 0900-1500 h, 17 June 2002 to 30 September 2002 and 25 April 2003 to 27 August 2003.

Mature males (mean \pm s.e.m. = 32.76 ± 0.62 mm; range of 20.9-54.0 mm) were selected haphazardly as test fish. Testing was performed as in Gabor & Ryan (2001) except in each trial, Amazon mollies were at least 10 mm SL \geq female sailfin mollies whereas Gabor & Ryan (2001) used size-matched females. For each trial, one male sailfin molly was first placed in a clear plastic cylinder (12 cm diameter x 30 cm) in the middle of the aquarium. Females (one sailfin, one Amazon) were simultaneously placed in the aquarium and allowed to swim freely. After a 10 min habituation time and careful removal of the plastic cylinder, I recorded the number of gonopodial thrusts (actual mating attempts) that males directed towards each female for 10 min after the first gonopodial thrust. Males were considered unresponsive if they performed less than five gonopodial thrusts. Unresponsive males were re-placed in their home tank to be re-tested in the future.

I used a two-tailed Wilcoxon sign-ranked test to compare the number of gonopodial thrusts directed toward conspecific and heterospecific females within populations. The number of gonopodial thrusts indicates actual male mate preference. I then calculated the strength of preference (SOP) for each male as the proportion of the total gonopodial thrusts directed towards sailfin mollies. A Mann-Whitney *U*-test was used to compare the pooled SOP for all sympatric and allopatric populations as well as for only naturally sympatric and allopatric populations. Non-parametric tests were used because our data was non-normally distributed after all attempts at transformation. Sample sizes vary for a few reasons; we could not collect the same number of males from each population, not all males responded, and some

populations experienced death in the lab before testing. All analyses were two-tailed with $\alpha = 0.05$.

RESULTS

Males from four of the sympatric populations showed no significant preference for either conspecific or heterospecific females (Table 1; Fig. 1.1). Males from the naturally sympatric Alfred Bonfil, Mexico population attempted to mate (number of gonopodial thrusts) significantly more with conspecific females than with heterospecifics (Fig. 1.1). Males from two of the allopatric populations showed no significant mating preference for heterospecifics or conspecific females (Table 1; Fig. 1). Males from the allopatric population from Lafayette, LA, however, attempted to mate significantly more with heterospecifics than with conspecific females (Fig. 1.1).

When the strength of preferences of males from all allopatric and sympatric populations were pooled, males from allopatry showed a significantly greater strength of preference for heterospecifics than did males from the pooled sympatric populations (Mann-Whitney *U*-test, $U = 795$, $z = -2.176$; $p = 0.0296$; Fig. 1.2a). Further, when strengths of preference of males from only naturally occurring sympatric and allopatric populations were pooled, there was again a significantly greater strength of preference for heterospecifics by males from allopatry than from sympatry (Mann-Whitney *U*-test, $U = 37$, $z = -3.515$; $p = 0.0004$; Fig. 1.2b).

DISCUSSION

Results of this study, when viewed in conjunction with previous studies provide evidence for a conflict in mate-quality and species recognition cues used by male sailfin mollies in making mating decisions. I tested male sailfin molly preference for conspecific and larger heterospecific females in populations that were sympatric with, and that were allopatric to Amazon mollies. Although a general conspecific preference is expected, males from sympatry showed no significant preference for conspecific females, except in one population (Population AB, Alfred Bonfil, MX; Fig 1.1). Males in allopatry also showed no significant conspecific mating preference, and one population (LA, Lafayette, LA; Fig 1) showed a heterospecific mating preference. My results are especially interesting when compared to similar trials using size-matched females by Gabor & Ryan (2001). Whereas Gabor & Ryan (2001) found that all six of their populations from sympatry showed a conspecific preference, only one of my sympatric populations showed a conspecific preference (Fig. 1.1). Further, Gabor & Ryan (2003) found that three of five allopatric populations also showed a conspecific preference, while none of my three allopatric populations demonstrated significant conspecific preferences with males from one allopatric population showing a significant preference for the larger heterospecifics (Fig. 1.1).

Male sailfin mollies have been tested for preference of size-matched conspecific and heterospecific females in previous studies that used different populations from the same geographic regions (Gabor & Ryan 2001; Ryan et al. 1996). These studies showed similar strengths of preference for conspecific females and suggest that male

preferences have remained relatively constant over time. Thus, as compared to the size-matched females used in Gabor & Ryan (2001) and Ryan et al. (1996), the changes in male mating preferences across the similarly located populations in my study demonstrated that the lack of clear mating preferences for conspecifics in most populations is a result of the treatment where Amazon mollies were larger than the female sailfin mollies.

While most populations lack significant mating preferences when the heterospecific female was larger, two populations showed significant preferences in the expected directions based on my predictions. Males from the Alfred Bonfil, Mexico site, which is deep within sympatry, preferred small conspecifics to larger heterospecifics more than males from other populations. Thus, males from this population may sacrifice mate-quality for conspecific matings. These males may be avoiding larger females and consequently avoiding mating with heterospecifics. In contrast, by preferring to mate with larger heterospecifics to conspecific females, males from the geographically distant allopatric site in Louisiana may be emphasizing mate-quality cues over species recognition cues.

Specifically, the data reported herein support two predictions from Pfennig (1998) that indicate a conflict in species and mate-quality cues; (1) a lack of significant preferences when high quality conspecifics resemble heterospecifics (Fig. 1.1), and a difference in the use of species recognition versus mate-quality recognition cues between sympatric and allopatric populations (Fig. 1.2a, b). When all sympatric populations and all allopatric populations are pooled (Fig 1.2a), there is a difference in strength of preference with males from sympatry showing a greater strength of

preference for conspecifics and males from allopatry showing a greater strength of preference for heterospecifics. Pooling only naturally occurring sympatric and allopatric populations, results in a similar but more pronounced difference in preference between sympatric and allopatric males (Fig. 1.2b). The change in strength of preference indicates that the introduced sympatric populations may still be in the process of evolving responses to Amazon mollies but heterospecific avoidance has not completely evolved in the 60 years since their introduction.

When a conflict in species recognition and mate-quality cues occurs, it may be resolved in different ways depending on the relative importance of both species recognition and mate-quality assessment. The resolution will depend on the costs of mating with heterospecifics and the frequency in which heterospecifics are encountered (Pfennig 1998, 2000). There may be a compromise of one type of cue for another but this is not a long-term solution. Selection will ultimately favor individuals that minimize recognition mistakes such as mating with heterospecifics and/or mating with low-quality mates (Ryan et al. 2003) especially when sperm is limited as it is in sailfin mollies (Aspbury & Gabor 2004). One outcome of such selection may yield traits used for species recognition differing from those used for mate-quality recognition. In this unisexual-bisexual species complex of mollies, I expect high-quality sailfin females to produce species recognition cues that are distinct from Amazon mollies. However, Dries (2003) suggests that Amazon mollies may garner matings due to the genes they share with sailfin mollies as a result of their hybrid origin. This may limit the amount of morphological divergence that can occur between the species due to their shared ancestry. Thus, males may require alternative

mechanisms to discriminate between females. Another evolutionary result of the conflict in cues may be for males to assess multiple species-specific cues, where different cues give different information about mate-quality (Candolin 2003). Thus, increasing male recognition of high-quality females while avoiding the risk of mating with a heterospecific (Pfennig 1998).

In this experiment, I examined male preference for conspecific and heterospecific females when the heterospecific females were larger in SL where larger SL equates to higher female fecundity. Naturally occurring variation in female size may be influenced by many factors: (1) Amazons that are larger in size may be older as both Amazons and female sailfin mollies continue to grow after reaching maturity. (2) However, other genetic and environmental factors may also influence female size such as foraging skills and competition. (3) Amazons may have a faster growth rate, allowing them to grow larger throughout their lifespan than female sailfin mollies and thus, fully exploit male preference for large size. I cannot differentiate between these hypotheses but my data, and that of Gabor & Ryan (2001), suggests that size related factors are important for the persistence of Amazon mollies.

Wymann & Whiting (2003) recently examined a conflict in mate-quality and species recognition cues in relation to size in two allopatric species of flat lizards (*Platysaurus broadleyi* and *P. capensis*) and found a similar conflict. They showed that these lizards used size as a mate-quality cue and exhibited a loss of conspecific preference when presented with a larger heterospecific female.

Size is widely regarded as indicating mate-quality in both males and females (review in Andersson 1994) although it may also be important to consider conflicts

involving mate-quality cues other than size. Thus far, no study has identified what cues, other than size male sailfin mollies use for identifying high-quality mates or for identifying conspecifics. Female sailfin mollies are, on average, smaller in sympatric populations than in allopatric populations (Gabor unpublished data). Thus, males from sympatry would be expected to recognize smaller females as conspecifics as they have in the sympatric population in Alfred Bonfil, Mexico. These males may be using small size as a species recognition cue in avoidance of large Amazon mollies.

In conclusion, there is a conflict in species recognition and mate-quality recognition cues used by male sailfin mollies in both sympatry and allopatry. This conflict may affect the evolution of male preferences for conspecific mates, sexually selected characters, and may lead to important evolutionary differences between sympatric and allopatric populations. Understanding male preferences and female cues will lead to a better understanding of mate choice processes and may help to explain the persistence of unisexual, gynogenetic Amazon mollies as they may be exploiting this conflict to garner matings.

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Table 1. Comparison of gonopodial thrusts among populations of male *Poecilia latipinna* as directed at female *P. latipinna* and *P. formosa* (Wilcoxon sign-ranked test comparing mean number of thrusts directed at female sailfin mollies, *P. latipinna*, and Amazon mollies, *P. formosa*, for males of each population tested). Population designations are as follows: LA, Louisiana; FL, Florida; MX, Mexico.

	Population	mean \pm s.e.m. number of thrusts directed at		<i>n</i>	<i>Z</i>	<i>p</i>
		<i>P. latipinna</i>	<i>P. formosa</i>			
Natural Sympatry	Vincente Guerrero, MX	22.20 \pm 14.34	6.80 \pm 3.07	10	-0.05	0.95
	Alfred Bonfil, MX	48.46 \pm 13.60	8.27 \pm 5.22	11	-2.31	0.02
	Venus, MX	31.44 \pm 13.04	11.00 \pm 4.07	8	-0.14	0.88
Introduced Sympatry	Martindale, TX	76.55 \pm 21.89	37.27 \pm 12.48	11	-1.51	0.13
	Comal Springs, TX	21.62 \pm 8.76	35.39 \pm 11.70	13	-1.50	0.13
Introduced Allopatry	Spring Lake, TX	30.96 \pm 8.78	31.96 \pm 8.68	25	-0.03	0.98
Natural Allopatry	Lafayette, LA	8.75 \pm 7.38	51.88 \pm 11.61	8	-2.10	0.03
	Destin, FL	9.00 \pm 4.55	29.50 \pm 9.43	6	-1.57	0.11

Figure Legends

Figure 1.1 Box plots representing male strength of preference estimated by the number of gonopodial thrusts directed towards *Poecilia latipinna* divided by the total number of gonopodial thrusts when the SL of *P. formosa* are ≥ 10 mm than female *P. latipinna*. Gray boxes are naturally sympatric populations, hatched boxes are introduced sympatric populations, spotted boxes are introduced allopatric populations and white boxes are naturally allopatric populations. The middle horizontal lines represent the medians for each population and the upper and lower lines of the boxes represent the first and third quartiles. The whiskers represent the range (Sokal & Rohlf 1995). Significance levels correspond to p - values from Wilcoxon sign-ranked tests comparing the number of thrusts directed at sailfin molly and Amazon molly females: * = $p < 0.05$; n.s. = $p \geq 0.05$. Data above the no-preference line ($y = 0.5$) indicate increasing strength of preference for female sailfin mollies, data below the line indicate an increasing preference for Amazon mollies. The left-most population is the population in 'deepest sympatry.' Populations to the right appear in order of increasing distance from deepest sympatry.

Figure 1.2 Box plots representing the pooled male sailfin molly strength of preference in sympatric and allopatric populations. (a) Data is pooled for all sympatric and all allopatric populations. (b) Data pooled for only naturally occurring sympatric and allopatric populations. Data above the no-preference line ($y = 0.5$) indicate an increasing strength of preference for females sailfin mollies, data below the line indicate an increasing preference for Amazon mollies. Significance is based

on a Mann-Whitney U -test on the number of gonopodial thrusts directed towards *P*.

latipinna and *P. formosa*: * = $p < 0.05$; n.s. = $p \geq 0.05$.

Figure 1.1

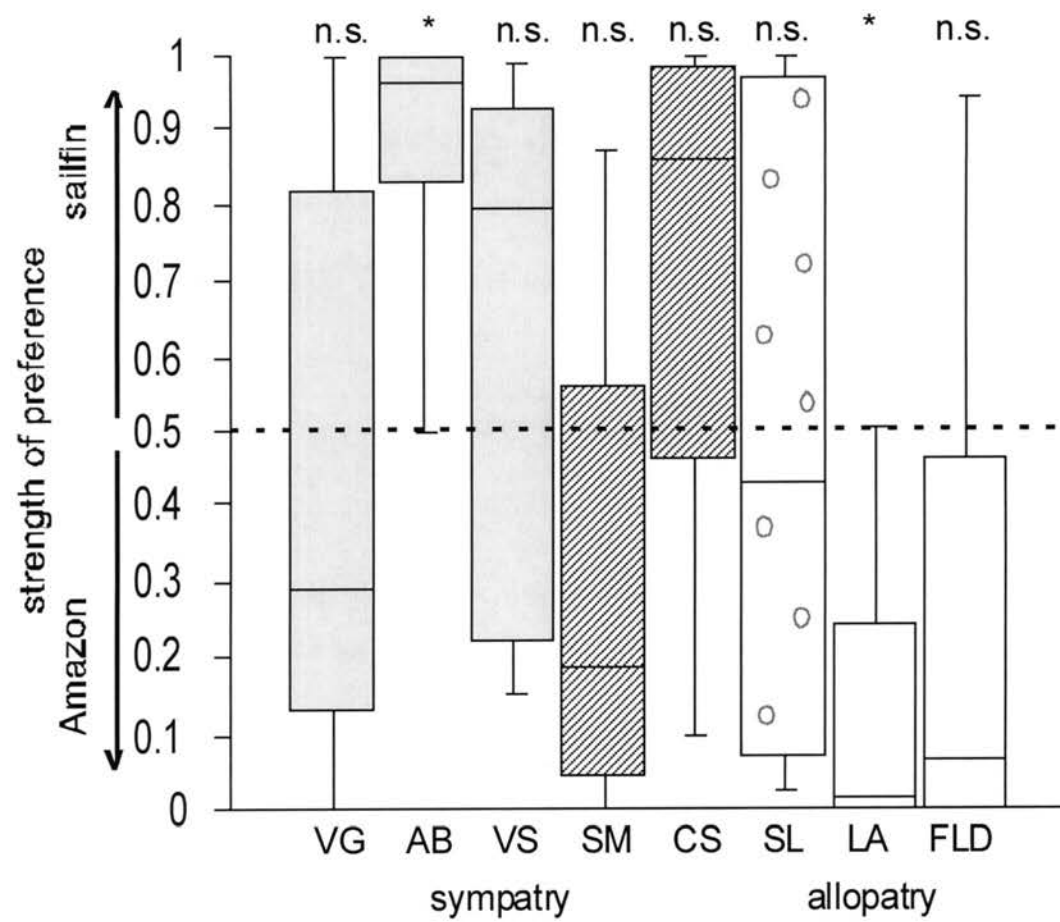
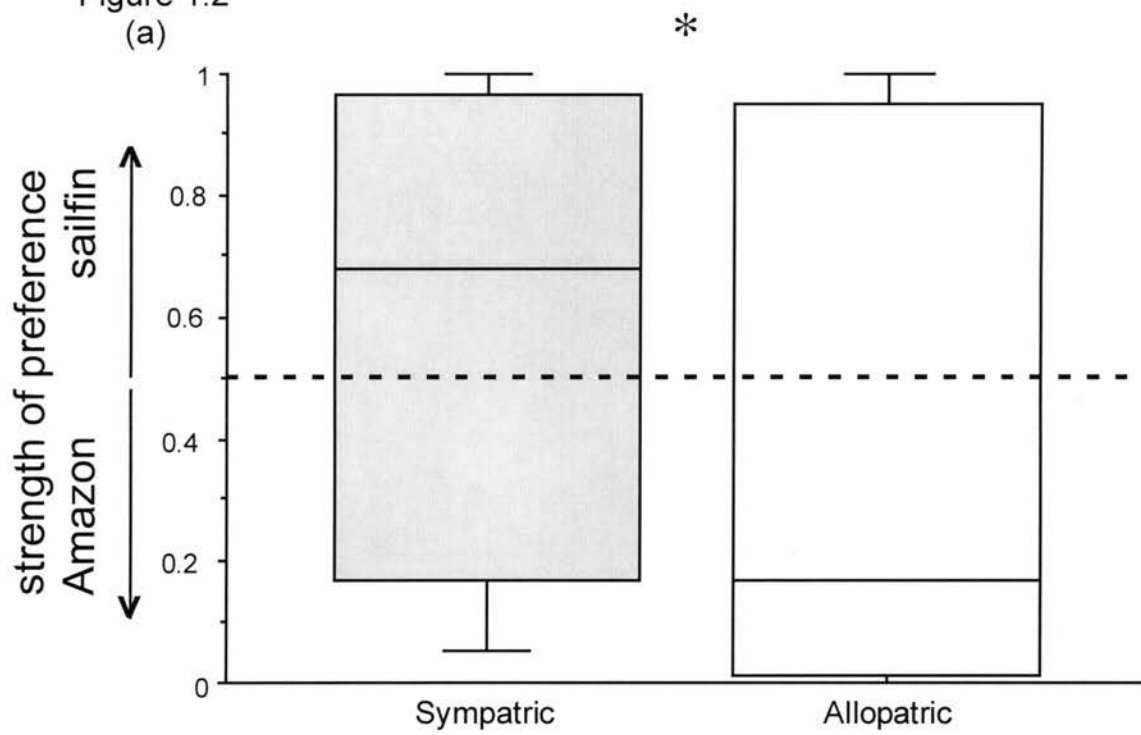
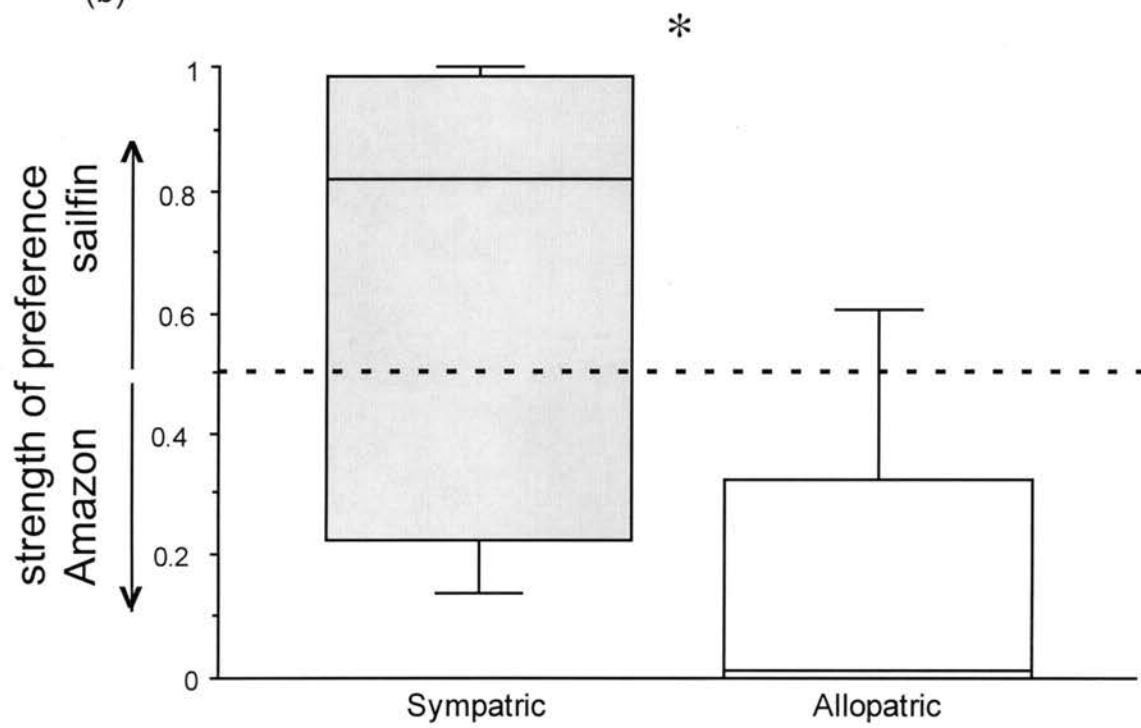


Figure 1.2
(a)



(b)



CHAPTER III

DO I KNOW YOU?

SPECIES RECOGNITION IN A UNISEXUAL-BISEXUAL SPECIES COMPLEX OF MOLLIES

When choosing a mate in a system where closely related heterospecifics are sympatric, individuals are expected to choose a genetically compatible conspecific and avoid heterospecifics (Dobzhansky 1937). To do this, animals may assess multiple morphological and behavioral traits, as well as weigh the relative importance of each trait. Candolin (2003) reviews the current hypotheses to explain the evolution of multiple cues including the multiple message hypothesis, the back-up signal hypothesis, and the species recognition hypothesis. While the multiple message and back-up signal hypotheses focus on multiple cues used solely for assessing mate-quality, the species recognition hypothesis proposes that cues used to identify a genetically compatible mate will differ from those used to identify a high-quality mate (Pfenning 1998, 2000).

Several studies have examined the possible use of multiple cues as a mechanism of species recognition. For the green tree frog (*Hyla chrysoscelis*), multiple cues each have individual importance. Female green tree frogs use pulse rate of male calls for species recognition, whereas call duration rate indicates male genetic quality and is used for mate-quality recognition (reviewed in Gerhardt 2001). Although female swordtail mollies (*Xiphophorus pygmaeus*) use vertical bars and chemical cues to assess species identity (Hankison & Morris 2003), they also prefer larger conspecific males, which could lead to mating with larger sympatric heterospecific males (*X. cortezi*) that overlap in size with *X. pygmaeus*. By testing female *X. pygmaeus* preference for vertical bars and chemical cues individually and in combination, Hankison & Morris (2003) found that both chemical cues and vertical bars were required for females to discriminate between relatively smaller conspecifics to larger

heterospecifics. Thus, by using multiple cues, females relied on a 'back-up' signal to avoid mating with heterospecifics or compromising between species and mate-quality recognition. The benefits of discriminating against a heterospecific and mating with a lower quality conspecific may be strong enough to balance the cost of assessing multiple cues.

Individuals may not only evaluate multiple traits but may also evaluate multiple components of a single composite trait. For example, female green swordtails (*Xiphophorus helleri*) prefer males with swords, extensions of the ventral caudal fin rays that have dorsal and ventral black stripes and yellow/green coloration between the two black stripes. Using digital video technology, Basolo & Trainor (2002) isolated each component of the male sword and found that females significantly preferred swords with black components to those without any black. Females also exhibited a preference for the yellow coloration between the black stripes, indicating that multiple sword components were evaluated by females.

It is especially relevant to determine the cues used in mate choice in species that risk mating with heterospecifics. One such example occurs in sympatric populations of *P. latipinna* and *P. formosa* where *P. formosa*, a unisexual gynogenetic species, must mate with males of closely related species (*P. latipinna* or *P. mexicana*) to initiate embryogenesis (Hubbs & Hubbs 1946; Kallman 1962; Darnell et al. 1967). However, inheritance in the resulting offspring is strictly maternal (Hubbs & Hubbs 1932; Balsano et al. 1989). *Poecilia formosa* is a result of specific hybrid events between *P. latipinna* and *P. mexicana* and has persisted in

natural environments for at least 100,000 years (Avise et al. 1991; Scharl et al. 1995).

Male *P. latipinna* prefer to mate with conspecific females (Gabor & Ryan 2001), but mate with *P. formosa* as well. Four non-mutually exclusive hypotheses may explain why males continue to mate with heterospecifics: (1) Female *P. latipinna* copy the mate choice of *P. formosa*, providing benefits for male *P. latipinna* that mate with *P. formosa* (Schlupp et al. 1994), (2) *Poecilia formosa* are more aggressive than female *P. latipinna* when interacting with male *P. latipinna* and thus illicit matings (Foran & Ryan 1994), (3) *Poecilia formosa* share genes with *P. latipinna* and *P. mexicana* and may be falsely recognized as conspecifics by males of these species (Dries 2003), (4) There is a conflict in species and mate-quality cues used by male *P. latipinna* when selecting mates that *P. formosa* may be taking advantage of. I have found (Chapter I) that in most populations surveyed, males presented with a choice to mate with relatively smaller conspecifics or relatively larger *P. formosa* no longer prefer conspecifics. These results suggest that large size is an indicator of mate-quality possibly because larger size indicates greater fecundity in *P. latipinna* (Travis et al. 1990; Trexler et al. 1997) and this cue conflicts with species recognition cues (Chapter I). At present, the cues important for species recognition in *P. latipinna* are unknown, while it is possible that both visual and chemical cues are important. Visual cues, however, are the likely source of differentiation as Gabor et al. (unpublished data) have found that males are not using chemical cues for species recognition.

The hybrid origin of *P. formosa* has resulted in a morphology intermediate between the parental species (Hubbs & Hubbs 1946; Dries 2003). *Poecilia formosa* has fewer dorsal fin rays (10-12) than *P. latipinna* (13-15), and the dorsal fin is positioned more anteriorly in female *P. formosa* than in *P. latipinna* (Hubbs & Hubbs 1932, Gabor unpublished data). There is also a lateral spotted pattern present on the body of *P. latipinna* that *P. formosa* lacks. To determine which visual traits males may be using for species recognition, I examined if these differences are important in mate choice by male *P. latipinna*.

My objective was to use model fish to examine the relative importance of individual visual cues used by male *P. latipinna* for species recognition. Examining individual cues is the first step towards obtaining a complete picture of species recognition under more complex scenarios. I formulated my models after *P. latipinna* models used by MacLaren et al. (2004). I first tested male preference for unaltered models of *P. latipinna* and *P. formosa* versus no stimulus. Second, I tested male preference for unaltered *P. latipinna* models versus unaltered *P. formosa* models. Finally, I tested males with models that isolate traits that differ between female *P. latipinna* and *P. formosa*. I predicted that males will prefer conspecifics and that they will have a stronger strength of preference for the isolated cues that best indicate conspecific sailfin females.

MATERIALS AND METHODS

Collection and Maintenance

The population of *P. latipinna* I used is from the Tamaulipas region of Mexico and is naturally sympatric with *P. formosa*. I collected both *P. latipinna* and *P.*

formosa (March 2003) by seine and dip-net and fishes were transported by car to Texas State University - San Marcos, TX. The fishes were maintained in a laboratory in 38 l aquaria (54 x 29 x 33 cm) at a constant temperature (22-25° C) and were maintained on a 14 L:10 D cycle with UV florescent lighting that simulates daylight (40 W Coralife Day-Max Aquarium daylight, 40 W Coralife Actinic 03 Blue, 40 W Coralife 10,000k high intensity purified super daylight, and 40 W regular fluorescent). Fishes were fed Spirulina and Freshwater flake food (Ocean Star International Inc.) twice daily and supplemented with live and freeze-dried brine shrimp. Only mature males, identified by the fusion of the anal fin into the gonopodium, were used in trials. Mature female *P. latipinna* and *P. formosa* that served as 'subject females' originated from the same population as the males that I tested.

Isolation of Species Recognition Cues

Construction of models

I took digital photos of 18 females per species from the sympatric population. Fishes were isolated in a small section (8 x 4 x 6 cm) of a 19 l aquarium (40 x 20.5 x 26.5 cm) with Plexiglass dividers. The aquarium was filled with 20 cm of water with white paper covering the back to provide a background for the photos. The aquarium was in a dark room, which excluded outside light and facilitated consistent lighting for all the pictures. A 15 W Sun-Glo full spectrum light (General Electric) was placed directly above the aquarium and a 60 W Plant Gro and Show (General Electric) light was placed in front of the aquarium to enhance definition of the images. Pictures were

taken with a digital camera (Nikon Digital CoolPix 950) placed 15cm from the aquarium. The setup of the tank allowed me to take pictures when fishes had all fins extended. In addition, all fishes were at a similar distance and angle to the camera.

To prepare the photos for construction of models, I used Adobe Photoshop 5.5 to cut-out each image. To control for size differences, each image was then 'free transformed' to 38 mm, the mean size of females (*P. latipinna* and *P. formosa*) for the population (mean \pm s.e.m. = 38.0 ± 0.37 mm; range from 30.1 – 45.4 mm). Although fishes may differ in characteristics on each lateral side, I used only one side to minimize this effect on male preference. I haphazardly selected the side used for each image based on the best image available for each fish. I then printed each fish image, horizontally flipped the image and printed these onto transparencies using a Hewlett Packard 7350 printer. I cut out the mirror images and glued (Elmer's) them together, fastening a piece of white paper cut in the shape of the fish's body (excluding the fins) between the two transparencies. The resulting two-dimensional models have detailed opaque bodies and transparent fins.

I randomly paired each of the 18 *P. formosa* models with one of the 18 model female *P. latipinna*. To isolate individual variables, I digitally manipulated pictures to make 'hybrid' models from the randomly paired models. For example, *P. formosa* #1 was paired with *P. latipinna* #4. To create a model 'hybrid' that isolated the dorsal fin differences, I cut the dorsal fin of *P. formosa* #1 from its picture and replaced it with the dorsal fin from *P. latipinna* #4. The fin was rotated to best fit the natural slope of the dorsal surface and positioned the same distance from the snout as it had been on the *P. latipinna* #4 image using a background grid for measurements. To create a

model ‘hybrid’ that isolated the lateral spotted pattern, the images of both fish (*P. formosa* #1 and *P. latipinna* #4) were aligned on a grid and Adobe Photoshop 5.5’s ‘rubber stamp’ tool (a cloning brush that samples from a set point on an image and paints to another) was used to transfer the *P. latipinna* #4 lateral spot pattern directly onto the *P. formosa* #1 body in the exact area. Finally, in order to control for potential shape differences, the dorsal fin and spotless body pattern of *P. formosa* #1 were transferred in the same manner onto the *P. latipinna* #4 image. My resulting ‘hybrid’ models consisted of (1) a *P. formosa* with a *P. latipinna* fin/placement (2) a *P. formosa* with *P. latipinna* lateral spotted pattern, and (3) a *P. latipinna* with *P. formosa* fin and lacking the lateral spotted pattern.

Animation

To animate the models, I created a motorized pulley system that was placed directly on top of the aquarium. I used a 120 v, AC motor with a two-speed switch that was taken from a fan. I integrated a common household dimmer switch to the motor and attached it to a wooden board (91.4 x 15.2 x 2.5 cm). Small rubber bands ran from the shaft of the motor (0.6 cm diameter) to a plastic spool (1.3 cm diameter) and elastic bands then ran to plastic disks (24.8 cm diameter) which served as the pulley. I attached a metal bar (11.4 cm) to the center of each pulley. Fishing line (16 cm) was tied to this L-shaped metal bar so when models were glued to the fishing line, they rotated in a circle (22.9 cm diameter) clockwise on each side of the tank. The speed of the stimulus models was constant within each trial

Time Trials

I ran trials in a 38 l aquarium (54 x 29 x 33 cm) that contained tan gravel and 24 cm of aerated and filtered water. A 15 W Sun-Glo full spectrum light (General Electric) was placed directly on top of the aquarium and two standard fluorescent lights were placed at an approximately 45 degree angle 5 cm from the back of each side of the aquarium. This lit both the model fish and the test fish. I placed the aquarium in a darkroom to block out all other light sources and covered the back-side with black plastic to prevent test fishes from being distracted by the environment around the tank. I lined the side walls of the testing chamber (35 cm from the sides of the tank) with white poster board to provide a uniform background for the models. I covered the front of the tank with one-way film to minimize disturbance of the fishes by outside activity. All fishes were fed prior to testing.

I divided the aquarium into three visual sections by marking on the one-way film. The outer sections (9 cm of each end of the aquarium) were the choice sections while the inner compartment was the no choice area. After attaching the models to the pulley system and turning on the motor, a male *P. latipinna* was placed in the center of the aquarium under a clear plastic cylinder (12 cm diameter x 15 cm) and allowed to acclimate for 10 min. After release, I recorded time spent by the male in each of the choice sections of the tank for 10 min. I reversed the sides of the models and allowed the male 10 min to re-acclimate with the models rotating before running the trial again. This controlled for any potential side bias of the test males. The initial left-right position of the transparencies was randomized between tests and the

transparencies were randomly selected from the 18 models of each species with no two pairs re-used.

Experiment 1: Model Control

To test male preference for unmanipulated models, I tested males ($n = 30$) with (1) *P. latipinna* model vs. no stimulus; (2) *P. formosa* model vs. no stimulus; and (3) *P. latipinna* model vs. *P. formosa* model. I randomized the order of the treatments between (1) and (2) and then tested treatment (3) so males were familiar with both species of model. Males were tested at an interval of 24 h between treatments.

Experiment 2: 'Hybrid' Preference

I tested male preference for 'hybrid' models by randomly pairing each 'hybrid' with one of the 18 unaltered *P. formosa* models. I chose to pair males with unaltered *P. formosa* because male *P. latipinna* would be expected to prefer unaltered *P. latipinna* models over any 'hybrid' model which does not have a full suite of *P. latipinna* characters. Alternatively, a male preference for any altered 'hybrid' model with at least one *P. latipinna* trait might be expected over an unaltered *P. formosa* model. Therefore, by pairing the 'hybrids' with unaltered *P. formosa* models, I expect males to have a stronger preference for those 'hybrids' that have cues that they recognize as *P. latipinna* characteristics over the unaltered *P. formosa* models. Males were tested ($n = 30$) in the following treatments; (1) *P. formosa* vs. *P. formosa* with *P. latipinna* fin/placement; (2) *P. formosa* vs. *P. formosa* with *P. latipinna* spotted pattern; and (3)

P. formosa vs. *P. latipinna* with *P. formosa* fin/placement and lack of spotted pattern (Fig. 2.1). I randomized all three treatments with an interval of 24 h between each treatment.

Statistical Analyses

A Wilcoxon sign-ranked test was used to compare the amount of time males spent on the right side within each trial among treatments for both experiments (Gabor 1999). To determine which trait elicited the greatest strength of preference (SOP) for conspecific females, I subtracted the amount of time spent with unaltered *P. formosa* models from the time spent with models altered with female *P. latipinna* traits and compared the SOP among the treatments using a Kruskal-Wallis test.

I also calculated male ‘responsiveness’ for each trial as the total time spent not in the middle (non-choice section) over the total time of each trial (1200 s) (Gabor & Page 2003). Responsiveness scores near one in trials with a high strength of preference indicate a strong choice for one of the stimuli. Responsiveness scores near one could also indicate a high degree of movement between the two stimuli if occurring in a trial with a low strength of preference. Responsiveness scores near zero indicate little interaction with either stimulus.

RESULTS

Males significantly preferred to associate with *P. latipinna* models over no stimulus (Wilcoxon sign-ranked test; $N = 30$, $Z = -3.114$, $p = 0.001$), *P. formosa* models over no stimulus ($n = 30$, $Z = -2.293$, $p = 0.021$, and *P. latipinna* models over

P. formosa models ($n = 30$, $z = -2.561$, $p = 0.010$) (Fig. 2.2). Males had no significant preferences for any 'hybrid' models over *P. formosa* models (Fin: $n = 30$, $Z = -0.062$, $p = 0.9508$; Spotted Pattern: $n = 30$, $Z = -0.854$, $p = 0.3933$; Shape: $n = 30$, $Z = -0.267$, $p = 0.7892$). There are also no significant differences in strength of preference between the three types of hybrids ($p = 0.7720$) (Fig. 2.3).

The responsiveness scores for the unaltered models versus no stimuli were ($n = 30$; mean \pm s.e.m.; *P. latipinna* = 0.488 ± 0.027 ; *P. formosa* = 0.473 ± 0.030). For the trials of unaltered *P. latipinna* versus unaltered *P. formosa* they were ($n = 30$; mean \pm s.e.m. = 0.532 ± 0.033). Responsiveness scores for the 'hybrid' trials were ($n = 30$; mean \pm s.e.m.; Fin = 0.580 ± 0.028 ; Pattern = 0.576 ± 0.034 ; Shape = 0.612 ± 0.025).

DISCUSSION

I found that when presented with unaltered models, male *P. latipinna* significantly recognized and preferred to associate with female *P. latipinna* models over no stimulus. Male *P. latipinna* also significantly preferred to associate with unaltered model *P. formosa* over no stimulus. The experiments demonstrating significant male preferences for models over no stimuli serve as a control to show that models generate the expected response in male *P. latipinna*. Male response to the models indicates that the models are reasonable facsimiles of real fishes. Males also significantly discriminated between and preferred to associate with female *P. latipinna* over *P. formosa* (Fig 2.2), as expected from previous work showing that

males prefer to associate with live conspecific females (Schlupp et al. 1991). This further demonstrates that males are appropriately responding to the stimuli.

I found that male *P. latipinna* did not significantly discriminate between unaltered *P. formosa* models and ‘hybrid’ models that isolated the individual species-specific cues of dorsal fin size and placement, lateral spotted pattern and body shape (Fig 2.3). Further, there was no difference in strength of preference for any of the altered models. These results indicate that although the unaltered *P. latipinna* and *P. formosa* models resulted in significant male preferences, my data did not support the hypothesis that the isolated visual cues are sufficient as single species recognition traits. While it is clear that unaltered models elicit expected responses, I do not know if the altered models accurately represented the traits tested.

The high responsiveness scores combined with high strength of preference for the unaltered *P. latipinna* over the unaltered *P. formosa* models indicates that in these trials, males are making a strong choice for the *P. latipinna* models. The low responsiveness scores in the trials testing unaltered models over no stimuli indicate males exhibit a high degree of movement between the choice compartments but still display a significant preference. In contrast, the ‘hybrid’ trials all had high responsiveness scores combined with low strength of preference. Males in these trials are not interacting with either stimuli and may be avoiding the models.

If male *P. latipinna* assess multiple cues concurrently, individual cues would be insufficient to elicit a response; instead, we would expect males to prefer the full suite of cues. My results are congruent with these expectations. Combinations of cues may be important as visual cues can interact in a complex manner. To examine cue

interactions, Kunzler & Bakker (2001) used computer-animated sticklebacks to test preferences of single and combined visual traits. Using virtual fish differing in red throat coloration, courtship intensity, body size, and combinations of these, Kunzler & Bakker (2001) found that male coloration increased female preference, whereas increased male courtship intensity did not. In addition, female stickleback preference increased as the number of traits available to judge male quality increased. I would expect similar results if male *P. latipinna* preference was tested for combinations of the cues I isolated in my current study. However, I have yet to determine which cues in combination would be important factors in yielding strong male preferences. Additionally, chemical cues alone also appear to be insufficient for males to recognize conspecifics (Gabor et al. unpublished data) although it is possible that a combination of visual and chemical cues are required.

The lack of a population level preference for any individual cue may be related to the amount of variation shown by these males. Differences in preference between individual males may be consistent and negate each other, resulting in no population level preference. Thus, the high level of variation in male *P. latipinna* preference may indicate a polymorphism in male preference with some males consistently preferring the dorsal fin shape and placement, while others consistently prefer the lateral spot pattern or general body shape. Hankison & Morris (2003) found an increase in variation when testing female *X. pygmaeus* preferences for chemical cues followed by chemical and visual cues. Although not significant, this difference in variation may be indicative of an interaction between the cues used for species recognition. They suggest testing repeatability to determine the role of variation in their experiment. I

did not test repeatability of male preference, however, it would be interesting to examine this given the conflict in species and mate-quality cues.

While I tested male preference for the known differences between female *P. latipinna* and *P. formosa*, there may be other cues males are using that I did not test. For example, morphometric analysis may reveal other important morphological differences between the species that would be important to assess. Although individual cues may not be sufficient for species recognition, this study provides the first step towards understanding the complex process of species recognition in a system where males risk mating with heterospecifics and suggests that male *P. latipinna* require a suite of cues for species recognition.

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FIGURE LEGENDS

Figure 2.1 Unaltered model female *P. latipinna* and *P. formosa* in the left column. In the right column, altered models of *P. formosa* with *P. latipinna* fin/placement, *P. formosa* with *P. latipinna* spotted pattern; and *P. formosa* fin/placement and lack of spotted pattern.

Figure 2.2 Box plots representing time spent by males with (a) unaltered *P. latipinna* models vs. no stimulus, (b) unaltered *P. formosa* models vs. no stimulus, and (c) unaltered *P. latipinna* models vs. unaltered *P. formosa* models. The middle horizontal lines represent the medians for each population and the upper and lower lines of the boxes represent the first and third quartiles (Sokal & Rohlf 1995). The whiskers represent the range. Significance levels are derived from *p*- values based on two tailed Wilcoxon sign-ranked tests comparing the time spent with each stimulus: * $p < 0.05$; ** < 0.01 ; n.s. $p \geq 0.05$.

Figure 2.3 Box plots representing the male strength of preference for models isolating the *P. latipinna* fin, pattern, and shape. Strength of preference is calculated as the amount of time spent with unaltered *P. formosa* models subtracted from the time spent with models altered with female *P. latipinna* traits. Data above the no-preference line ($y = 0.5$) indicate an increasing strength of preference for altered models, data below the line indicate an increasing preference for unaltered *P. formosa* models.

Figure 2.1

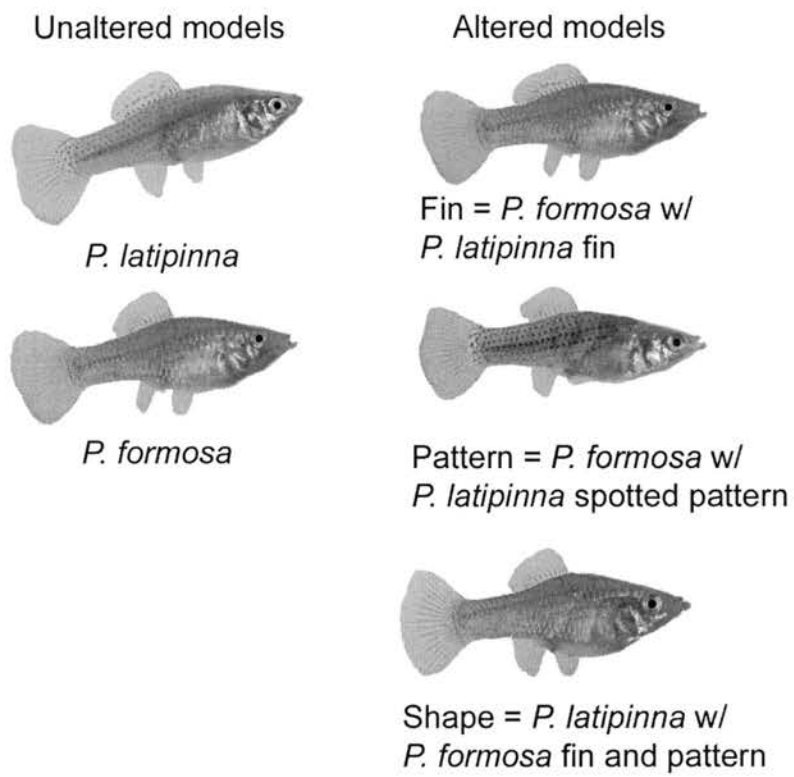


Figure 2.2

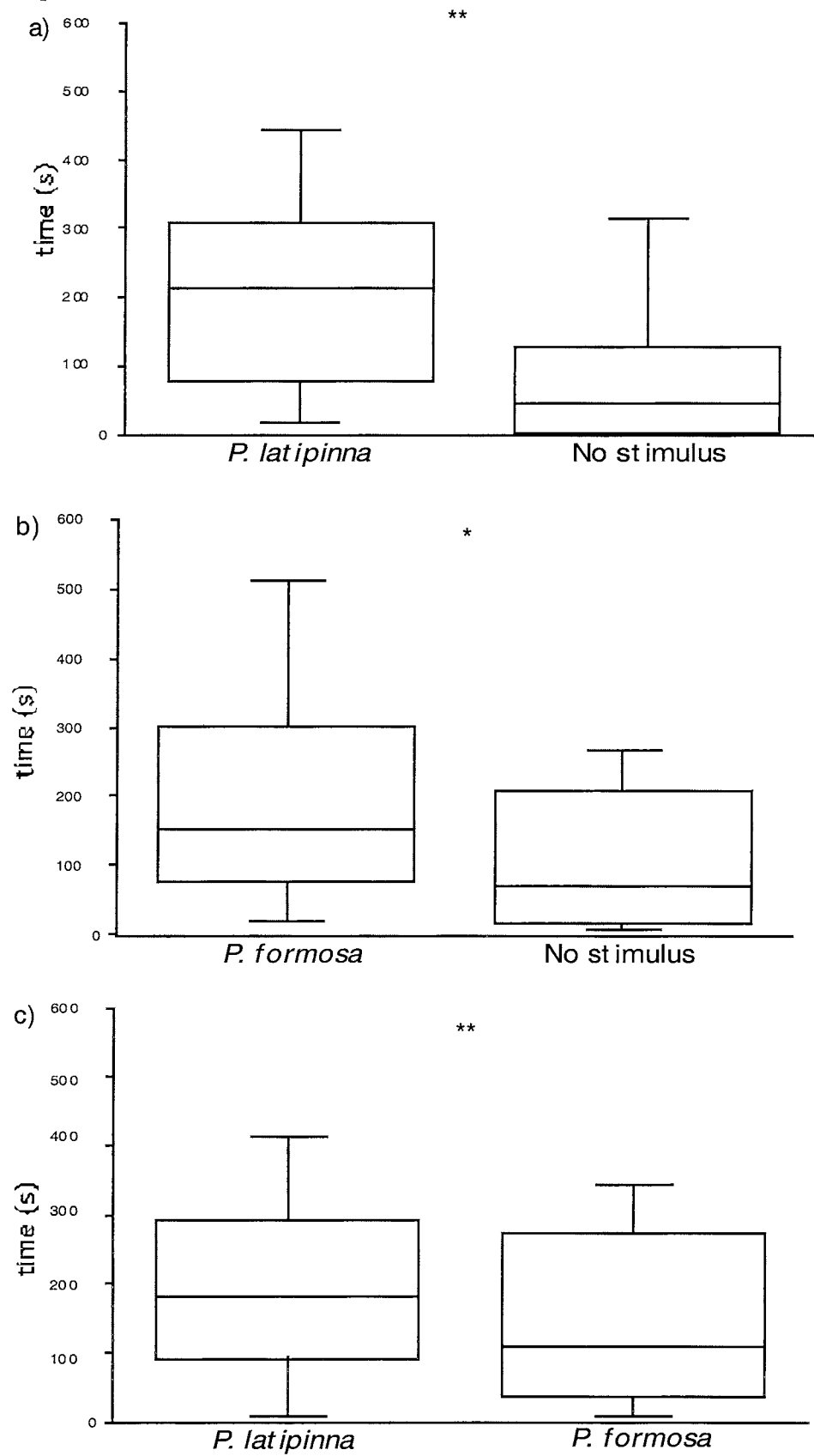
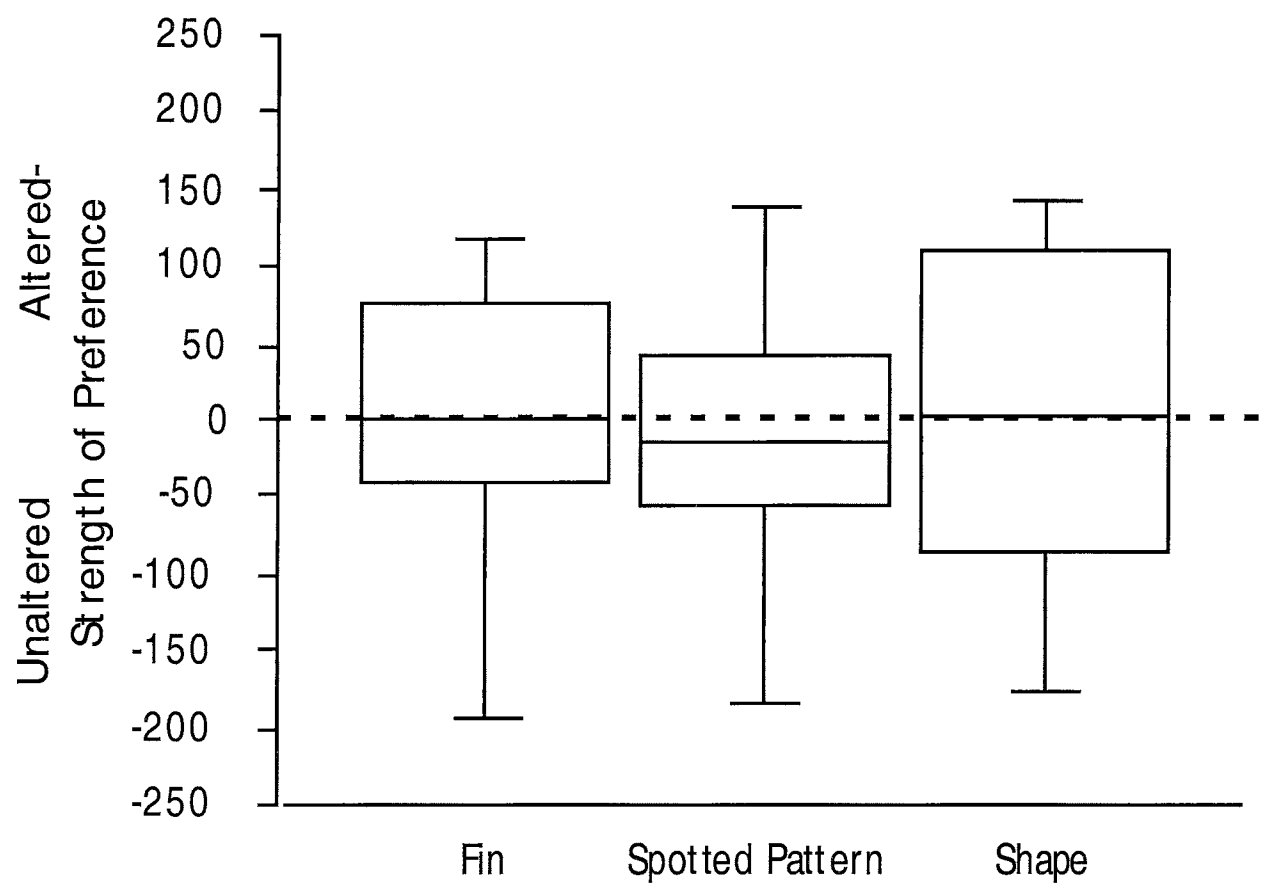


Figure 2.3



VITA

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