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The Evolutionary Persistence of the Gynogenetic Amazon Molly, *Poecilia formosa*

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The Evolutionary Persistence
of the Gynogenetic Amazon Molly, *Poecilia formosa*

by

Laurie Ann Dries, B.A.

Dissertation

Presented to the Faculty of the Graduate School of
The University of Texas at Austin
in Partial Fulfillment
of the Requirements
for the Degree of

Doctor of Philosophy

The University of Texas at Austin
May, 2000

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Dedication

For Zeke and the corroboree;
it's the journey not the destination

Sex and the Single Species

Introduction

Ten thousand year ago, a fish said to her mate,
" I have to bear fry, and for you I can't wait!"
So she went to the corner and stood under a light,
'Til a sailfin swam by, and she winked at the sight.
She acted real coy and gave him a nod,
So he nestled up close with his sleek gonopod.
He gave a few thrusts while watched by his uncle,
"My fitness is rising; and what a fine caudal peduncle!"

But on him the joke was played out that night,
For she tossed his genes out left and right.
She used only for his sperm,
And from then on her own species she spurned.
"Now I know where the action is!
Since I discovered gynogenesis!
Now my sisters are like my mother,
Who are like my daughters and one another."

Every problem she ran into,
She chopped up with her axe or blender.
The only one she couldn't hatchet,
Was that of Muller's nasty ratchet.
But twice the daughters came to dinner,
Compared to her rival, *Poecilia latipinna*.
So a big advantage was accrued her,
"Til one day males began to elude her.

The question asked in this dissertation,
Is " How can this be a stable situation?"
Formosa needs *Latipinna*, that is true,
But twice the daughters *Formosa* is due.
If that keeps up, there are two ways to go:

Formosa dies fast, or *Formosa* dies slow.
But for ten thousand year, *Formosa*'s held on,
So my dissertation asks: " What's going down?"

Materials and Methods

I raised some fish in big septic tanks,
For access to these, I give Hillis thanks.
He didn't help much, but stayed out of my way,
An advisor like that doesn't come every day.

Results

When *Formosa* is rare, male sailfins are hot,
But when *Formosa* is common, sailfins are not.
So males become choosy when sharing their spuge,
And schools of *Formosa* no longer are huge.

Discussion

Gynogenesis works, that's what I say,
And is rather appealing at the end of the day.
When males get tiring and my wits' at its end,
Sexual parasitism sounds like a whim.

Literature Cited

I read the best of Crow and Bull,

And found the rest all rather dull.

But though I read some Kondrashov,

It's the little things that pissed me off

David M. Hillis

Acknowledgements

This dissertation project was funded with the generous contributions of a National Science Foundation Dissertation Improvement Grant, Sigma Xi Scientific Research Society, American Society of Fisheries – Texas Chapter, University of Texas Department of Zoology Stengl Endowment Fund, and the Dries Alliance for funding Dispensation (D.A.D.). This dissertation would have been impossible without the help of numerous people whose generosity and patience was boundless. In particular I would like to thank my co-supervisor, Dr. David M. Hillis, for his dedication to his unexpected role as my mentor. He remains unequaled in his support, encouragement, humor, tolerance, musical taste, softball skills, canoeing finesse, knowledge of the natural history, and dedication to the bow. You have been my wellspring of inspiration, and I learned perhaps even too much from you at times. My co-supervisor Jim Bull deserves my gratitude for his participation in my supervision and sharing his is truly amazing breadth of knowledge. I benefited in countless ways from his dedication to thoughtful, clean, and simple, but elegant scientific study design. He has succeeded in converting me into another member of the Brilliance of Bull Cult I am immensely grateful to both David and Jim for the time and effort spent on attending my seminars and touting my work to friends and colleagues at scientific meetings. I would like to thank Mike Ryan for his insightful contributions to my dissertation work, and for providing the opportunity to conduct research in his laboratory during my undergraduate years. I owe him many thanks for getting me "out from behind bars". To Stan Rand I owe hours of creative scientific discussion while patiently waiting for the frogs to call. His contributions to my dissertation committee were endless and irreplaceable. To Mike Singer I owe my presence in science. My friends and family provided encouragement, support and liberal access to their ears as sounding boards. The past and present members of the Hillis/Bull/Cannatella lab family were a rock of moral support through the years. Thank you Barb, Marty, Matt, John, Ken, Keith, Anna, Kris, Mark, Kelly, Wayne, and Catfish. Thank you to all of the undergraduates who tolerated uncomfortable habitats and weather conditions to help me with my research, particularly Jesse Gevirtz and Erica Powell. Thank you to John Crutchfield, Tony Alexander, and the Brackenridge Field laboratory where the majority of fieldwork was conducted. They protected the fish in my experimental ponds from numerous unexpected weather and perturbations. Finally, I would not have survived the final throes of this project or had the strength and persistence to finish this thesis without T.P. Wilcox.

The Evolutionary Persistence
of the Gynogenetic Amazon Molly, *Poecilia formosa*

Publication No.____________

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The University of Texas at Austin, 2000

Co-Supervisors: David M. Hillis, James J. Bull

One of the central questions in evolutionary biology over the last two decades has been the persistence of sexual reproduction. Theoretical studies have identified a range of genetic and ecological mechanisms that favor sexual reproduction and predict that asexually reproducing lineages should not persist over evolutionary time. Gynogenetic lineages are unisexual (all-female), reproduce asexually, and face an additional obstacle to evolutionary persistence; reproduction is dependent on sperm from a related sexual species. Therefore a gynogenetic lineage must coexist with a species it can parasitize sexually. Simple population models incorporating reproductive success and mate discrimination predict gynogenetic lineages should rapidly become extinct. More complex population models predict evolutionary persistence in the presence of ecological niche-partitioning or density-dependent mating. *Poecilia formosa*, the Amazon molly, is a gynogenetic lineage of livebearing fish that coexists with the sexual species, *P. mexicana* or *P. latipinna*. This thesis describes three studies that empirically investigate factors that could affect evolutionary persistence of gynogenetic *Poecilia formosa*: mate discrimination, interspecific competition for mates, and frequency-dependent reproductive success. The first study asks if the ability of *P. formosa* to attract heterospecific *P. latipinna* and *P. mexicana* males is related to its hybrid origin or is a result of coevolution as posited by previous researchers. The results from laboratory behavioral tests indicate mate attraction ability is present at the moment of hybridization and it is not necessary to assume a coevolutionary process. The second study measures antagonistic behavior of *P. formosa* and *P. mexicana* females and shows they do not differ. The final study uses semi-natural experimental ponds to ask whether reproductive success of *P. formosa* is negatively frequency-dependent and if it is sufficient to explain co-existence of *P. formosa* with its *P. latipinna* host. The results indicate when frequency of *P. formosa* is high, its reproductive success is low and vice versa. This causes their frequency in a population to oscillate, which prevents extinction of themselves and their hosts. This is the first empirical study to show the importance of frequency-dependent reproductive success in the evolutionary persistence of gynogenetic lineages.

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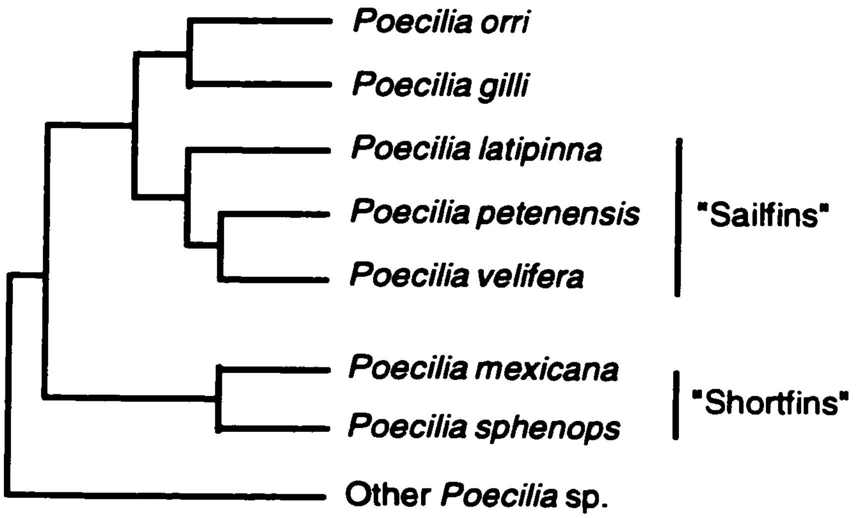
Chapter 1: Natural History of *Poecilia*

PHYLOGENETIC HISTORY

Poecilia is a group of fishes that belongs to the monophyletic clade Poeciliinae within Poeciliidae (Parenti 1981, Rosen & Bailey 1963). The poeciliid clade is defined by several morphological characteristics related to osteology and placement of fins and sensory cells (Parenti & Rauchenberger 1989). The *Poecilia* clade includes 43 described species distributed in North, Central, and South American and the West Indies (Parenti & Rauchenberger 1989, Rosen & Bailey 1963). Within *Poecilia* there are two clades of mollies, the sailfin or highfin mollies, which includes *P. latipinna*, *P. petenensis*, and *P. velifera*, and the shortfin mollies including *P. mexicana*, *P. gilli*, *P. latipunctata*, *P. orri*, and *P. sphenops* (Breden et al. 1999, Darnell & Abramoff 1968)(Fig. 1.1). The moniker sailfin refers to the exaggerated size of a male's the dorsal fin, exemplifying the extent of sexual dimorphism typical of this group. *Poecilia formosa* is a unisexual lineage of mollies that originated as a result of hybridization between *P. mexicana* and *P. latipinna* (Avise et al. 1991, Monaco et al. 1982, Turner et al. 1980a, Abramoff et al. 1968, Hubbs & Hubbs 1932). In this chapter I will focus on three of these species, *P. latipinna*, *P. mexicana*, and *P. formosa*.

Figure 1.1 Phylogeny of mollies in the genus *Poecilia*

The phylogenetic relationships among most species in the group *Poecilia* within Poeciliidae are depicted in this figure. The tree was drawn based on two studies that used molecular data. The placement of *P. mexicana* was taken from Schartl et al. (1996), the remainder of the tree was taken from Breden et al. (1999). *Poecilia formosa* is not shown because this lineage arose from hybridization between *P. latipinna* and *P. mexicana* and represents a reticulation event. Some *Poecilia* species are not shown because they were not included in either study or are not placed within the *Poecilia* clade.



(Breden et al. 1999, Schartl et al. 1996)

LIFE HISTORY AND ECOLOGY

There is a diverse array of life history patterns among the species within *Poecilia* (summarized in Reznick & Miles 1989). In general mollies and guppies are small (20 - 70 mm), abundant fishes, that are primarily surface-dwellers in small, shallow waters of temperate and tropical zones throughout the New World (Meffe & Snelson 1989, Rosen 1973). Mollies have an important position in many aquatic communities; not only do they serve as prey for piscivorous fishes, snakes, and wading birds (Trexler 1994, Mushinsky & Hebrard 1977, Hunt 1953), they are a likely link between producers and secondary consumers (Meffe & Snelson 1989, Hubbs 1964).

Habitat

Poecilia latipinna (sailfin molly) inhabits freshwater sloughs and briny estuaries associated with the Atlantic Ocean and the Gulf of Mexico from North Carolina to Tampico, Mexico (Trexler 1986, Miller 1983, Lee et al. 1980, Simanek 1978). It was introduced from sites in Louisiana and Florida to the San Marcos, Comal, and Guadalupe Rivers of central Texas in the early forties and has remained to the present (Brown 1953). *Poecilia mexicana* inhabits inland freshwater streams and coastal lagoons of the Atlantic slope from the Río Grande drainage to the Río San Carlos in northeastern Mexico (Miller 1983, Menzel & Darnell 1973a). *Poecilia latipinna* and *P. mexicana* historically lived in sympatry in the lagoons around Tampico; however, many of these habitats have been destroyed as a result of development and expansion of the city. It is unclear if there are currently any areas of sympatry of these two species. *Poecilia formosa* (Amazon molly) are found from Brownsville, Texas to Tampico, Mexico and in the San Marcos River in Martindale, Texas where they were introduced from Brownsville, Texas (Kallman 1962). They co-exist with *P. latipinna* in coastal populations from Brownsville, Texas south to Laguna Tampochoco east of Tuxpan, Mexico, and with *P. mexicana* in inland streams of northeastern Mexico (Miller 1983, Menzel & Darnell 1973a).

Poecilia latipinna and *P. formosa* are tolerant of a wide range of water conditions. In Texas, they are found in vegetated shallow pools and narrow sloughs (Pietsch 1969, Hubbs 1964). The water is often very turbid due to floating silt and organic matter (Pietsch 1969, personal observation) and variable in temperature and salinity. The waters of the Río Grande drainage vary from freshwater to nearly marine levels of salinity and can experience temperatures between 26°C and 31°C (unpublished data). *Poecilia latipinna* can tolerate elevated salinity and temperature to extremes beyond those found in natural waters (Trexler 1986, Large 1985, Gunter 1950). *Poecilia mexicana* is found in riffles and shallow pools of creeks and rivers at the base of the Sierra Madre but also inhabits the margins of other waters (Balsano et al. 1989). It, too, is tolerant of turbid water but the effects of high salinity are unknown (Menzel & Darnell 1973a). *Poecilia formosa* inhabits the same macro- and microhabitats of their Texas host species, *P. latipinna* (Schlupp 1996, Hubbs 1964). In Mexico, the abundance of *P. formosa* differs with microhabitat (Balsano et al. 1981). A higher percentage of individuals were found downstream in slightly deeper waters of side channels than in the shallow waters at the edges of the main river channel.

Diet

There is a broad range of diet among the species including both herbivores and omnivores. Some authors have characterized *P. latipinna*, as a phytoplanktivore or phycophagous (Wetzel 1971, Pietsch 1969, Hubbs 1964, Harrington & Harrington 1961) and others have documented a diet of periphyton, including, plants, invertebrates and detritus (Harrington & Harrington 1982, Hubbs 1964). Given this contradiction and the willingness of recently field-caught fish to consume live brine shrimp and mealworms, they appear be opportunists, eating whatever is abundant perhaps while retaining a preference for phytoplankton and algae. *Poecilia mexicana* is clearly an omnivore with a diet that includes algae, vascular plants, organic detritus, diatoms, and desmids (Menzel & Darnell 1973a, Darnell 1962). *Poecilia formosa* is primarily herbivorous but occasionally feeds on invertebrates (Balsano et al., 1981). Though all three species have been reported as ecologically similar (Rasch & Balsano 1989, Balsano et al. 1985, Hubbs 1964), Balsano et al. (1989) suggest there is loose niche partitioning among *P. formosa* and *P. mexicana*. I suspect the diet of *P. formosa* overlaps with both *P. latipinna* and *P. mexicana* but the extent is unclear and may depend on the identity of the host species.

Growth Patterns

Growth patterns of adults and juveniles have been studied in detail only in *P. latipinna* (Travis 1994, Trexler et al. 1992, Snelson 1989, 1985, 1984, 1982, Hubbs 1964). However, *P. mexicana* and *P. formosa* are likely to have similar characteristics because these patterns are typical of poeciliins in general (reviewed in Snelson, Jr. 1989). Offspring are developmentally well advanced at birth and begin feeding and growing immediately (Snelson, Jr. 1989). Juvenile *P. latipinna* have higher growth rates than adults (Snelson, Jr. 1984, 1982, Trexler 1985) and increase the most in size during the warm months of spring and summer (Hubbs 1964). Social context does not affect growth rates of juvenile males but juvenile females raised in pairs appear to coordinate their development and grow more slowly than isolated females (Farr & Travis 1989). As juveniles begin sexual maturation growth rate decreases and the magnitude of decrease differs between females and males (Farr & Travis 1989). Growth rate of adult females decreases exponentially as they age but it is always faster than that of adult males (Ricker 1979).

Sexual maturation in *P. latipinna* males is coupled with a more substantial reduction in growth rate than that seen in females (Snelson, Jr. 1989, 1985, 1982); males grow up to 50% more slowly than females (Snelson, Jr. 1982). Adult male body size is influenced by Y-linked alleles that determine the age of sexual maturation (Travis 1994) and does not appear to be affected by social factors such as aggression (Farr & Travis 1989), but is influenced by environmental conditions (Trexler 1989, 1986). Large males born late in the year typically overwinter before sexual maturation begins while small males mature more quickly than large males and are capable of siring broods before the winter season (Snelson, Jr. 1989). This was confirmed by some of the juvenile males collected in November for the studies in this thesis. These juveniles were reared in outdoor ponds also matured at large body sizes the following June (Chapter 5). Body size, in conjunction with habitat quality, affects mortality in both juveniles and adults; larger fish have lower mortality and there is a minimum size for survival over the winter (Trexler et al. 1992). It is probable adult male body size in *P. mexicana* is determined in a similar manner because this genetic mechanism is typical of poeciliins in general (Snelson, Jr. 1989).

Reproductive Seasons

Poecilia latipinna reproduces seasonally with some variation in timing of onset and cessation across its extensive geographic range. In Florida, reproduction begins as early as February, may continue through the winter, and typically includes two peaks, one in May and another in August (Smith 1988, Large 1985, Snelson 1984, Wetherington 1982, Grier 1973). The reproductive seasons in natural populations of both *P. latipinna* and *P. formosa* in Texas follow a similar pattern but may begin as early as April and extend until November at more southern latitudes (Hubbs 1964). *Poecilia formosa* and *P. latipinna* from the same populations have similar interbrood intervals (Hubbs & Dries in press) further indicating that reproductive seasons are similar.

REPRODUCTIVE MORPHOLOGY AND PHYSIOLOGY

Females

In female poeciliids production, fertilization and development of eggs occur in the follicular organ, which is comparable to the follicles and uterus in mammals. The follicular organ is connected to the environment by the gonoduct, the urinary/vaginal tract, leading to the urogenital opening through which mature embryos are born (reviewed in Constantz 1989). There is no post-parturition parental care by mollies; offspring are precocious when they are born (Snelson, Jr. 1989).

Ploidy and Oogenesis

All sexually reproducing species of *Poecilia* are diploid with 44 – 46 chromosomes (Angus 1989). *Poecilia latipinna* and *P. mexicana* are strictly diploid, gynogenetic *P. formosa* are primarily diploid (2n = 46) but triploid strains (3n = 69) are common in the Soto La Marina drainage in northeastern Mexico (Rasch & Balsano 1974, Menzel & Darnell 1973b, Balsano et al. 1972). Gametogenesis in females of sexual species follows the typical pattern of meiosis but gynogenetic *P. formosa* produce genetically identical ova through a process called apomixis (Rasch et al. 1982, Monaco et al. 1984, Uzzell 1970) which is not found in any other clonal vertebrate (Dawley 1989). During apomixis eggs are produced mitotically. Premeiotic cells are duplicated by mitosis, the DNA is duplicated once more but homologous chromosomes do not pair, there is no recombination, and there is no random segregation. This cell divides creating diploid ova genetically identical to the mother and to each other (Uzzell 1970). These ova require sperm to initiate embryogenesis but in general no syngamy occurs and the genes of the sperm are not incorporated into the diploid offspring (Monaco et al. 1984, Hubbs & Hubbs 1923). Occasionally the exclusion of the sperm genome fails resulting in gynogenetic triploid offspring (Rasch & Balsano 1989) or the appearance of small microchromosomes in cells of gynogenetic females (Schartl et al. 1995). Although pigment genes presumably on these chromosomal fragments are expressed, there is no evidence of recombination between the microchromosomes and the chromosomes of the gynogenetic female (Schartl et al. 1995).

Fecundity

Poeciliid females are typified by birth of live young and the number of offspring a female *P. latipinna* or *P. formosa* can produce is directly related to her body size (Travis & Trexler 1987, Trexler 1985, Thibault & Schultz 1978, Constantz 1974, Hubbs 1971, 1964, Krumholz 1948). Their reproductive potential increases as they age because they continue to grow throughout most of their lifetime; larger, older females can, and generally do, produce more offspring until they begin to senesce (reviewed in Travis 1994). *Poecilia latipinna, P. mexicana* and *P. formosa* females of the same size produce equal numbers of eggs (Travis 1994, Monaco et al. 1978, Hubbs 1964).

Superfetation

Superfetation is a characteristic of offspring production of some species of poeciliids (reviewed in Reznick & Miles 1989). In these species females carry embryos in various stages of development and give birth to multiple broods in succession with very short interbrood intervals. *Poecilia latipinna*, *P. mexicana*, and *P. formosa* have been characterized as both superfetators and non-superfetators by different authors (Snelson, Jr. et al. 1986, Monaco et al. 1983, Thibault & Schultz 1978, Hubbs 1964, Turner 1937, 1940). Monaco et al. (1983) reported evidence of superfetation in females of all three species. In their study seventeen (8%) of females examined (6 *P. mexicana*, 4 *P. formosa*, 7 *P. latipinna*) carried embryos in two distinctly different stages of development. However, the number of embryos at an early developmental stage was small relative to those at a more advanced stage in all but 2 cases. Thibault & Schultz (1978) suggested such early stage embryos represent aborted offspring, not an instance of superfetation.

Hubbs (1964) also reported similar evidence of superfetation in six *P. formosa* females. However, he argues that the cause of apparent superfetation in *P. latipinna* and *P. formosa* differs from other poeciliids. He bases this conclusion on two factors. One, *P. formosa* and *P. latipinna* females produce a clutch of eggs that are yolked simultaneously and, once fertilized, embryonic development and birth occur before yolk deposition of the next clutch of eggs (Turner 1937). This precludes fertilization of some eggs while others are in later stages of development. Two, the presence of a small number of early embryos is a result of a skewed sex ratio caused by an overabundance of females in the population. Some females remain unmated after birth of offspring and these females would rely on stored sperm to fertilize subsequent broods. An incompletely fertilized clutch of eggs would result when the amount of stored sperm is depleted.

In my samples I have never found a female with embryos of drastically different developmental stages (Table 1.1).

Table 1.1 Proportions of Embryos in Egg Clutches of *Poecilia* Females

This table lists the number of embryos, number of fully yolked eggs, and proportion of embryos within a clutch of eggs for *P. latipinna* and *P. formosa* females collected from Olmito Creek in South Texas. Female standard length in millimeters is given (SL mm). There is no significant difference in proportion of embryos/clutch of eggs between *P. latipinna* and *P. formosa* females. All of the embryos within a brood were in the same developmental stage, as defined by Monaco et al. (1983).

Species of Female	Body size (SL mm)	Embryos	Mature eggs	% Embryos
P. latipinna	40	0	34	0
P. latipinna	35	0	5	0
P. formosa	44	5	3	62
P. formosa	43	0	30	0
P. formosa	40.5	18	2	90
P. formosa	40	15	0	100
P. formosa	37	14	16	47

A recent study of the lengths of interbrood intervals in both *P. latipinna* and *P. formosa* from several populations supports the hypothesis that broods are distinct and females are not superfetators (Hubbs & Dries, in press). Moreover, I re-examined the data reported by Monaco et al. (1983) and have concluded that some "single" interbrood intervals may have been multiple intervals that were not detected by the authors (Table1.2). The two cases of nearly equal numbers of offspring at two developmental stages consist of offspring in the penultimate or ultimate stage including birth. The extreme developmental differences between these stages, the variable time periods encompassed by putative interbrood intervals, and the large brood sizes suggest to me that the evidence for superfetation is weak and Hubbs' interpretation is probably correct. All of these data indicate there is intraspecific variation in this trait (Reznick & Miles 1989) with non-superfetation as the general rule in these species.

Table 1.2 Absence of Superfetation in *Poecilia* Females

This table shows the brood births *P. latipinna*, *P. mexicana*, and *P. formosa* females. The first four columns are data from Monaco et al. (1983) of specific identity of representative laboratory-reared females, birth dates and numbers of fry born to these females, and interbrood intervals. (1) Indicates the categorizations of these data according to Monaco et al. (1983). (2) Indicates categorizations according to this paper (Dries 2000). These data can support both the presence and absence of superfetation in these species.

Table 1.2 Absence of Superfetation in *Poecilia* Females

Species	Fry Birth Date	Fry_1	Interbrood $Interval_1$ (days)	Broods	Fry_2	Interbrood $Interval_2$ (days)	Broods
P. latipinna	8 July 80	16		3	45	0	1
	9 July 80	21	1				
	14 July 80	8	5				
P. mexicana	26 Oct. 80	7		3	21	26	1
	4 Nov. 80	8	9				
	21 Nov. 80	6	17				
P. formosa	12 Dec. 79	9		3		41	2
	16 Dec. 79	7	4		16		
	22 Jan. 80	12	37		12		
P. formosa	14 Nov. 79	5		2		0	1
	29 Nov. 79	7	15				
P. formosa	17 Jan. 80	12		6	32	46	2
	9 Feb. 80	4	23				
	24 Feb. 80	16	15				
	4 Mar. 80	12	8		20	32	
	31 Mar. 80	6	27				
	4 Apr. 80	14	5				

Embryo Provisioning

There is wide variation in the nutritional investment of poeciliid females into their embryos. In some species females are strictly lecithotrophic (ovoviviparous) providing only yolk to the eggs, which is the sole source of nourishment for embryos throughout development (Wourms 1981). In other species, females are matrotrophic (viviparous); they produce yolk but also provide nourishment to the embryos until birth (Wourms 1981) through vascular connections in the follicular tissues (Wourms et al. 1988, Turner 1947.) *Poecilia latipinna* females in some populations are facultatively matrotrophic and the amount of nourishment provided by the mother to her embryos is dependent on several factors (Trexler 1997, 1985). Low food availability and low salinity results in larger ova presumably due to greater yolk deposition. Low food availability coupled with large brood size results in a greater level of matrotrophy; females provide more nourishment in addition to yolk to their embryos. This plasticity in offspring provisioning may allow females to adjust their fecundity in response to environmental and physiological factors. It is not known whether *P. formosa* or *P. mexicana* females are also facultatively matrotrophic.

Multiple Paternity

Female poeciliids are known to store sperm in the folds of the follicular organ (Constantz 1984, Hubbs 1964, Turner 1937). When isolated from males, *P. formosa* and *P. latipinna* females can produce broods of offspring in the laboratory for a span of up to 6 months by relying on stored sperm (Hubbs & Dries in press, Thibault & Schultz 1978). Storage of sperm and multiple copulations create the potential for multiple paternity which has been reported in *P. latipinna* from Florida (Trexler et al. 1997, Travis et al. 1990). The proportion of females likely to carry a multiply sired brood can vary across populations, seasons, and body sizes (Trexler et al. 1997, Travis et al. 1990). Larger females are more likely to carry a brood of offspring sired by different fathers than smaller females (Trexler et al. 1997). The increased probability of multiple paternity for larger females could be a consequence of their larger brood size in general, but this remains undocumented. The existence and extent of multiple paternity in *P mexicana* and *P. formosa* is unknown. If it does exist it could cast light on the dynamics of the parasitic relationship between *P. formosa* and its hosts, *P. latipinna* and *P. mexicana.*

Males

Adult male poeciliids are typified by the presence of an intromittant organ called the gonopodium. During sexual maturation rays 3, 4, and 5 of the anal fin of a juvenile male lengthen and thicken (Constantz 1989, Rosen & Gordon 1953, Cummings 1943). These changes are accompanied by specialization of muscles, bones, and ligaments to create the gonopodial suspensorium. This structure provides skeletal support and confers rotational mobility to the organ (Lodi 1979, Schultz 1963, Rosen & Tucker 1961, Rosen & Gordon 1953). In some species projections and hooks form on the distal end of the gonopodium and may play a role in stabilizing the male's position in the water column during copulation

(Rosen & Bailey 1963, Rosen & Tucker 1961, Meek & Hildebrand 1916). The fully developed gonopodium of male P. *latipinna* and *P. mexicana* is bilaterally symmetrical and short relative to body length (Chambers 1987, Rosen & Bailey 1963). On the ventral margin of gonopodial ray 3 is a fleshy palp with extensive vascularization (Chambers 1987). The palp may be a sensory structure that facilitates copulation by providing information on spatial position that cannot be obtained visually because the gonopodium is below and behind the eye in these mollies (Constantz 1989)

Spermatogenesis

Male poeciliids have fused, paired testes connected to a single duct (Constantz 1989). Sperm are produced in the Sertoli cells, and transferred to the tissues forming the inner surface of the lumen of the reproductive duct (Rosen & Bailey 1963). Four to five thousand sperm are combined into bundles, called spermatozeugmata, by a sticky, gelatinous secretion (Kallman 1975, Hoar 1969, Kadow 1954).

Male poeciliids were traditionally assumed to continuously produce an overabundance of sperm (Turner 1937). However, the most recent studies of species that inhabit temperate regions, indicate males experience quiescence of testes during the winter months and presumably do not produce sperm (Grier 1981). Monaco et al. (1981) investigated testicular maturation and sperm production in *P. mexicana* of the Río Purificación in Mexico and found no seasonal effects or differences associated with body size or social position. Apparently the semi-tropical nature of northeastern Mexico allows *P. mexicana* to breed year-round. To my knowledge there are no similar quantitative studies of *P. latipinna* males so it is unclear whether they experience seasonal recrudescence. Given the extensive range of the species and geographic variation in length of breeding season (Snelson 1984, Travis 1994, Hubbs 1964) I suspect *P. latipinna* males of southern Texas populations are less likely to experience a distinct change in reproductive condition because of the generally warm climate.

Males produce more than enough sperm to fertilize all of the eggs a female can produce (Monaco et al 1981, Thibault & Schultz 1973). However, there is no evidence these sperm are always readily available. There could be a refractory period between copulation bouts during which males are not able to transfer sperm to the female even if those sperm have already been produced. Some authors have suggested this is related to forward gonopodial stretching when no female is near often between bouts of mating attempts; the behavior may serve to release and/or transfer sperm from the body to the end of the gonopodium (Bowden 1969).

Sexual Dimorphism and Mate Recognition Behavior

Several poeciliids are well-known model systems for the study of the evolution of mate recognition and discrimination (reviewed in Meffe & Snelson 1989). A survey of variation within genera, species and even within sex reveals suites of preferences and behavioral interactions that indicate the complexity of solutions to the problem of finding a mate. *Poecilia latipinna*, *P. mexicana* and *P. formosa* have been the subjects of numerous studies addressing a variety of hypotheses of the proximate action and ultimate outcome of sexual selection.

Males

Poecilia latipinna and *P. mexicana* have marked sexual dimorphism; males are more colorful than females (Snelson, Jr. 1985, Baird 1968, Hubbs 1942) and some possess traits likely to have evolved under the force of sexual selection (Ptacek & Travis 1996, Travis 1994, Fisher 1958).

Within males of either species there are alternate mating strategies correlated with male size; larger males have exaggerated morphological characteristics, like the enlarged dorsal fin of *P. latipinna*, are more colorful, and rely on courtship rituals to attract females. Smaller males lack the flashy characteristics of large males and employ a sneaker strategy; they forego courtship and attempt to force copulation with females. Intermediately sized males have partial development of the dorsal fin and coloration and employ some or all of the behavioral characteristics of other males (Farr 1989, Travis & Woodward 1989, Woodhead & Armstrong 1985, Snelson, Jr. 1985, Luckner 1979, Simanek 1978, Baird 1974, Hubbs 1942). The typical behavior pattern of male *P. latipinna* is to approach a female from below and behind and nibble at her gonopore. The gonoporal nibbling probably plays a role in conspecific mate recognition (Schlupp et al. 1991) and in assessment of a female's reproductive condition (Travis & Woodward 1989, Farr & Travis 1986). If the male finds the female suitable, he will either attempt to copulate if he is small, or he will proceed with courtship if he is large. The courtship display of large males precedes initial copulation attempts and consists of swimming in front of the female, erecting the large dorsal fin, and sigmoid curving of the body in an attempt to elicit her cooperation (Ptacek & Travis 1996, Parzefall 1969). Periods of courtship are interspersed with gonoporal nibbling and attempts by the male to insert the gonopodium into the female's gonopore (Ptacek 1998, Ptacek & Travis 1996, Parzefall 1969). Small males attempt to copulate by rotating the gonopodium forward under the pelvic fins and thrusting it into the gonopore of the female. Larger males spend a greater proportion of mating behaviors on courtship rather than gonopodial thrusting while smaller males focus on gonoporal nibbling and gonopodial thrusting (Ptacek & Travis 1996, Travis & Woodward 1989).

Male size and mating strategy are not strictly correlated in *P. latipinna* as in some other poeciliids (i.e. guppies: *P. reticulata*, and *Xiphophorus*: swordtails). The variation in male size and mating strategy in *P. latipinna* represents a continuum in which populations differ in ways that defy simple predictions (Travis 1994). In general small males are less than 30 mm in standard length, intermediate males are 30 – 45 mm and large males are greater than 45 mm (Snelson, Jr. 1985). Social context has a role in determining the particular combination of mating behaviors employed by a male (Travis & Woodward 1989). The size of a particular male relative to other males in his population determines which mating strategies are employed and in what proportions (Ptacek & Travis 1996). The largest and smallest males tend to employ strictly courtship or sneaking strategies respectively, while males of intermediate size adjust their mating strategy in accordance with the size of other males in the population (Ptacek & Travis 1996). The rates of gonopodial thrusting and gonoporal nibbling vary among males of different populations but the patterns of variation indicates these behaviors can change independently of each other and of size (Ptacek & Travis 1996).

Poecilia mexicana males exhibit mating strategies similar to those of *P. latipinna*. Larger *P. mexicana* males approach females from above with the head pointed downward, dorsal and caudal fins erect, but lack a sigmoid courtship display (Balsano et al. 1985, Parzefall 1969). Subsequently these males change position to below and behind the female and nibble at the gonopore and attempt copulation. In some instances males will attempt to stop the flight of a female by pushing her with the snout placed just posterior to her throat and lifting her upward. Small *P. mexicana* males employ the same sneaker behavioral pattern as *P. latipinna* males (Schlupp et al. 1991, Balsano et al. 1985, Parzefall 1969).

Early studies documented the mate preferences of males of *P. mexicana* and *P. latipinna* (Schlupp 1991, Hubbs 1964) and clearly showed the ability of individuals of both species to recognize conspecific mates (Ryan et al. 1996, Schlupp et al. 1991, Balsano et al. 1985, Hubbs 1964). Males of both *P. latipinna* and *P. mexicana* prefer larger, receptive females (Schlupp et al. 1991, Balsano et al. 1985, Ptacek & Travis, 1996), and receptivity state can reverse male preference for conspecific females to a preference for gynogenetic *P. formosa* females (Schlupp et al. 1991). Larger *P. latipinna* males from Florida populations have stronger preferences than smaller males (Ptacek & Travis 1996). Even more surprising is that *P. latipinna* males have also been reported to copy the mate choices of other conspecific males, even if the other male is observed near a gynogenetic *P. formosa* female (Schlupp & Ryan 1997).

Aggressive interactions among males can determine dominance hierarchies in both *P. mexicana* and *P. latipinna* (Dries Chapter 5, Balsano et al. 1985, Hubbs 1964). Studies of male behavior in laboratory aquaria and natural populations indicate these hierarchies do not influence access to mates (Balsano et al. 1985, Baird 1968, Hubbs 1964). In natural populations, when a female is receptive all the males in the vicinity will attempt to mate with her creating a mating frenzy. During these frenzies the dominance hierarchies are not enforced and males do not behave aggressively towards one another (Balsano et al. 1989, Baird 1968, Hubbs 1964). *Poecilia mexicana* males form dominance hierarchies that are not strictly based on size (Balsano et al. 1985). Quartets of males of the same size formed linear dominance hierarchies in the laboratory but only four of 20 males in a natural population formed a similar social structure. The remaining males in the natural population were subordinate to the dominant males but their social position was not linear within the group of subordinates (Balsano et al. 1985). Dominant males in natural populations defended home ranges without regard to the females present. They spent more time defending their home range from other males than interacting with females, but did not exclude other males from access to females. Subordinate males were allowed to interact and mate with females within a dominant male's home range. Numerous males would attempt to copulate with the same receptive female causing a mating frenzy without regard to the home range resident (Balsano et al. 1985). Balsano et al.'s (1985) results clearly indicate home range in *P. mexicana* functions only in competition for food not competition for mates.

In *P. latipinna* size-based linear dominance hierarchies form in the laboratory and in natural populations when density is low but are not apparent when density is high (Dries Chapter 5, Baird 1968). Aggression is associated primarily with feeding or home range defense, and secondarily with competition for mates (Baird 1969). Hubbs (1964) contends *P. latipinna* males from southern Texas populations have territories while Baird (1968) described spatial distribution that consists of large home ranges occupied by large males. These observations apply only to larger males and do not address the possible spatial distribution of males of different sizes. Additional data on social structure of *P. latipinna* males in natural populations is lacking and would greatly enhance our understanding of the dynamics of mixed populations of *P. latipinna* and *P. formosa*.

Females

In 1989, intraspecific male competition was considered the primary mechanism of sexual selection in *P. latipinna* and *P. mexicana* (reviewed by Farr 1989). Female choice was thought to be a secondary force in the outcome of mate choice (Farr 1989). Since then numerous additional studies of these two species, and of *P. formosa*, have elucidated much greater complexity in female mate recognition and social behaviors in these species (Ptacek 1998, Ptacek & Travis 1996, Ryan et al. 1996).

Poecilia latipinna, *P. mexicana*, and *P. formosa* females behave differently based on stage of their reproductive cycle. If a female is a virgin or has given birth within the previous 8 days she becomes receptive to advances of males (Ptacek & Travis 1997, Sumner et al. 1994, Travis 1989, Farr & Travis 1986, Snelson, Jr. et al. 1986, Parzefall 1973). These stages coincide with the presence of mature, yolked ova (Farr & Travis 1986, Snelson, Jr. et al. 1986, Thibault & Schultz 1978) and when the oviduct is open (Parzefall 1973). Thus, it represents the stage when probability of fertilization is highest. A receptive female will remain in close proximity to males during courtship and copulation attempts (Parzefall 1973). Subsequently, she will facilitate intromission by tilting her body laterally (Ptacek 1998, Luckner 1979). In *P. latipinna*, the females may swim in tandem with a courting male (Parzefall 1969). Unreceptive females can behave aggressively to males, chasing and biting as well as swimming away (Balsano et al. 1985, Baird 1968).

Pheromones are produced by the sexual females of *P. latipinna* and *P. mexicana* and by unisexual *P. formosa* females (Sumner et al. 1994, Liley & Stacey 1983, Parzefall 1973, Amouriq 1967). Liley and Stacey's (1983) study of ovariectomized and hypophysectomized females indicated the pheromone is produced within the ovary under the control of ovarian hormones but its exact composition is uncertain. The timing of its production and/or release are linked to the ovarian cycle (Sumner et al. 1994, Farr & Travis 1986, Parzefall 1973, Brett & Grosse 1982) and appears to signal the presence of mature ova ready to be fertilized (Farr & Travis 1986, Monaco et al. 1978). The pheromone stimulates male sexual behavior (Sumner et al. 1994, Farr & Travis 1986, Brett & Gross 1982, Crow & Liley, 1979) and plays a role in conspecific mate recognition of *P. latipinna* and *P. mexicana* (Schlupp et al. 1991). It is unclear whether the substance evolved as a receptivity signal to males, or is an example of an existing metabolic byproduct of the ovarian cycle that males have evolved to exploit. There have been no studies to determine the strength and longevity of the pheromone or to determine how far it travels once released. Likewise, there have been no studies of the actual signal perceived by males and the responses it provokes, or exploring the possibility of chemical signals emitted by courting males that facilitate female choice. This information is critical if we want to understand the evolutionary origin and significance of chemical signals in these fishes.

In addition to the behaviors based on physiology, female mollies have numerous additional preferences which all suggest female choice is as important as male competition in mate recognition and reproductive success. *Poecilia mexicana*, *P. latipinna*, and *P. formosa* females all prefer larger males as mates (Marler & Ryan 1997) and will aggressively compete with one another for access to these males (Dries Chapter 5, Foran & Ryan 1994). *Poecilia mexicana* and *P. latipinna* females prefer conspecific males (Ptacek 1998). *Poecilia formosa* has a preference for *P. latipinna* males rather than distantly related *Xiphophorus multilineatus* males and this preference can be strengthened by experience (Marler et al. 1997). Male mate preference in *P. formosa* is also influenced by ontogenetic experience. Körner et al. (1999) found that *P. formosa* females reared with *P. mexicana* males preferred to associate with videotaped images of *P. mexicana* rather than *P. latipinna* males. The same was true for *P. formosa* reared with *P. latipinna* males; they preferred images of *P. latipinna* males.

Poecilia latipinna females prefer larger males that also possess symmetrical vertical bars that are displayed during courtship (Schlüter et al. 1998). However, these preferences are not rigid; females will copy the mate choice of other conspecific females and of gynogenetic females (Witte & Ryan 1998, Schlupp et al. 1994). Male body length influences the willingness of *P. latipinna* females to copy the mate choice of other conspecific females. Copying occurs when the difference in male size is slight and doesn't take precedence over the preference for larger males (Witte & Ryan 1998). Small differences in male size don't appear to play a role in the willingness to copy the mate choice of gynogenetic *P. formosa* females. *Poecilia latipinna* females will switch their preference to a previously unattractive mate if she observes that male in close proximity to, or courting, a *P. formosa* female (Schlupp et al. 1994). This behavior has been put forth as a factor that maintains the stability of a mixed population of *P. formosa* and *P. latipinna* (Schlupp et al. 1994). Even though a male can attract a conspecific female by consorting with a gynogenetic female, copulation is not required to reap this benefit. In fact, the copying behavior was documented in the absence of physical contact between males and gynogenetic females. Theoretically, males that attract conspecific females merely by courting or shoaling with gynogenetic females will experienced increased reproductive success simply because he did not squander additional time copulating with the gynogenetic female. Copying behavior of females does not appear to be a primary factor in the maintenance of these populations.

Females prefer to associate with groups of other fish rather than remain in isolation (Gabor 1999, Schlupp & Ryan 1996, Baird 1968) and also prefer larger rather than smaller fish regardless of gender (Gabor 1999). Although females prefer groups of conspecific females in the laboratory, larger groups that include gynogenetic *P. formosa* are preferred over smaller groups (Schlupp & Ryan 1996). Social structure among females in natural populations appears to be dependent on female size rather than specific identity (Baird 1968). Aggressive interactions among females have been documented in all three species (Dries Chapter 4, Balsano et al. 1981, Foran & Ryan 1994). Females will bite, chase, and butt other females when no males are present and will actively attempt to block access to a male if present (Dries Chapters 4 & 5, Foran & Ryan 1994). *Poecilia mexicana* and *P. formosa* females will behave aggressively towards males, especially if at the center of a mating frenzy (Balsano et al. 1985).

The majority of the behavioral studies reviewed here were designed to investigate a particular type of behavior under controlled laboratory conditions. Which behaviors are expressed in natural populations consisting of large numbers of mollies is yet to be determined, especially for mixed populations of *P. latipinna* and *P. formosa.* The field studies of Balsano et al. (1985) and Baird (1968) are notable exceptions and illustrate how important field studies can be in understanding the significance of behavior seen in the laboratory. Behavioral interactions between *P. mexicana* and *P. formosa*, and *P. latipinna* and *P. formosa* in laboratory aquaria revealed the presence of linear dominance hierarchies among males. However, in natural populations, this social structure is not as prevalent and is influenced by density of fishes. Moreover it does not determine male access to conspecific mates (Balsano et al. 1985, Baird 1968). Laboratory studies alone could not have made this distinction.

SUMMARY

This chapter presented a review of the natural history of livebearing fishes in the group *Poecilia*, in particular *P. latipinna*, *P. mexicana*, and *P. formosa*. I have tried to provide a thorough background of all aspects of natural history related to the behavioral and ecological projects presented in this thesis. The phylogenetic tree presented lacks inclusion of all taxa within the group *Poecilia*, but hopefully a complete phylogeny will be forthcoming in the near future. Various aspects of life history, reproductive physiology, and behavior are still unknown for the species I used in my investigations. I hope that future studies will fill in the gaps because good ecological investigations depend upon natural history information. The sheer number of biologists that have elucidated the information given here should emphasize the enormous amount of time and effort required to document various aspects of the lives of these common, rather unglamorous species of fishes.

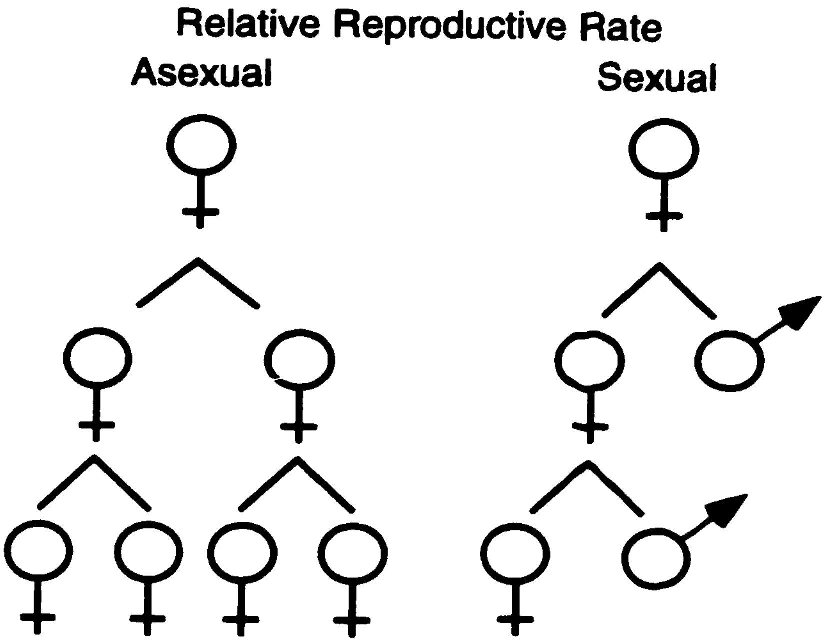
Chapter 2: The Evolution of Sex and the Persistence of the Gynogenetic Amazon Molly, *Poecilia formosa*

THE EVOLUTION OF SEX

One of the central questions in evolutionary biology over the last three decades has been why sexual reproduction is so prevalent (Bell 1982, Williams 1975, Maynard Smith 1976, Crow & Kimura 1965). Consider one of the more obvious advantages of asexual reproduction; an asexual population can increase in size faster than a sexual population (Williams 1975, Maynard Smith 1976). Asexual lineages are composed entirely of females, each of whom can reproduce independently while a sexual species is composed of females and males that depend upon each other for reproduction. Imagine a population where half of the individuals are asexual females, and half are sexual, one-fourth males and one-fourth females. Assuming that each female produces two offspring per generation regardless of reproductive mode, and that all females are identical in reproductive physiology and health, the proportion of asexual individuals in the population will increase faster than the proportion of sexual individuals (Maynard Smith 1978). This occurs because each asexual female produces two female offspring but each sexual female produces one male and one female in each generation. The asexual females have greater rate of reproduction relative to the sexual females (Fig. 2.1), which has been called the "twofold cost of producing males" (Maynard Smith 1978, 1971a, 1971b).

Figure 2.1: **Relative Reproductive Advantage of Asexual Reproduction**

This cartoon illustrates the relative reproductive rate advantage of asexual reproduction ("twofold cost of producing males"). Asexual lineages are composed entirely of females, sexual species of males and females. If asexual and sexual females each have only two offspring, the number of asexual individuals will increase while the number of sexual individuals with remain the same. Each asexual female can reproduce while each sexual female requires a male to reproduce. Since half of sexual offspring are male there are no additional females in the next generation producing offspring.


Relative Reproductive Rate
Asexual
Sexual

Theoretically, the relative reproductive rate advantage of asexual females should cause them to replace the sexual individuals in the population (Bell 1982, Maynard Smith 1978, Williams 1975). If this were true, why do we not observe a predominance of asexual reproduction among multicellular organisms?

The striking disadvantage of asexual reproduction is that it lacks genetic recombination. Processes by which the disadvantage accrues can be loosely divided into two categories, genetic and ecological. Genetic processes that have garnered the most discussion, such as Muller's Ratchet (Felsenstein 1974, Muller 1964), deterministic mutations (Kondrashov 1988), and background trapping (Rice 1998), focus on the nature of mutations and their evolutionary effects. All of these processes predict that the ultimate consequence of numerous deleterious mutations, dearth of advantageous mutations, or mutations trapped in poor genetic backgrounds is a decrease in average fitness of a population over time.

Genetic Models of Asexual Disadvantage

Muller's Ratchet describes a stepwise accumulation of deleterious mutations in a population that cannot be reversed because there is no genetic recombination (Muller 1964). An asexual population has numerous individuals that deviate from genetic uniformity because some individuals have acquired deleterious mutations. Some individuals may have none, one, two, or very few mutations while others may have a large number accumulated over numerous generations. Consider individuals with the same number of mutations of one mutational class, and the population as groups of individuals belonging to several mutational classes. When random genetic drift causes individuals with no deleterious mutations, or the lowest mutational class, to die without reproducing, the population will consist of individuals with at least one mutation. The only way an individual with no deleterious mutations could arise is through genetic recombination, a genetic process that does not occur during asexual reproduction. The lowest mutational class is lost forever from the population, the ratchet has increased a step and the average fitness of the population has decreased.

Kondrashov's (1988) concept of decreasing fitness due to asexual reproduction also assumes an accumulation of deleterious mutations, but the deleterious effect of each mutation is slight and the overall effect of numerous mutations is multiplicative rather than additive. He envisions a threshold level of genetic contamination above which additional deleterious mutations cause drastic reductions in fitness. The deterministic mutation model does not rely on the effects of genetic drift, as does Muller's Ratchet, and therefore, can be applied to large populations. Lynch and Gabriel (1990) developed the idea of deterministic mutations to include an interaction between mutation and drift called "mutational melt-down". They argue that even if an asexual population is large, the accumulation of deleterious mutations will cause population size to decrease (Gabriel et al. 1993). As population size becomes smaller, random genetic drift plays a larger role until it overcomes selection, sealing the evolutionary fate of the population.

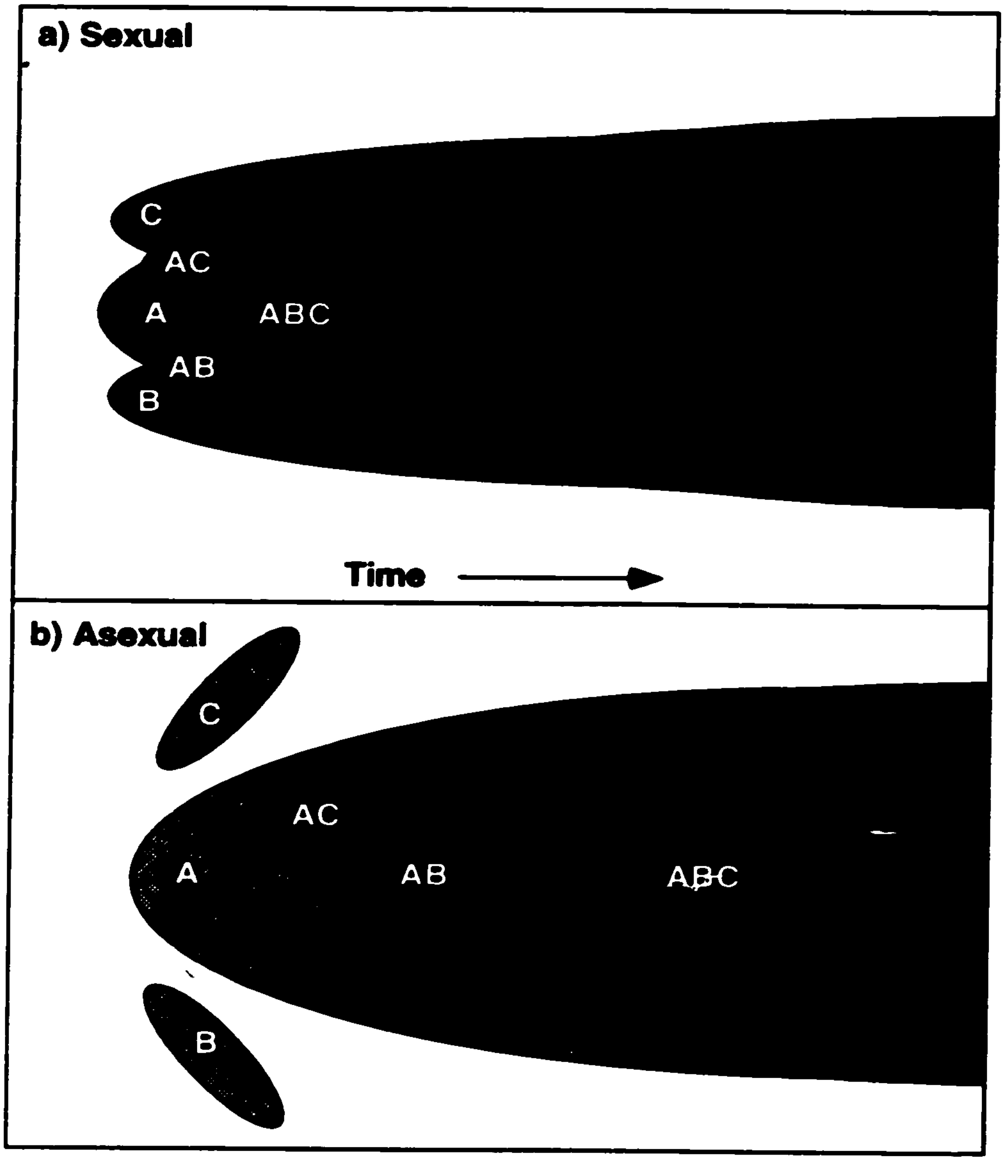
Wagner & Gabriel (1990) presented a model that suggests Muller's ratchet can be countered by compensatory mutations when mutation rates are high. They posit if the expression of phenotypic characters depends on a complex set of interactions among genes, the effects of deleterious mutations can be balanced by compensatory back mutations that restore an advantageous phenotype without recreating the original gene sequence. As long as deleterious mutations have conditional as well as unconditional effects, there can be compensatory mutations and asexual reproduction is predicted to persist in large populations where the mutation rate is slow. However, there may be an upper limit to how many mutations an organism can tolerate, regardless of the quality of effects. The accumulation of two types of mutations necessitates an ever-increasing degree of linkage disequilibrium, leaving the paired mutations susceptible to a runaway process (Fisher 1930) which would fix the deleterious as well as the compensatory allele in the population.

Models that focus on the fate of beneficial mutations in the absence of recombination also predict a decrease in average fitness over time. A beneficial mutation that arises in an individual has a small probability of becoming fixed in the population (Crow & Kimura 1965). John Maynard Smith (1978) clearly described the reason for this by pointing out the effects of asexual reproduction on the rate at which an allele can spread in a population. In a population of sexually reproducing individuals a beneficial mutation can be transmitted to numerous individuals of different genetic backgrounds because recombination can change gene combinations with every generation and even with every brood of the same two parents. The mutation can confer greater fitness to a wider variety of individuals and increase its probability of fixation in the population (Fig. 2.2a). The spread of a beneficial mutation in an asexual population is slower because a beneficial mutation that arises is destined to remain in the same genetic background until another beneficial mutation arises within a descendent of the original parent. The rate of accumulation of beneficial mutations can be so slow that there is a high probability a particular mutation will be lost from the population through random genetic drift before another arises; this is especially true in small populations (Fig. 2.2b). Maynard Smith (1978) argues that, in fact, the rate of spread of beneficial alleles would only be slow in large populations; both the sexual and asexual populations will suffer equally from increased effects of random genetic drift when their population sizes are small (Maynard Smith 1978).

Figure 2.2: Rate of Evolution in Asexual and Sexual Populations

This figure represents the rate at which independent advantageous mutations are combined into a single genotype in sexual and asexual populations. The x-axis represents time in generations. The y-axis represents the population size. The size of each shaded area represents the portion of the population that carries the allelic combinations. a) This cartoon shows the appearance and spread of three different, advantageous mutations that arose independently in a sexually reproducing population. Alleles A, B, and C arise at the same time in different individuals in the population. Alleles A and C, and A and B are combined (AC, AB) and occur in individuals of the next generation through genetic recombination. After several more generations, alleles A, B, and C are combined into the same genotype (ABC) by genetic recombination and shared by many individuals. b) This cartoon shows the appearance and spread of three different, independent advantageous mutations in an asexually reproducing population that lacks genetic recombination. Alleles A, B, and C arise at the same time in different individuals and spread among the offspring of the clonal lineage in which they arose. The allelic combinations AC, AB, and ABC arise after more generations than in a sexual population because each allele must arise independently in each clonal lineage to create advantageous allelic combinations

Rate of Evolution in Sexual vs. Asexual Populations



(After Crow & Kimura 1965)

Rice (1998) developed the concept of background trapping to describe why beneficial mutations would be lost in large asexual populations. His thesis is that the accumulation of deleterious mutations creates a population where the majority of individuals have genotypes of poor allelic combinations. When a beneficial mutation arises, it is more likely to occur in an individual with a poor genetic background than in one of the few individuals with good genetic backgrounds. An individual with a poor background will leave fewer offspring, impeding the spread of any beneficial mutations they might carry. The majority of beneficial mutations will be lost from the population before they can be fixed.

Ecological Models of Asexual Disadvantage

The rates of evolution expected in asexual and sexual populations are intimately tied to ecologically based models for the advantages of sexual reproduction (Maynard Smith 1978, Williams 1975). The key concept of ecological models is that it is advantageous for organisms to be adapted to the environment in which they live. A single mutation isn't strictly deleterious or beneficial; it is the combinations of alleles at multiple loci that are critical to the survival of an organism and determine the average fitness of a population. Sexual reproduction is thought to enhance the ability of a population to maintain adaptation to its environment or adapt to changing environments. Genetic recombination in this context is a two-edged sword; it can group multiple advantageous mutations into a single, superior genotype within a generation, but it can also break apart such a group. It is here that the rate of evolution becomes an important factor. In a changing environment, introduction of new genotypes through recombination is an advantage because it provides more variation per generation upon which selection can act. A species with sexual reproduction can respond to selection more quickly than an asexual lineage (Williams 1975, Crow & Kimura 1965).

Based on the logic of the ecological models asexuality should be favored in stable, unchanging environments. If a habitat requires specific multi-locus allele combinations that exist in the asexual species, lack of recombination is an enormous short-term advantage (Zweifel 1965). Any alleles that favor recombination will be selected against (Kimura & Crow, 1965). On the other hand, asexuality should be rare in biologically complex and variable environments (Williams, 1975). In a continuously changing environment, the ideal genotype for the current conditions is also changing. At any one point in time, the genetic reality lags behind the genetic ideal; the current genotype is not the ideal for the new environmental conditions. Genetic recombination reduces the lag in a sexual population relative to an asexual population. However, this does not guarantee the survival of a sexual population; a sufficiently high rate of environmental change will cause the lag to be insurmountable for both sexual and asexual populations (Maynard Smith 1978).

There are numerous predictions of the evolutionary fate of asexual populations that can be made based on the theoretical work described here. It is clear both genetic and ecological factors can be important in population dynamics and the models described above implicitly consider asexual lineages that reproduce independently of sexual species. How can they be applied to sperm-dependent asexual lineages?

EVOLUTIONARY PERSISTENCE OF GYNOGENETIC LINEAGES

Gynogenetic lineages are unisexual (all-female) species that are rare among vertebrates but have been found in salamanders (*Ambystoma jeffersonianum* complex) and fishes (*Poecilia formosa*, triploid *Poeciliopsis monacha-lucida*, *Menidia clarkhubbsi*, *Carrassius auratus lansgdorfii*, *C. auratus gibelo*, *Phoxinus eos-neogaeus*, *Rutilus alburnoides*, *Cobitis taenia* complex). These lineages face an additional obstacle to evolutionary persistence because this form of asexual reproduction is sperm dependent (Hubbs & Hubbs 1932, 1946). Gynogenetic females produce eggs of full ploidy but sperm is required to initiate embryogenesis (Monaco et al. 1984, Hubbs & Hubbs 1932). Ironically, the genes contained in the sperm are not incorporated into the gynogenetic offspring (Monaco et al. 1984, Rasch et al. 1982, Turner 1982, Turner et al. 1983); males who mate with gynogenetic females gain no direct fitness advantage.

Theoretical Models

How does gynogenesis fit into the models described in the previous section? Gynogenetic lineages clearly face an additional obstacle to evolutionary persistence; they cannot persist in isolation from a sexual species. A gynogenetic lineage must co-exist with a sexual host species whose males can be sexually parasitized sexually. Simple population models that incorporate reproductive success and mate discrimination would predict gynogenetic lineages should rapidly become extinct. If all factors are equal and mate discrimination by males of the sexual species is absent, the reproductive rate advantage of the gynogenetic lineage would cause the proportion of gynogenetic females to increase rapidly, eventually driving the sexual species to extinction through competitive exclusion (Kiester et al. 1981, Clanton 1934). Their own extinction would soon follow. On the other hand, if males discriminate between conspecific sexual females and gynogenetic females, and prefer the former, the gynogens would decrease in the population and eventually become extinct through lack of reproduction (Kawecki 1988, Stenseth et al. 1985). Under these simple models, only a perfect balance between the higher reproductive rate of the gynogens and the mate-discrimination abilities of the sexual males would allow a gynogenetic lineage and its sexual host species to co-exist through evolutionary time. In a population of finite size with stochastic variation, the eventual predicted outcome for the gynogenetic lineage is rapid extinction, regardless of the exact values of mate discrimination or reproductive rate advantage.

The existence of gynogenetic lineages beyond that predicted by genetic theory (Gabriel et al. 1993, Lynch & Gabriel 1990) indicates the simple models are insufficient descriptions of reality. More complex models predict evolutionary persistence of gynogenetic lineages if there is ecological niche partitioning (Kirkendall & Stenseth 1990, Vrijenhoek 1984), if mate discrimination is weak (Kawecki 1988, Moore & McKay 1971), or if mating is density dependent (Stenseth et al. 1985, Moore & McKay 1971) or frequency dependent (Stenseth et al. 1985). Many of these theoretical models are based on the *Poeciliopsis* hybridogenetic species complex of livebearing fishes for which there is abundant natural history information (Vrijenhoek 1994, Kawecki 1988, Stenseth et al. 1985, Moore & McKay 1971). These landmark studies provide valuable insight into potentially important factors in the dynamics of mixed populations of gynogenetic lineages and their host species. Therefore I will digress to briefly describe hybridogenesis so that its similarities with and differences from gynogenesis are clear before I proceed.

Hybridogenesis

Hybridogenesis is another type of sperm-dependent reproduction that occurs in unisexual lineages that is similar to gynogenesis that occurs in unisexual lineages (Schultz 1969). Hybridogenetic females produce haploid eggs that require syngamy with haploid sperm for embryogenesis (Miller & Schultz 1959). The genes contributed by the male's sperm are expressed in the hybridogenetic offspring (Schultz 1973); however, these genes are excluded during oogenesis (Schultz 1973). As in gynogenesis, there is no recombination and eggs are clonal copies of the mother's genome (Vrijenhoek et al. 1978, Schultz 1961, 1966). Despite the cytogenetic differences between gynogenesis and hybridogenesis, their requirements at the organismal level are very similar; females must obtain sperm from males of a sexual species. For hybridogenetic *Poeciliopsis monacha-lucida*, and the gynogenetic fish *Poecilia formosa*, the acquisition of sperm requires more than a release of eggs and milt into the water column. The intimate contact of copulation between sexual males and clonal females is necessary because initiation of embryogenesis occurs within the reproductive follicular organ of the female.

Hereafter I will discuss the models as they relate to gynogenetic lineages and only make explicit reference to hybridogenetic lineages when the predictions would differ.

Ecological Niche-Partitioning

Vrijenhoek (1984, 1989 and references therein), Stenseth et al. (1985), and Kirkendall & Stenseth (1990) produced models that predict stable co-existence og a gynogenetic lineage and its sexual host species if the sexual and gynogenetic individuals occupy different niches within the same habitat. The reliance on different resources would allow the gynogenetic lineage to enjoy its reproductive rate advantage without competitively excluding their sexual hosts. Further niche partitioning among different clonal lineages would also reduce competition within the gynogenetic females. This idea led Vrijenhoek to formulate the "Frozen-Niche Variation" hypothesis (1989, 1984). He suggests the ability to utilize different niches is inherited at the moment of hybridization; multiple clonal lineages originate from multiple hybridization events. Thus, niche preference of a particular clonal lineage is frozen at the time of origination. This hypothesis is more applicable to hybridogenetic lineages because new clonal strains originate frequently; they can even be generated in the laboratory by crossing the sexual progenitor species (Schultz 1973). In a gynogenetic lineage that originated from a single (or very few) hybridization(s), clonal diversity is low and new variation is not introduced by the male genome. Thus, the opportunity for niche partitioning among clones is small. In the gynogenetic *Poecilia formosa*, less than ten clones have been found among several populations (Turner et al. 1983, 1980b, Kallman 1962). However, niche partitioning is still a possible factor in the co-existence of gynogenetic lineages and their sexual host species.

Weak Male Mate Discrimination

Weak mate discrimination in males can theoretically lead to stable co-existence of gynogenetic lineages and their sexual hosts (Kawecki 1988, McKay, 1971, Moore & McKay, 1971). Moore & McKay (1971) suggest the interaction between male mate discrimination and male dominance hierarchies woulld contribute to weak mate preference in subordinate males. Although males prefer conspecific females, the dynamics of the hierarchical system among males would restrict access of subordinate males to conspecific females. These males would become less choosy and mate with gynogenetic females, especially during mating frenzies. This model is contradicted by empirical studies of mixed populations of *P. latipinna* and *P. formosa* (Baird 1968, Hubbs 1964), and *P. mexicana* and *P. formosa* (Balsano et al. 1985). These studies show that dominance hierarchies among males do not restrict access to females.

Kawecki's mathematical model (1988) suggests the time available for accurate identification of conspecific females is a critical component in weak male mate discrimination. He argues subordinate males have less time for correct identification because dominant males aggressively chase them from the vicinity of conspecific females. Since subordinate males have less time, they mistake gynogenetic for conspecific females more often than dominant males. It is primarily the subordinate males who mate with gynogenetic females because they err by make hasty decisions rather than lose the potential opportunity to mate with a conspecific female. Woodhead and Armstrong (1985) gave a similar explanation for insemination of gynogenetic females based on empirical studies of male mate discrimination in *P. latipinna*. They concluded smaller, subordinate males were younger and inexperienced in identification and therefore made more mate discrimination errors. However, they presented no data supporting this conclusion and a more recent study (Ryan et al. 1996) provides evidence that rejects these conclusions. Small and large males did not differ in their strong preference for conspecific females.

Density-Dependent Mating

In theory, gynogenetic lineages can persist if mating is density-dependent (Moore & McKay 1971). If habitat size is constant and the sexual male to female ratio is one to one, interactions among males could be determined by the habitat structure. Mixed populations of gynogenetic and sexual species can be stable if intermale competition increases as density increases and vice versa. At low densities the encounter rate between males is very low reducing competition between males for conspecific females. All males have the opportunity to mate with their preferred female and few would mate with gynogenetic females. When densities are high, more males are denied access to conspecific sexual females, mate discrimination weakens and more gynogenetic females are mated. This explanation assumes the density of gynogenetic individuals does not affect the density of sexual individuals and therefore implicitly requires niche partitioning.

Frequency-Dependent Mating

Another approach to unraveling the mystery of stability in asexual populations is to examine the conditions under which gynogenesis and sexuality are Evolutionarily Stable Strategies (Maynard Smith & Price 1973). An ESS is a strategy that once established cannot be invaded by another strategy. Stenseth, Kirkendall and Moran (1985) created a mathematical model exploring the conditions under which sexual reproduction is expected to be an evolutionarily stable and therefore safe from invasion by a gynogenetic lineage. A sexually reproducing population at equilibrium is susceptible to invasion when the reproductive rate of gynogenetic females is at least half that of sexual females. A decrease in the relative reproductive advantage of this magnitude would reduce the threat of competitive exclusion and subsequent extinction of the sexual species. The only biologically realistic conditions that would reduce the reproductive rate advantage of gynogenetic females are if few individuals have offspring or if brood sizes are small.

In addition to conditions that favor invasion and establishment of gynogenetic females in a population of sexual organisms, this model describes the conditions necessary for stable co-existence. Long-term evolutionary co-existence of a gynogenetic lineage with its sexual host species can occur when birth rates, mortality rates, and/or male mate discrimination are density or frequency dependent.

Summary

The controversy over the advantages of sexual reproduction has produced a broad and varied theoretical framework around which empirical studies can be built. The models formulated to understand the evolutionary persistence of asexual gynogenetic lineages are the basis of the studies presented in this thesis. The factors identified as important in long-term co-existence of gynogenetic lineages and their sexual hosts include ecological niche partitioning, weak male mate discrimination, density-dependent male mate discrimination, and frequency-dependent birth rates, mortality rates, and reproductive success. Using the gynogenetic *Poecilia formosa* and its sexual host species, *P. latipinna* and *P. mexicana*, I have investigated male mate discrimination and frequency-dependent reproductive success. I present the results of these studies in the following chapters; chapters three and four describe laboratory studies designed to understand the basis of the imperfection male mate discrimination, and chapter five describes a study of frequency dependence in semi-natural experimental populations. Hopefully my focus on more than one possible factor will convince you of the complexity of population dynamics in mixed populations of gynogenetic lineages and their sexual host species. At the very least I hope to show that empirical tests of these models are feasible and that numerous interesting hypotheses remain to be tested.

Chapter 3: Are Amazon Mollies Red Queens?

INTRODUCTION

Gynogenetic lineages are clonal, all-female species in which reproduction is apomictic (Monaco et al. 1984). Gynogenetic females produce diploid eggs that require sperm to initiate embryogenesis (Monaco et al. 1984, Hubbs & Hubbs 1932) but fusion of gametes does not occur. None of the genetic material from the sperm is incorporated into the chromosomes of the gynogenetic offspring (Monaco et al. 1984, Rasch et al. 1982, Turner 1982, Turner et al. 1983). Since there are no males in these lineages, gynogenetic females must attract and mate with males of another species to reproduce successfully. Under these unusual circumstances, selection on gynogenetic females is expected to favor those who attract heterospecific sexual males. However, selection is also expected to favor males who avoid mating with gynogenetic females because their genes are not transferred into the offspring (McKay 1971). Theoretically these selection pressures can form the basis of a coevolutionary arms race between the gynogenetic females and sexual males (Vrijenhoek 1994, Bell 1982, Van Valen 1973).

The Amazon molly, *Poecilia formosa*, is a gynogenetic lineage of livebearing fish that exploits males of the closely related species, the sailfin molly, *P. latipinna*, and the Atlantic molly, *P. mexicana*, for sperm (Breden et al.

1999, Schartl et al. 1996, Hubbs 1964, Hubbs & Hubbs 1932, Balsano et al. 1985, 1981). This species complex has been studied extensively in an attempt to understand the evolution of conspecific mate recognition and discrimination, and the persistence of asexual reproduction, presumably because the gynogenetic females are easily identifiable agents and targets of selection (Körner et al. 1999, Landmann et al. 1999, Ptacek 1998, Witte & Ryan 1998, Marler et al. 1997, Ptacek & Travis 1996, Marler & Ryan 1997, Ryan et al. 1996, Foran & Ryan 1994, Schlupp & Ryan 1996, Schlupp et al. 1991, 1994, 1998, Woodhead & Armstrong 1985, Hubbs 1964, Gabor & Ryan unpublished). The most recent studies have documented the ability of males to discriminate between gynogenetic and conspecific females with a preference for the latter (Ryan et al. 1996, Schlupp et al. 1991, Hubbs 1964, Gabor & Ryan unpublished). The presence of discrimination against the gynogens by sexual males leads to the prediction that gynogenetic lineages should become extinct because they would not be able to reproduce (Clanton 1934). While numerous studies have documented male discrimination against *P. formosa*, the preferences are not absolute; though infrequent, males still make mistakes (Ryan et al. 1996, Schlupp et al. 1991).

Based on the imperfection of male mate discrimination, some studies explain the persistence of *P. formosa* in the context of a host/parasite coevolutionary arms race (Schlupp et al. 1998, 1994, Schartl 1996, Schlupp & Ryan 1997, Schlupp et al. 1991). In this context the persistence of the gynogenetic lineage is due to the evolution of characteristics that thwart male mate discrimination and recognition (Schlupp et al. 1991). These characters then impose selection on males favoring the evolution of stronger mate discrimination that, in turn, imposes selection on the gynogens to evolve even greater ability to attract heterospecific males. Parasites in coevolutionary scenarios have been dubbed "Red Queens" (Bell 1982, Van Valen 1973) after the character of Lewis Carroll's Alice in Wonderland (1871), who is forever running to stay in the same place. In this case, the gynogenetic females are Red Queens because they are locked in an evolutionary battle with the sexual males in order to maintain their ability to obtain sperm. An implicit assumption of the Red Queen scenario as it applies to *P. formosa* is that this gynogenetic lineage has evolved strategies to thwart the mate recognition system of the sexual males since its time of origination.

The hybrid origin of *P. formosa* suggests an alternative explanation for the prevalence of male mate discrimination errors: namely shared genetic history. The gynogenetic *P. formosa* lineage is a result of a single hybridization between a male *P. latipinna* and a female *P. mexicana* (Schartl et al. 1996, Avise et al. 1991, Turner et al 1980a, Abramoff et al. 1968). Thus, *P. formosa* shares a genetic history with both species whose males it exploits for sperm. It is possible *P. formosa* attracts these males, or *P. latipinna* and *P. mexicana* males err and choose gynogens, because they all share some of the genes that underlie mate attraction and discrimination. This idea was verbally described by Lima et al. (1996) to explain the ability of hybridogenetic females to attract heterospecific, sexual males. Likewise, gynogenetic *P. formosa* may persist because at the moment of hybridization their mate attraction abilities were "frozen". This verbal model is similar to the Frozen Niche-Variation model (Vrijenhoek 1989, 1984) and I will refer to it as the Frozen Mate-Attraction model.

To distinguish between the coevolutionary Red Queen model and the Frozen Mate-Attraction model the ideal experiment would consist of an examination of the ability of males to discriminate between extant *P. formosa* and *P. formosa* from the time of origination, and of behavioral differences between these females. It is impossible to travel back in time to investigate the original gynogenetic females. However, it is possible to use analogs for them by creating sexual hybrid females using standard crosses between several virgin males and females of the parental species mentioned above. If *P. formosa* has evolved strategies that thwart male mate recognition since its origination, males should prefer to mate with gynogenetic females and avoid mating with sexual hybrid females. By the same logic *P. formosa* should differ behaviorally from hybrid females. If the attractiveness of *P. formosa* is a consequence of their hybrid origin, males should have no preference for either female and females should not differ in behavior. Lack of male preference for gynogenetic or sexual hybrid females would not directly determine if *P. formosa* and *P. latipinna* are locked in a coevolutionary arms race. It would however, indicate that evolution is not necessary to explain the attractiveness of*P. formosa* to *P. latipinna* males. The purpose of this study is to determine if hybridization (the absence of evolution) is sufficient to explain the relative attractiveness of *P. formosa* to *P. latipinna* and *P. mexicana* males.

MATERIALS AND METHODS

Crosses

I collected 39 fry from a stock population of field caught *Poecilia mexicana* (Rio Tigre, Tamaulipas, Mexico), 18 fry from a stock population of *P. latipinna* (Tampico, Tamaulipas, Mexico), and 157 *P. formosa* fry from the San Marcos River (Martindale, Texas). I reared fry of each species in separate outdoor ponds at the Brackendridge Field Laboratory (University of Texas at Austin) to obtain virgin males and females. Ponds were equipped with removeable 1/16" mesh net liners that captured all of the fish in the pond without draining the water. All fish in the ponds were examined monthly, and sexually maturing males and females were removed and placed in separate holding ponds until they were used for cross breeding. Males in the process of sexual maturation are easily identified by inspection of the anal fin (Ptacek & Travis 1996, Rosen & Gordon 1953, Cummings 1943). As males mature, the anal fin rays gradually compress and elongate until they form an intromittant organ, the gonopodium (Constantz 1989, Cummings 1943, Grobstein 1940). Thus all males were removed before they could inseminate females.

Forty-seven virgin *P. formosa* females were removed from the outdoor ponds and taken to the lab for use in behavioral experiments. Fifteen virgin *P. mexicana* females and 4 *P. latipinna* males were placed in one outdoor pond to produce hybrid fry (designated M_f/L_m; f = female, m = male). Seven virgin *P. latipinna* females and 8 *P. mexicana* males were also placed in an outdoor pond to produce hybrids (designated L_f/M_m). Each pond was checked monthly for hybrid fry. If present, fry were removed placed in separate outdoor ponds according to cross type and checked every thirty days for sexually mature virgin males and females. This produced a total of 38 virgin hybrid females (18 M_f/L_m, 20 L_f/M_m) and 50 males (15 M_f/L_m, 35 L_f/M_m). These fish were brought to the laboratory and maintained in 7.6-liter aquaria under a 12:12hr day:night light cycle. All fish were fed Tetramin flake food and live brine shrimp daily to satiation.

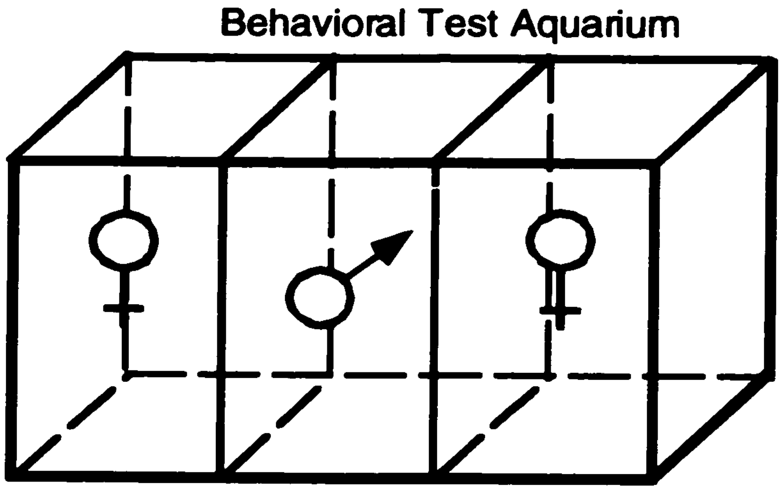
Behavioral Tests

To evaluate male mate discrimination I conducted a total of 39 tests of the responses of males to virgin hybrid and virgin gynogenetic females (23 *P. latipinna* males 16 *P. mexicana* males). There were two types of tests for each species of male, one using hybrid females whose mothers were *P. mexicana* (M_f/L_m) and the other using hybrids whose mothers were *P. latipinna* (L_f/M_m). I also conducted 33 tests of behavioral interactions between virgin hybrid and virgin gynogenetic females. I used virgin females to ensure they were matched for receptivity because this has been shown to affect male and female behavior (Sumner et al. 1994, Schlupp et al. 1991, Travis 1989, Travis & Woodward 1989, Farr & Travis 1986, Farr et al. 1986). Female behavior was tested with and without a male present. Twenty-three of these tests examined behaviors in the presence of a *P. latipinna* male, 9 in the absence of males. Each male and each pair of females were used once for each type of test. The tests were videotaped using a Magnavox Newvicon video camera for permanent record.

Each test was conducted in a 1.9-liter aquarium divided into three compartments by clear plastic removable walls that allowed circulation of water throughout the aquarium (Fig. 3.1). In tests without a male, a virgin hybrid and a virgin gynogenetic female were matched for size and each placed in one of the outer compartments. The virginity of both females ensured they were matched for receptivity. In each test with a male, two size-matched, virgin females were placed in opposite ends of the aquarium and a male was placed in the center compartment. All fish were allowed to acclimate for two days after which the dividers were removed and the fish observed for 15 minutes. The number of occurrences of five behaviors were monitored during the tests with males, gonopodial thrusts by the male to each female, bites, butts and chases directed by one female to the other female, and blocks by one female obstructing the access of the male and other female to each other. Blocks and gonopodial thrusts were not counted in the tests without males.

Figure 3.1: Behavioral Test Aquarium

Cartoon of the behavioral test aquarium used in all experiments. The aquarium is divided into three sections by two clear plastic dividers that allow water flow between sections. A male was placed in the center section and a female was placed on either side in the outer sections. After an acclimation period of two days the dividers were removed and fish were allowed to swim freely in the aquarium throughout the experiment.



In mate discrimination tests males were asked to respond to heterospecific females; some males responded very little or not at all. This could be interpreted as a true lack of preference for the stimulus fish or a lack of male motivation to mate. To distinguish between these two possibilities, a control test using two receptive, conspecific females was conducted immediately following the initial test. The fish were allowed to acclimate for 15 minutes, then males were allowed to interact with the conspecific females and gonopodial thrusts to each were counted. If a male did not respond with at least 5 thrusts to the conspecific females, the male was deemed unmotivated to mate and the first test was omitted from further analysis.

Male response to hybrid females would not necessarily be comparable with that to gynogens if the hybrids are sterile. To evaluate this possibility, hybrid females that did not receive thrusts during a behavioral test were placed in individual aquaria with either a hybrid male or a sailfin male. Those females that received thrusts were placed in aquaria without males. The aquaria were checked daily for the presence of fry until the death of the female. Several hybrid females produced fry, confirming their fertility. In addition, hybrid offpsring from a previous study of *P. latipinna/ P. mexicana* were all sexual and fertile (Abramoff et al. 1968). Based on all of these factors I am assuming the hybrid females used in this study are fertile and thus, that males respond to them accordingly.

Statistical analysis

Non-parametric and distribution-free statistical methods were used to analyze the results of all experiments because the underlying distribution of the behaviors examined here is unknown (Sokal & Rohlf 1995). Non-parametric methods are less sensitive to violations of the assumptions of normality and homogeneity of variances implicit in many parametric tests (e.g. Student's t-test, ANOVA) (reviewed in Potvin & Roff 1993, Sokal & Rohlf 1995). While the ability of non-parametric analyses to detect effects may be weaker than that of parametric methods, power and reliability do not suffer when assumptions are violated as in parametric analyses (Potvin & Roff 1993, Seaman & Jaeger 1990, Tukey 1962). In fact in some cases the non-parametric method is more powerful than its parametric counterpart (Potvin & Roff 1993). Non-parametric methods may increase the probability of accepting the null hypothesis when it is, in fact, false (Type II error). However, the majority of behavioral studies of these fishes have detected significant levels of differences, using similar sample sizes and the rank tests employed here (Körner et al. 1999, Landmann et al. 1999, Ryan et al. 1996, Foran & Ryan 1994, Schlupp & Ryan 1997, Schlupp et al. 1998, 1994, 1991, Marler et al. 1997, Marler & Ryan 1997, Ptacek & Travis 1996). In addition, the jackknife and bootstrap procedures allow further examination of the confidence intervals surrounding observed differences and thus, evaluate the power of the rank tests for these data sets (Potvin & Roff 1993).

Raw data from all experiments were analyzed using Wilcoxon's signed-ranks test for paired designs. Data from multiple experiments were analyzed separately according to hybrid female type (M_f/L_m and L_f/M_m) and after pooling according to female reproductive mode (sexual hybrid vs. gynogenetic). Two statistical approaches were used to evaluate differences in behaviors between the types of hybrid females and the gynogens with which they were paired, to support pooling of the data. Possible differences in the number of thrusts of males were examined using Mann-Whitney *U*-tests and jackknife permutation (Potvin & Roff 1993, Efron 1979, Quenouille 1949). Differences in the strength of male response were examined by converting the raw data into proportions of thrusts, which were then compared using bootstrap resampling (Efron & Tibshirani 1993, Quenouille 1949). Comparisons of female behavior between experiments were conducted using Mann-Whitney *U*-tests and jackknife permutation. Jackknife and bootstrap methods are used to create pseudodata sets from the original data and construct a distribution of possible values (Efron & Gong 1982, Quenouille 1949). The value observed from the original data is compared to this distribution to evaluate its position within the 95% confidence interval (Efron et al. 1996, Sokal & Rohlf 1995). The jackknife permutation technique used in this study consisted of creating two pseudodata partitions of equal size as the two partitions in the original data set by shuffling all of the original data points without replacement. A single partition of the original data consists of the observed responses of one of the two types of females. This procedure was conducted 1000 times to create a distribution of differences between the mean values of pseudodata partitions. This distribution was used to determine the position of the observed difference of means relative to the jackknifed distribution (Hillis et al. 1996, Potvin & Roff 1993). The bootstrap procedure consisted of randomly resampling the original data set with replacement to create 1000 pseudodata sets of the same size as the original. The bootstrap distribution was used to determine the position of the strength of response observed in this study and a previously published study (Ryan et al. 1996), as in the jackknife analyses.

Data were analyzed using the Statview statistical analysis program for the Mactintosh (SAS Institute, Incorporated 1998). I wrote the bootstrap and jackknife programs and analyzed the data using UNIX operating system on a DEC Alpha computer.

RESULTS

Male Preference

Poecilia latipinna

P. latipinna males did not show a difference in preference for hybrids or gynogenetic *P. formosa*. They did not direct a significantly greater number of thrusts towards hybrids of either type (M_f/L_m or L_f/M_m), or to gynogens even when they were motivated to mate (Hybrids pooled: $z = {}^{-}0.059$, $p = 0.953$; M_f/L_m hybrids: $z = {}^{-}0.539$, $p = 0.589$; L_f/M_m hybrids: $z = {}^{-}0.270$, $p = 0.787$)(Table 3.1, Fig. 3.2). The data from experiments with each type of hybrid female were pooled because *P. latipinna* males did not differ significantly in the mean number or proportion of thrusts directed to either type of hybrid (number: $U = 60.5$, $z = 0.000$, $p > 0.999$; proportion: $U = 64.0$, $z = {}^{-}0.123$, $p = 0.902$) or to the gynogens with which the hybrids were paired (number: $U = 46.5$, $z = {}^{-}1.055$, $p = 0.291$; proportion: $U = 52.0$, $z = {}^{-}0.862$, $p = 0.389$)(Table 3.1).

Table 3.1 Descriptive Statistics for Male Mate Preference.

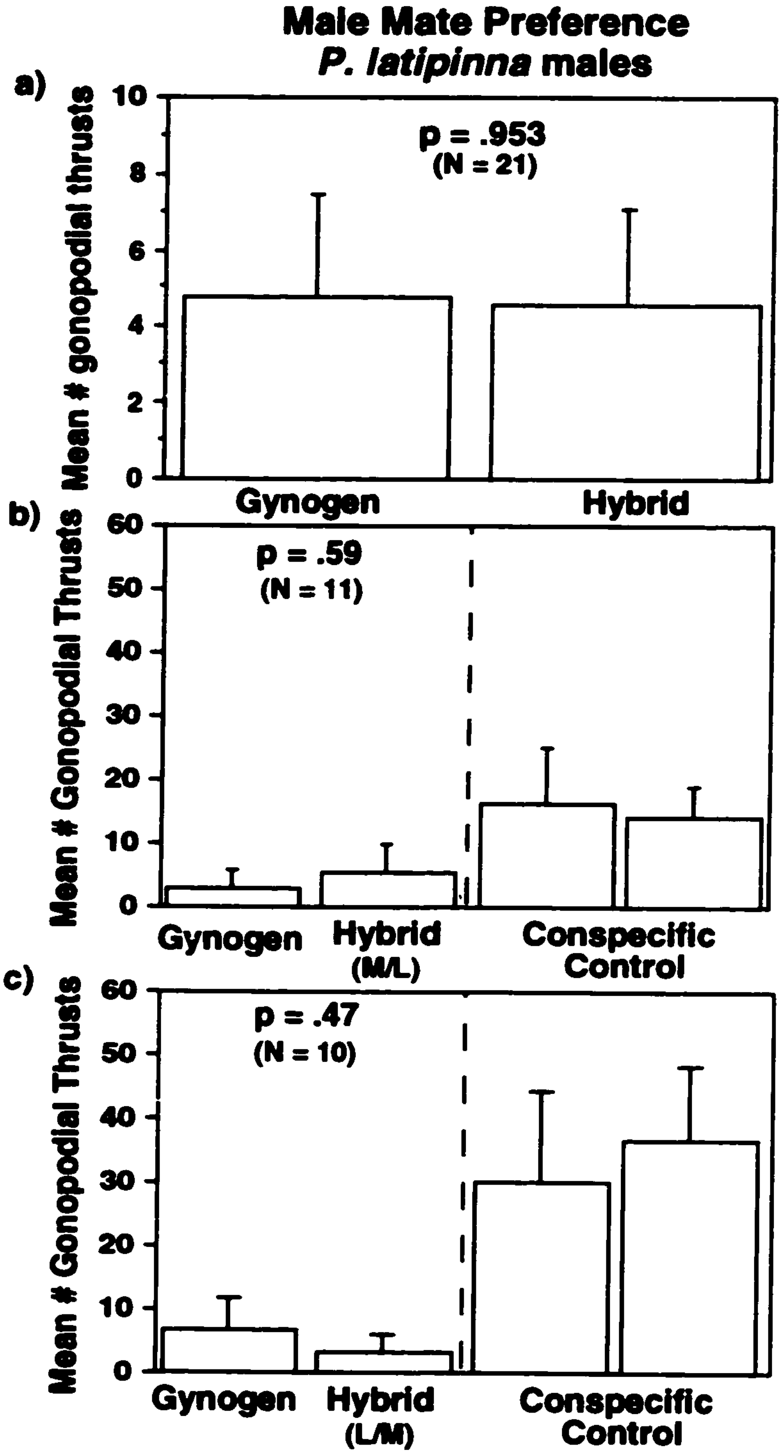
This table shows descriptive statistics for the number of thrusts directed by *P. latipinna* and *P. mexicana* males to sexual hybrid females of two types (M/L: *P. mexicana* mother/ *P. latipinna* father, L/M: *P. latipinna* mother/ *P. mexicana* mother) and to gynogenetic *P. formosa* females paired with hybrids. The statistics for the pooled results of both experiments are presented also (ALL). Listed are the arithmetic average (Mean), standard deviation (S.D.), standard error (S.E.), and number of tests (N).

Male Behavior

Male	***P. latipinna***			***P. mexicana***		
Hybrids	ALL	M/L	L/M	ALL	M/L	L/M
Mean	4.6	5.5	4.3	18.9	34.4	3.5
S.D.	11.8	14.7	7.9	38.1	49.8	9.5
S.E.	2.6	4.4	2.3	9.5	17.6	3.4
N	23	11	12	16	8	8
Gynogens	ALL	M/L	L/M	ALL	M/L	L/M
Mean	4.8	3.1	6.3	10.7	20.3	1.1
S.D.	12.4	9.3	14.1	20.8	26.7	2.8
S.E.	2.7	2.8	4.1	5.2	9.4	1.0
N	23	11	12	16	8	8

Figure 3.2: *Poecilia latipinna* Male Mate Discrimination

Results of male mate discrimination tests showing the number of gonopodial thrusts of *P. latipinna* males directed towards gynogenetic *P. formosa* females versus sexual hybrid females. The means and one standard error are shown in each case. Probability values are two-tailed probabilities of Wilcoxon signed-ranks tests. (a) The mean number of thrusts by males to gynogenetic and sexual hybrid females of both types. (b) The mean number of thrusts by males to gynogenetic and sexual hybrid females, type M/L (*P. mexicana* mother/*P. latipinna* father). The number of thrusts directed by these same males to a pair of conspecific females is shown as the conspecific control. (c) The number of thrusts by males to gynogenetic and sexual hybrid females, type L/M (*P. latipinna* mother/*P. mexicana* father). The number of thrusts directed by these same males to a pair of conspecific females is shown as the conspecific control.


Male Mate Preference
P. latipinna males
a)
Mean # gonopodial thrusts
10
8
6
4
2
0
p = .953
(N = 21)
Gynogen
Hybrid
b)
Mean # Gonopodial Thrusts
60
50
40
30
20
10
0
p = .59
(N = 11)
Gynogen
Hybrid
(M/L)
Conspecific
Control
c)
Mean # Gonopodial Thrusts
60
50
40
30
20
10
0
p = .47
(N = 10)
Gynogen
Hybrid
(L/M)
Conspecific
Control

In addition, the jackknife analyses also indicate the observed difference in male response to hybrid females of each type is not significantly different than zero ($p = 0.758$)(Fig. 3.3). Neither is the response to the two groups of gynogenetic females with which the hybrids were paired ($p = 0.416$). These results indicate pooling of the data from experiments with both hybrid types is appropriate. Bootstrap resampling results indicate the 95% confidence intervals of male response for both hybrid (0.005-0.083) and gynogenetic females (0.017-0.19) (Fig. 3.4). When the previously documented mean proportion of thrusts directed toward gynogenetic females by *P. latipinna* males (Ryan et al. 1996) is compared with the bootstrap distribution of response to hybrid females, it also lies within the 95% confidence interval. This suggests the similarity in male response to hybrids and gynogenetic females in this study was not an anomaly, but rather is consistent with male behavior observed in other studies using similar protocols. More striking, the corresponding strength of preference for conspecific females (0.93) is completely outside the bootstrap distribution of response to hybrids. This strongly suggests, given the opportunity to choose between hybrid and conspecific females, males would respond as if the hybrid females were gynogenetic females; they would prefer conspecific mates.

Figure 3.3: Jackknife Analysis of the Significance of *Poecilia latipinna* Male Mate Discrimination

These graphs show the results of 1000 jackknife resampling analyses to determine the significance of the observed difference in male response to hybrid and gynogenetic females. Results of 1000 jackknife resampling replicates of the number of gonopodial thrusts of *P. latipinna* males directed towards gynogenetic *P. formosa* females versus sexual hybrid females. Each bar represents the frequency of the difference between mean number of thrusts towards each female type in each replicate. The gray bars represent 95% of the distribution, the white bars indicate the 5% tail. The arrows indicate the position within the jackknife distribution of the difference of means observed in the real data set. (a) The difference in mean number of thrusts to sexual hybrid females of both types (M/L: *P. mexicana* mother/*P. latipinna* father, L/M: *P. latipinna* mother/*P. mexicana* father) from jackknife replicates. The observed difference of 2.0 is not significantly different from zero ($p = 0.758$). (b) Jackknife resampling was also conducted using the responses of males to the two groups of gynogenetic females with which the hybrid females were paired. This analysis serves merely as a control for the level of responses of males across the two types of test. The distribution of differences in mean number of thrusts to the two groups of gynogenetic females is shown. The observed value is not significant ($p = 0.416$) and indicates males did not differ in their level of response to gynogenetic females in both tests.

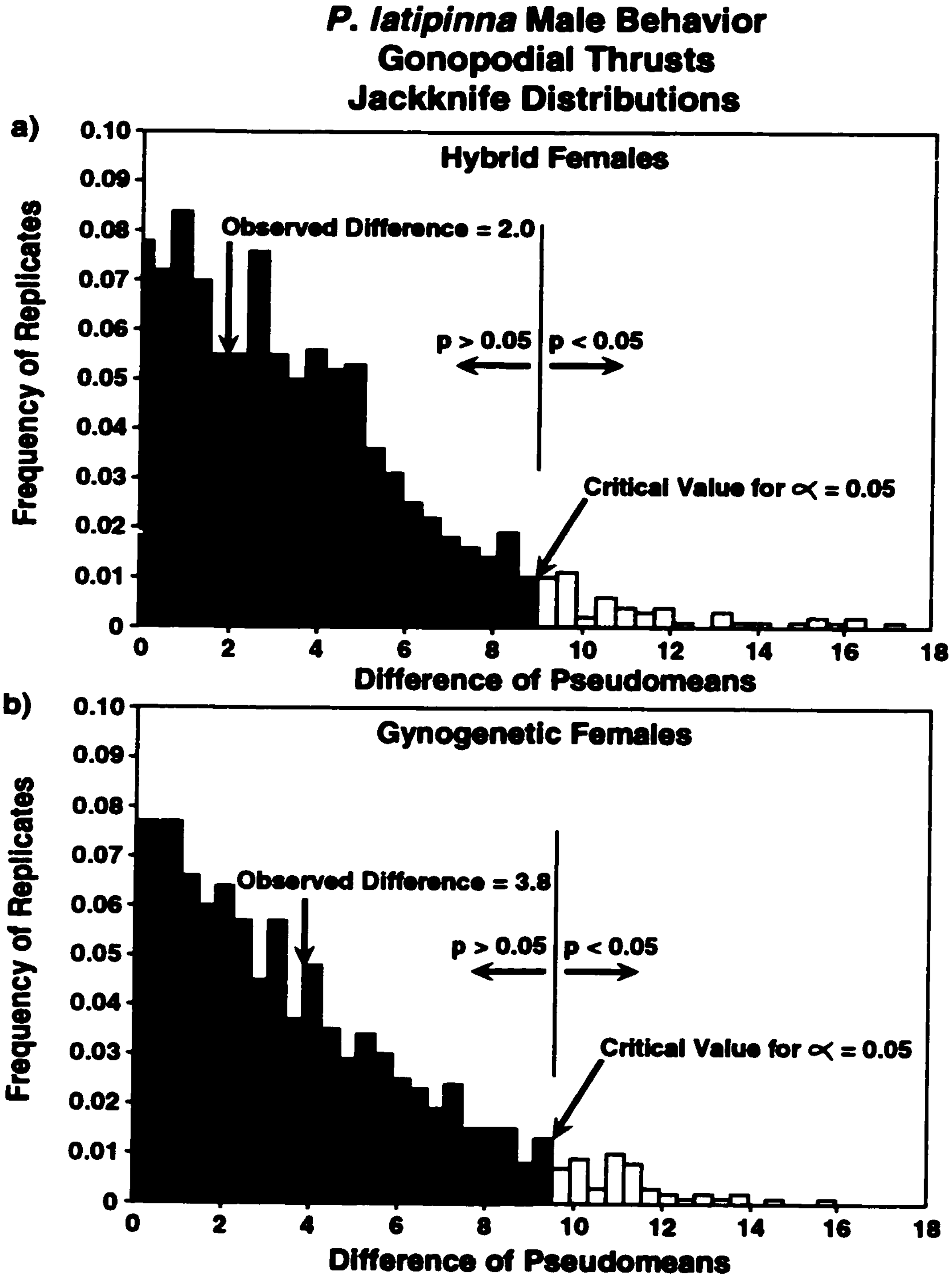
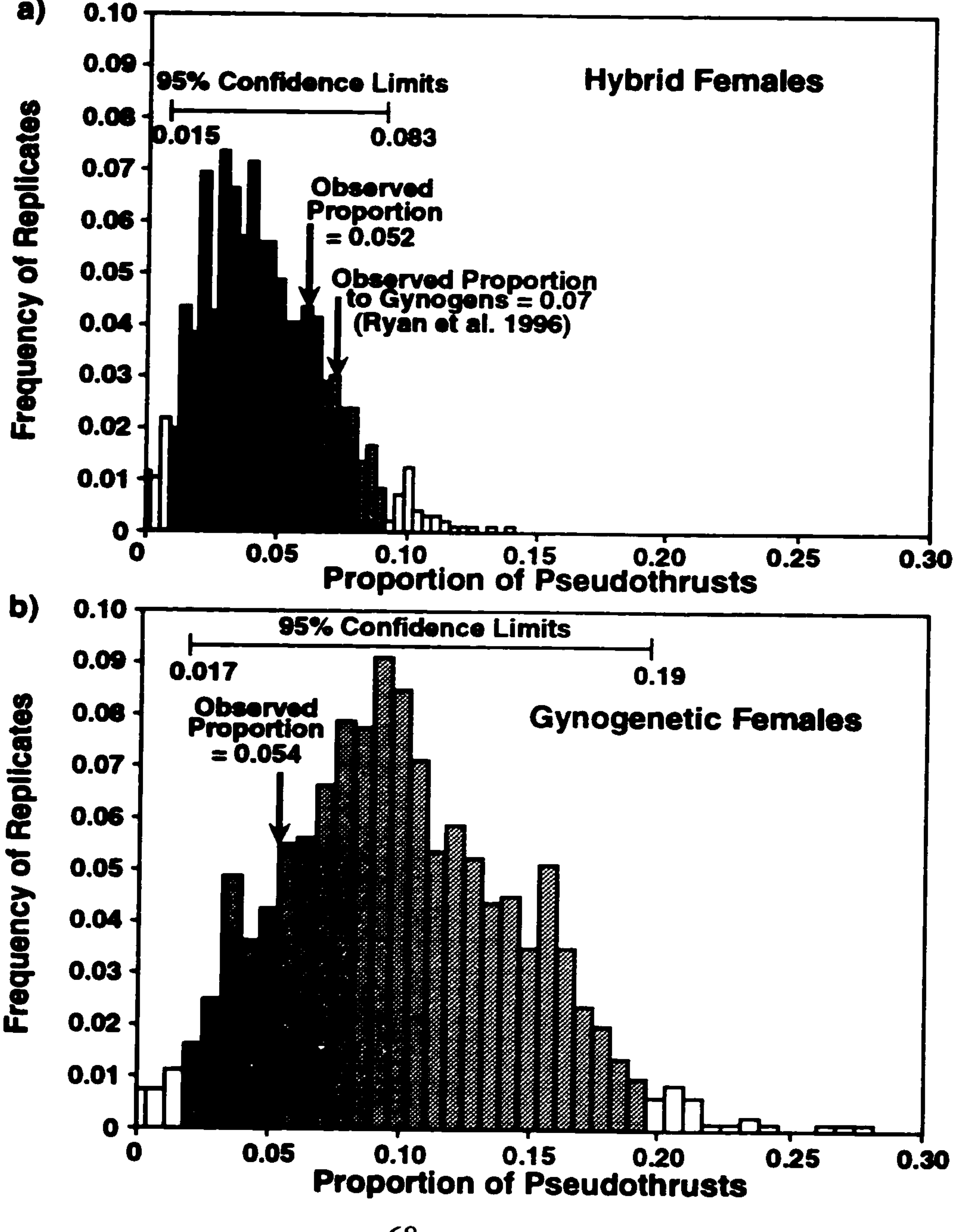

P. latipinna Male Behavior
Gonopodial Thrusts
Jackknife Distributions
a)
Hybrid Females
Observed Difference = 2.0
p > 0.05
p < 0.05
Critical Value for ∝ = 0.05
Frequency of Replicates
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
2
4
6
8
10
12
14
16
18
Difference of Pseudomeans
b)
Gynogenetic Females
Observed Difference = 3.8
p > 0.05
p < 0.05
Critical Value for ∝ = 0.05
Frequency of Replicates
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
2
4
6
8
10
12
14
16
18
Difference of Pseudomeans

Figure 3.4: **Bootstrap Calculation of Confidence Limits for *Poecilia latipinna* Male Mate Discrimination**

Results of 1000 bootstrap resampling replicates of the proportion of gonopodial thrusts of *P. latipinna* males directed towards gynogenetic *P. formosa* females and sexual hybrid females. Each bar represents the frequency of the mean proportion of thrusts directed toward females in each replicate. The gray bars indicate the confidence limits for the observed means. The arrows indicate the position within the bootstrap distribution of the proportion of thrusts observed in the real data set and a previously published data set. (a) The position of the observed mean proportion of thrusts to sexual hybrid females of both types (M/L: *P. mexicana* mother/*P. latipinna* father, L/M: *P. latipinna* mother/*P. mexicana* father) in the bootstrap distribution is shown. The 95% confidence limits of the observed proportion are 0.005 to 0.083. (b) The position of the observed mean proportion of thrusts to the gynogenetic females with which the hybrids were paired is shown within the bootstrap distribution. The 95% confidence limits of the observed proportion are 0.017 to 0.19.

P. latipinna **Male Behavior**
Bootstrap Distributions
Gonopodial Thrusts

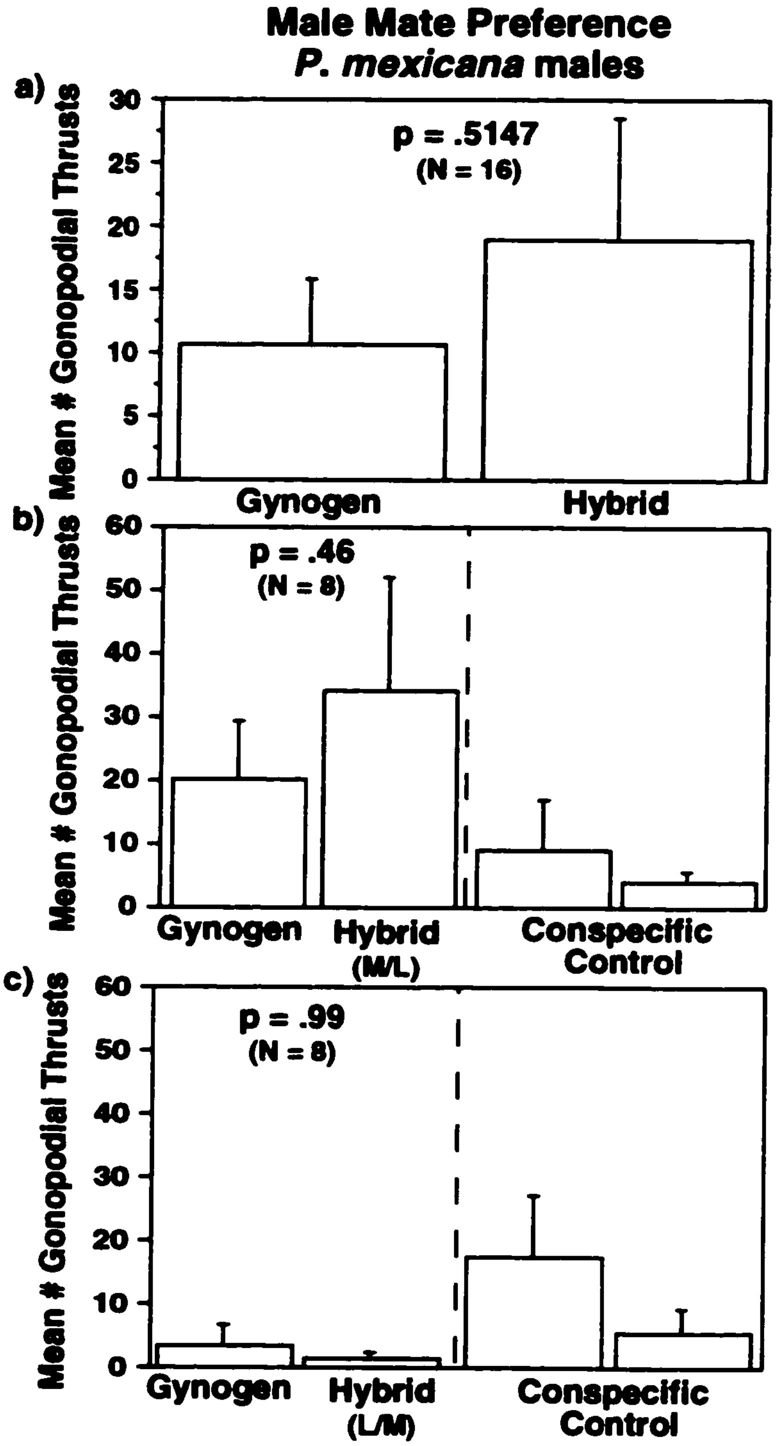


Poecilia mexicana

Poecilia mexicana males showed no difference in preference for hybrid or gynogenetic females (Hybrids pooled: $z = {}^{-}0.652$, $p = 0.5147$; M_f/L_m hybrids: $z = {}^{-}0.734$, $p = 0.463$; L_f/M_m hybrids: $z = 0.000$, $p > 0.999$)(Fig. 3.5, Table 3.1). When response to each type of hybrid was analyzed separately, males did not direct a significantly larger number of thrusts to either hybrid (*P. mexicana*: $U = 17$, $z = {}^{-}1.736$, $p = 0.083$) or either set of gynogens with which hybrids were paired ($U = 15$, $z = {}^{-}0.210$, $p = 0.834$)(Table 3.1). The proportion of thrusts to either type of hybrid, or the two sets of gynogens used in the hybrid tests, also did not differ (hybrids: $U = 20.5$, $z = {}^{-}1.208$, $p = 0.227$ gynogens: $U = 22.5$, $z = {}^{-}0.998$, $p = 0.318$).

Figure 3.5: *Poecilia mexicana* Male Mate Discrimination

Results of male mate discrimination tests showing the number of gonopodial thrusts of *P. mexicana* males directed towards gynogenetic *P. formosa* females versus sexual hybrid females. The means and one standard error are shown in each case. Probability values are two-tailed probabilities of Wilcoxon signed ranks tests. (a) This graph shows the responses of males to gynogenetic and sexual hybrid females pooled across experiments. (b) The mean number of thrusts by males to gynogenetic and sexual hybrid females, type M/L (*P. mexicana* mother/*P. latipinna* father) is shown. The conspecific control indicates the response of these same males to a pair of conspecific females. (c) The mean number of thrusts by males to gynogenetic and sexual hybrid females, type L/M (*P. latipinna* mother/*P. mexicana* father) is shown. The response of males to a pair of conspecific females is shown as the conspecific control.


Male Mate Preference
P. mexicana males
a)
Mean # Gonopodial Thrusts
30
25
20
15
10
5
0
p = .5147
(N = 16)
Gynogen
Hybrid
b)
Mean # Gonopodial Thrusts
60
50
40
30
20
10
0
p = .46
(N = 8)
Gynogen
Hybrid
(M/L)
Conspecific
Control
c)
Mean # Gonopodial Thrusts
60
50
40
30
20
10
0
p = .99
(N = 8)
Gynogen
Hybrid
(L/M)
Conspecific
Control

The results of the jackknife analyses show the observed difference in number of thrusts is not significantly different from zero for hybrid or gynogenetic females (hybrids: p = 0.285, gynogens: p = 0.062)(Fig. 3.6). These results suggest pooling of data for both types of hybrids is appropriate. The 95% confidence interval generated by the bootstrap analyses of male response to hybrid females (0.075 to 0.40) includes the previously documented strength of *P. mexicana* male response to gynogenetic females (Ryan et al. 1996)(Fig. 3.7). This indicates the level of male response in this study is similar to that of previous studies and also suggests that *P. mexicana* males would prefer conspecific females to hybrid females. The confidence intervals around *P. mexicana* male response include a wider range of values than those for *P. latipinna* males, further illustrating the generally weaker mate discrimination against gynogens of *P. mexicana* males relative to *P. latipinna* males (Ptacek 1998, Ryan et al. 1996).

Figure 3.6: **Jackknife Analysis of the Significance of *Poecilia mexicana* Male Mate Discrimination**

These graphs show the results of 1000 jackknife resampling analyses to determine the significance of the observed difference in male response to hybrid and gynogenetic females. Results of 1000 jackknife resampling replicates of the number of gonopodial thrusts of *P. mexicana* males directed towards gynogenetic *P. formosa* females versus sexual hybrid females. Each bar represents the frequency of the difference between mean number of thrusts towards each female type in each replicate. The gray bars represent 95% of the distribution, the white bars indicate the 5% tail. The arrows indicate the position within the jackknife distribution of the difference of means observed in the real data set. (a) The difference in mean number of thrusts to sexual hybrid females of both types (M/L: *P. mexicana* mother/*P. latipinna* father, L/M: *P. latipinna* mother/*P. mexicana* father) from jackknife replicates. The observed difference of 30.9 is not significantly different from zero ($p = 0.285$). (b) Jackknife resampling was also conducted using the responses of males to the two groups of gynogenetic females with which the hybrid females were paired. This analysis serves merely as a control for the level of responses of males across the two types of test. The distribution of differences in mean number of thrusts to the two groups of gynogenetic females is shown. The observed value is not significant ($p = 0.062$) and indicates males did not differ in their level of response to gynogenetic females in both tests.

P. mexicana Male Behavior
Gonopodial Thrusts
Jackknife Distributions

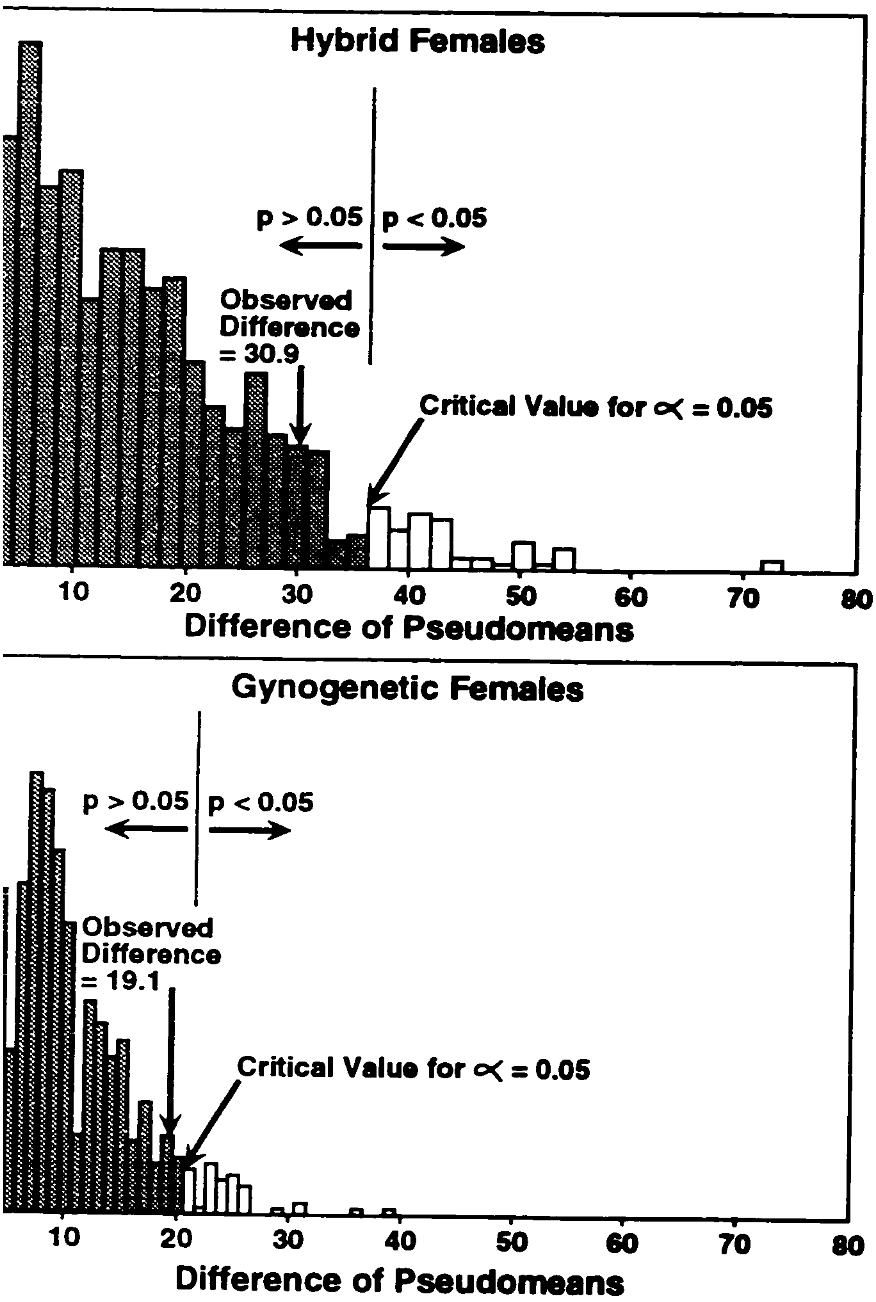


Figure 3.7: Bootstrap Calculation of Confidence Limits for *Poecilia mexicana* Male Mate Discrimination

Results of 1000 bootstrap resampling replicates of the proportion of gonopodial thrusts of *P. mexicana* males directed towards gynogenetic *P. formosa* females and sexual hybrid females. Each bar represents the frequency of the mean proportion of thrusts directed toward females in each replicate. The gray bars indicate the confidence limits for the observed means. The arrows indicate the position within the bootstrap distribution of the proportion of thrusts observed in the real data set and a previously published data set. (a) The position of the observed mean proportion of thrusts to sexual hybrid females of both types (M/L: *P. mexicana* mother/*P. latipinna* father, L/M: *P. latipinna* mother/*P. mexicana* father) in the bootstrap distribution is shown. The 95% confidence limits of the observed proportion are 0.075 to 0.40. (b) The position of the observed mean proportion of thrusts to the gynogenetic females with which the hybrids were paired is shown within the bootstrap distribution. The 95% confidence limits of the observed proportion are 0.09 to 0.425.

Female Behavior

Gynogenetic ***P. formosa*** did not differ from sexual hybrid females in antagonistic behavior overall when males were absent (L_f/M_m hybrids: $z = {}^{-}0.770$, $p = 0.441$)(Table 3.2, Fig. 3.8), or when males were present (Hybrids pooled: $z = {}^{-}0.593$, $p = 0.553$)(Fig. 3.9). When each type of behavior was analyzed separately, hybrid females and gynogens did not differ significantly when males were absent (Bonferroni adjusted $\propto = 0.012$)(Bites: $z = {}^{-}1.014$, $p = 0.311$; Butts: $z = {}^{-}0.944$, $p = 0.345$; Chases: $z = 0.0$, $p > 0.999$)(Fig. 3.8) or when males were present (Bites: $z = {}^{-}0.191$, $p = 0.848$, $N = 24$; Butts: $z = {}^{-}0.314$, $p = 0.743$; Chases: $z = {}^{-}0.734$, $p = 0.463$; Blocks: $z = {}^{-}0.803$, $p = 0.422$)(Fig. 3.9). Jackknife permutation analysis also indicates the observed difference between L_f/M_m hybrid and gynogenetic females when males were absent is also not significant ($p = 0.897$)(Fig. 3.10).

Table 3.2 **Descriptive Statistics for Gynogenetic and Hybrid Female Behavior**

This table shows descriptive statistics for the number of antagonistic behaviors exhibited by sexual hybrid females of two types (M/L and L/M) and gynogenetic *P. formosa* females paired with those hybrids when males were present. The table also includes statistics for behaviors exhibited by sexual hybrid females of one type (L/M) and the gynogenetic females with which they were paired when males were absent. The statistics for the pooled results of both experiments are presented also (Both). Listed are the arithmetic average (Mean), standard deviation (S.D.), standard error (S.E.), and number of tests (N).

	Female Behavior			
Male	**Present**			**Absent**
Hybrids	M/L	L/M	Both	L/M
Mean	16.9	11.0	13.5	15.9
S.D.	12.9	18.6	16.4	40.6
S.E.	4.1	5.0	3.3	13.5
N	10	14	24	9
Gynogens	M/L	L/M	Both	L/M
Mean	16.5	14.6	15.4	18.2
S.D.	10.2	21.9	17.7	33.9
S.E.	3.2	5.8	3.6	11.3
N	10	14	24	9

Figure 3.8: *Poecilia formosa* and Hybrid Female Behavior - Male Absent

Results of female behavioral tests showing the number of antagonistic behaviors exhibited by gynogenetic *P. formosa* and sexual hybrid females when males were absent from the aquarium. The means and one standard error are shown in each case. Probability values are two-tailed probabilities of Wilcoxon signed ranks tests. (a) The total number of antagonistic behaviors exhibited by gynogenetic *P. formosa* females and sexual hybrid females, type L/M (*P. latipinna* mother/*P. mexicana* father). (b) The number of each of three types of antagonistic behavior exhibited by gynogenetic *P. formosa* females and sexual hybrid females, type L/M (*P. latipinna* mother/*P. mexicana* father). Blocks are not presented because attempts by one female to block mating behavior by a male to another female cannot be counted in the absence of males.

Female Antagonistic Behavior
Male Absent

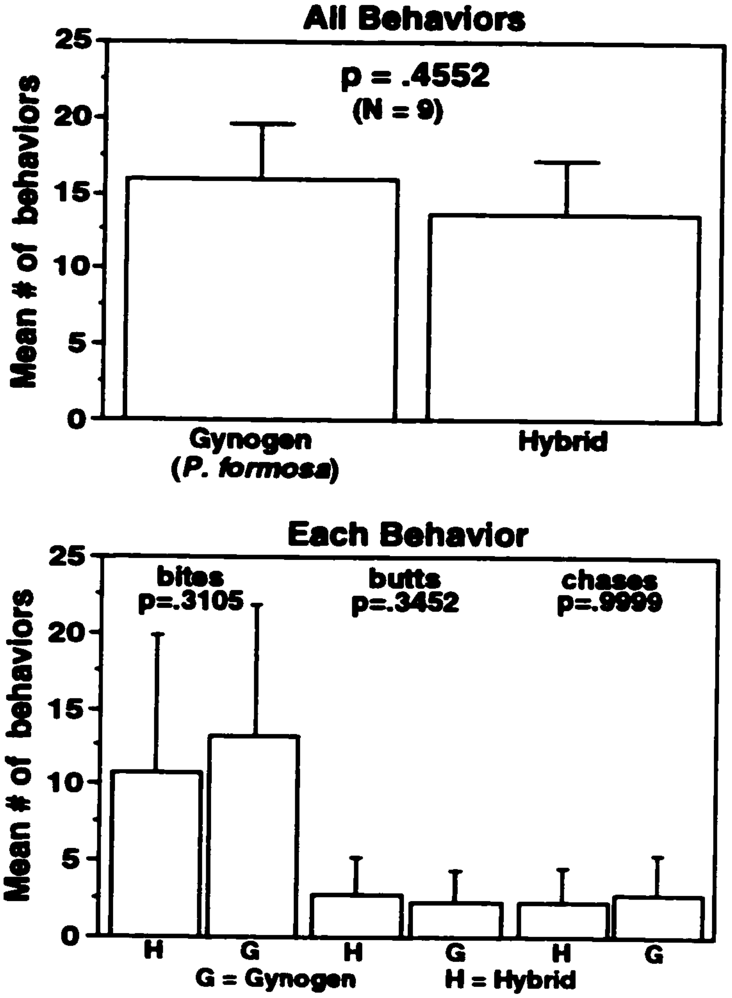
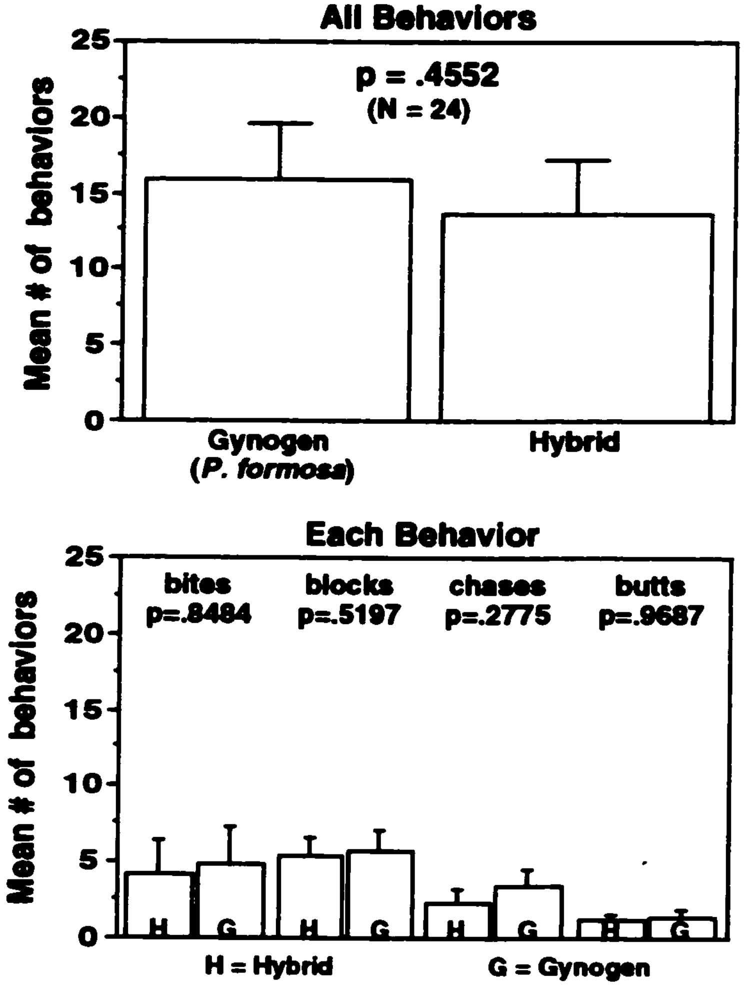


Figure 3.9: *Poecilia formosa* and Hybrid Female Behavior - Male Present

Results of female behavioral tests showing the number of antagonistic behaviors exhibited by gynogenetic *P. formosa* and sexual hybrid females when a *P. latipinna* or a *P. mexicana* male was present in the aquarium. The means and one standard error are shown in each case. Probability values are two-tailed probabilities of Wilcoxon signed ranks tests. (a) The total number of antagonistic behaviors exhibited by gynogenetic *P. formosa* females and sexual hybrid females of two types, L/M (*P. latipinna* mother/*P. mexicana* father) and M/L (*P. mexicana* mother/*P. latipinna* father). (b) The number of each of four types of antagonistic behavior exhibited by gynogenetic *P. formosa* females and sexual hybrid females.

Female Antagonistic Behavior
Male Present

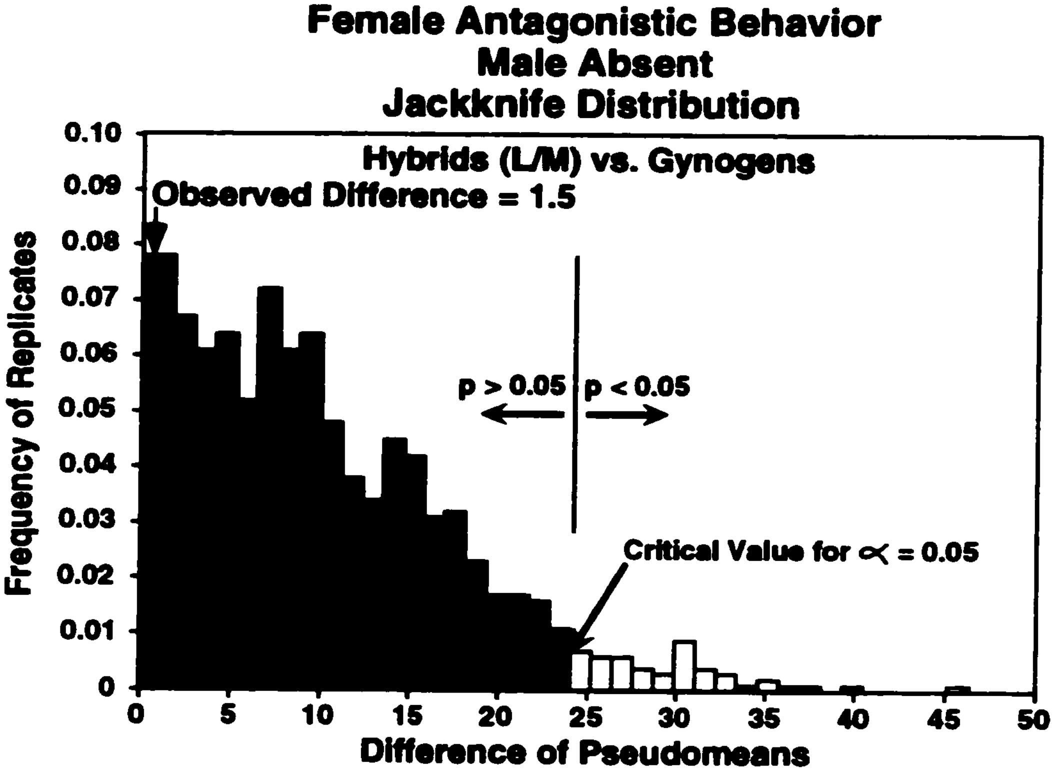


All Behaviors

Each Behavior

Figure 3.10: Jackknife Analysis of the Significance of *Poecilia formosa* and Hybrid Female Behavior - Male Absent

Results of 1000 jackknife resampling replicates of the number of antagonistic behaviors of *P. formosa* and L/M hybrid females (*P. latipinna* mother/*P. mexicana* father) in the absence of males. Each bar represents the frequency of the difference between mean number of behaviors of each female type in each replicate. The gray bars represent 95% of the distribution, the white bars indicate the 5% tail. The arrow indicates the position within the jackknife distribution of the difference of means observed in the real data set. The observed difference is not significantly different than zero (p = 0.897).


Female Antagonistic Behavior
Male Absent
Jackknife Distribution
Hybrids (L/M) vs. Gynogens
Observed Difference = 1.5
p > 0.05
p < 0.05
Critical Value for ∝ = 0.05
Frequency of Replicates
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
5
10
15
20
25
30
35
40
45
50
Difference of Pseudomeans

To evaluate whether pooling of data from the two hybrid types was appropriate, I compared behaviors of L_f/M_m and M_f/L_m hybrid females using two statistical approaches, the non-parametric Mann-Whitney *U*-test and jackknife permutation. The Mann-Whitney analyses detected no significant differences overall when all types of behavior were analyzed together (all behaviors: $U = 43.5$, $z = {}^{-}1.158$, $p = 0.1138$)(Table 3.3, Fig. 3.11).

Table 3.3 Descriptive Statistics for Hybrid Females

This table shows descriptive statistics for the number of four agonistic behaviors exhibited by sexual hybrid females of two types (M/L and L/M) and gynogenetic *P. formosa* females paired with those hybrids when males were present. The table also includes statistics for behaviors exhibited by sexual hybrid females of one type (L/M) and the gynogenetic females with which they were paired when males were absent. The statistics for the pooled results of both experiments are presented also (Both). Listed are the arithmetic average (Mean), standard deviation (S.D.), standard error (S.E.), and number of tests (N).

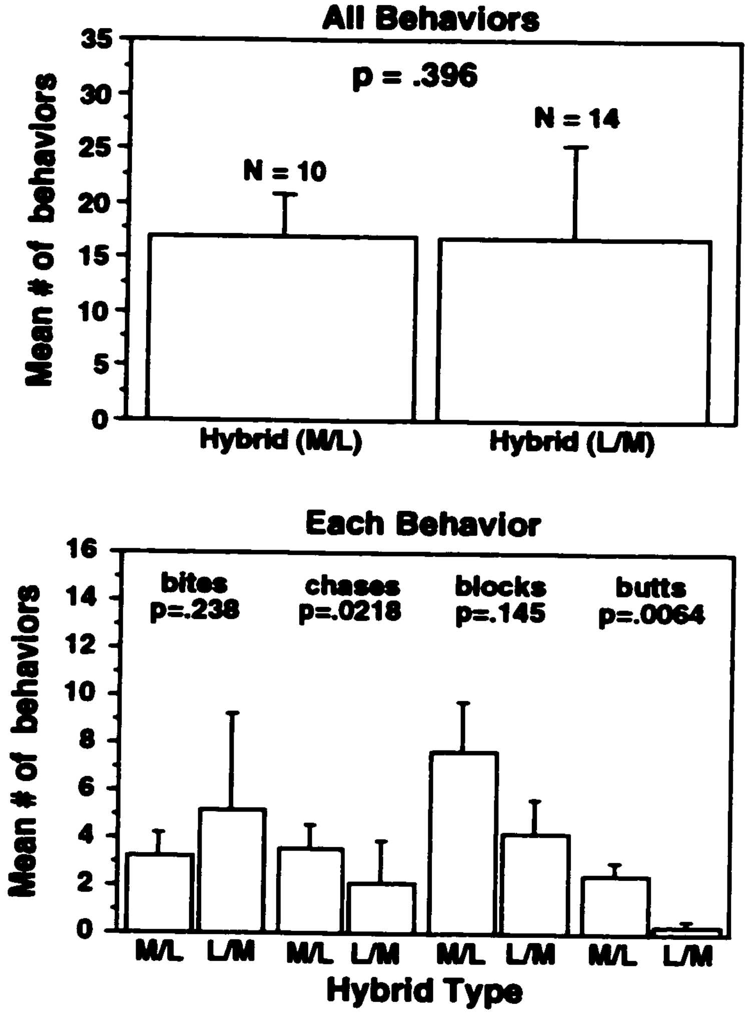
Female Behavior

Male	**Present**				**Absent**		
Hybrids	Bites	Butts	Chases	Blocks	Bites	Butts	Chases
M/L N=10							
Mean	3.2	2.4	3.6	7.7			
S.D.	3.3	2.1	3.1	6.7			
S.E.	1.0	0.7	0.9	2.1			
L/M N=14							
Mean	4.6	0.57	2.0	3.8	10.8	2.8	2.3
S.D.	13.4	1.1	5.6	4.2	27.2	7.2	6.3
S.E.	3.6	0.3	1.5	1.1	9.1	2.4	2.1
Both N=24							
Mean	4.0	1.3	2.7	5.4			
S.D.	10.3	1.8	4.7	5.6			
S.E.	2.1	0.4	1.0	1.1			
Gynogens	Bites	Butts	Chases	Blocks	Bites	Butts	Chases
M/L N=10							
Mean	1.3	1.3	5.1	8.8			
S.D.	1.3	1.7	5.1	7.0			
S.E.	0.4	0.5	1.6	2.2			
L/M N=14							
Mean	7.0	1.4	2.6	3.7	13.2	2.2	2.8
S.D.	15.1	2.9	6.9	5.1	26.8	6.3	8.0
S.E.	4.0	0.8	1.8	1.4	8.7	2.1	2.7
Both N=24							
Mean	4.6	1.3	3.6	5.8			
S.D.	4.7	4.2	6.2	6.4			
S.E.	1.0	0.5	1.3	1.3			

Figure 3.11: **Hybrid Female Behavior- Male Present**

Results of a comparison of the number of antagonistic behaviors exhibited by each type of sexual hybrid female when paired with a gynogenetic female, when a *P. latipinna* or a ***P. mexicana*** male was present. The means and one standard error are shown in each case. Probability values are two-tailed probabilities of Mann-Whitney *U*-tests. (a) The total number of antagonistic behaviors exhibited by L/M sexual hybrid females (***P. latipinna*** mother/ ***P. mexicana*** father) and M/L sexual hybrid females (***P. mexicana*** mother/***P. latipinna*** father). (b) The number of each of four types of antagonistic behavior exhibited by each type of sexual hybrid female.

Female Antagonistic Behavior
Male Present



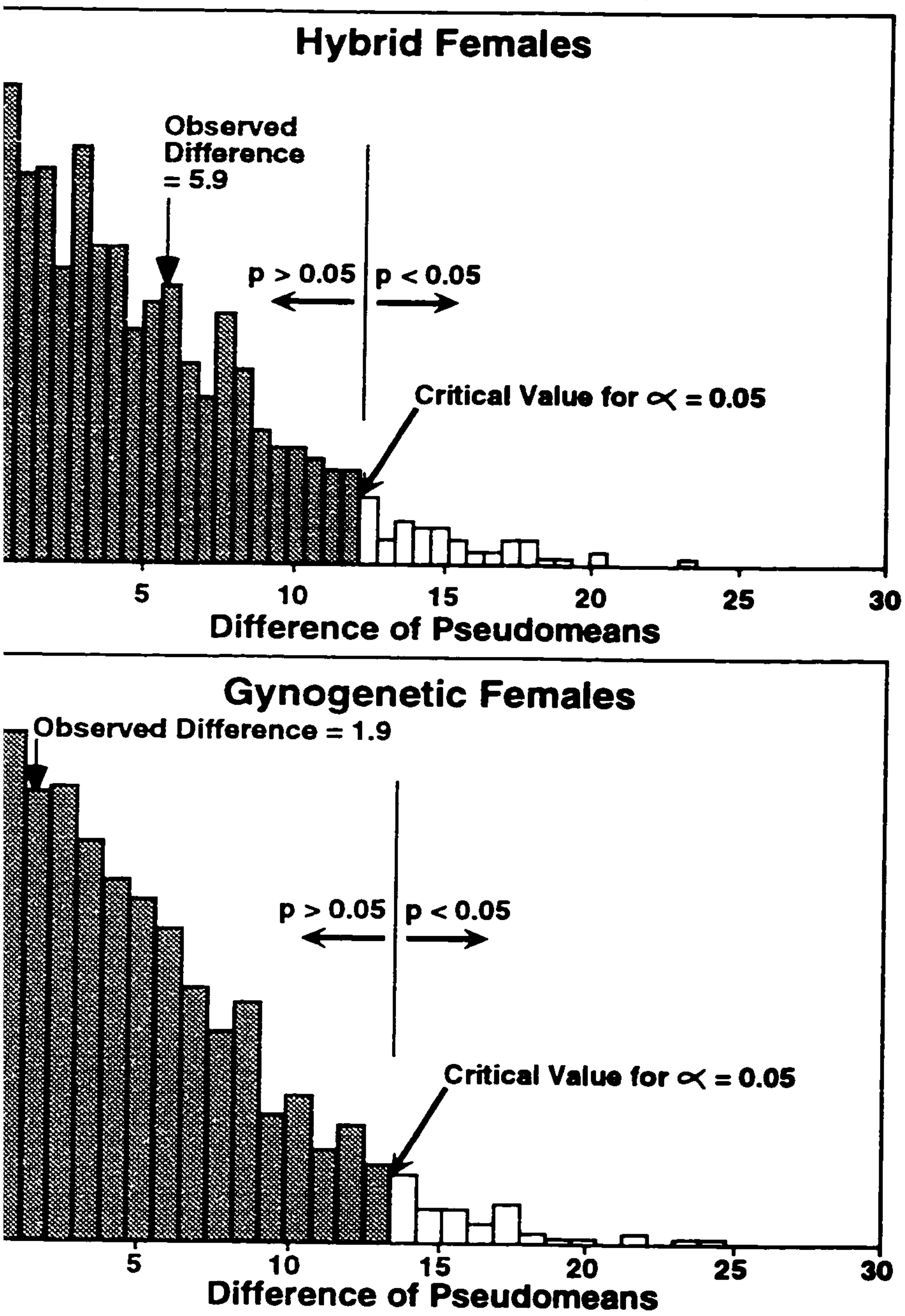
However, when each behavior was analyzed separately there were significant differences in the number of butts and chases (Butts: $U = 21$, $z = {}^{-}2.729$, $p = 0.006$; Chases: $U = 28$, $z = {}^{-}2.295$, $p = 0.022$) but not in number of bites or blocks (Bites: $U = 46$, $z = {}^{-}1.178$, $p = 0.239$; Blocks: $U = 41.5$, $z = {}^{-}1.457$, $p = 0.145$) (Bonferroni adjusted significance criterion). The M_f/L_m hybrid females exhibited more butts and bites than L_f/M_m hybrids but this did not translate into any overall difference in behavior. There were no differences in behavior of the gynogenetic females with which the hybrids were paired (All behaviors: $U = 43$, $z = {}^{-}1.364$, $p = 0.172$). The results of jackknife permutation tests indicate the observed behavioral difference between hybrid female types is not significantly different than zero ($p = 0.348$) in the presence of males (Fig. 3.12). Neither is the difference between the gynogenetic females with which the hybrids were paired ($p = 0.767$). All of these results indicate pooling of data from both types of hybrid females is appropriate.

The results of all of the experiments suggest *P. formosa* females do not behave differently than hybrid females when competing for males. Moreover, males do not discriminate between hybrid and *P. formosa* females and they retain their strong preference for conspecific females.

Figure 3.12: Jackknife Analysis of the Significance of *Poecilia formosa* and Hybrid Female Behavior - Male Present

Results of 1000 jackknife resampling replicates of the number of antagonistic behaviors of hybrid females of both types (M/L: *P. mexicana* mother/*P. latipinna* father, L/M: *P. latipinna* mother/*P. mexicana* father). Each bar represents the frequency of the difference between mean number of behaviors of each female type in each replicate. The gray bars represent 95% of the distribution, the white bars indicate the 5% tail. The arrow indicates the position within the jackknife distribution of the difference of means observed in the real data set. (a) The observed difference between the two types of hybrid females is not significantly different from zero (p = 0.348). (b) The difference in behavior of gynogenetic *P. formosa* females paired with the two types of hybrids is also not significant (p = 0.767). Both results support pooling of the data from the two experiments.

Female Antagonistic Behavior
P. latipinna male present
Jackknife Distributions



DISCUSSION

Has a coevolutionary arms race caused *P. formosa* to evolve an ability to circumvent male mate recognition? Are Amazon mollies Red Queens? Alternatively has *P. formosa* evolved their attractiveness to sexual heterospecific males without a coevolutionary response in the sexual species? Although both of these paradigms could apply to *P. formosa*, this study supports a "Frozen Mate-attraction" model where the ability to attract males is present at the instant of hybridization. *Poecilia latipinna* and *P. mexicana* males do not discriminate between sexual hybrid and gynogenetic *P. formosa* females, and gynogenetic and hybrid females do not differ in antagonistic behaviors associated with competition for male mates. Both of these results contradict the predictions of a coevolutionary arms race scenario as suggested by Schlupp et al. (1991) and the simpler hypothesis of evolution in *P. formosa* only. This is not meant to imply there are no differences between gynogenetic and sexual hybrid females, but rather that differences too small to be detected in this study, if they exist, do not appear to be used by males to distinguish between acceptable and unacceptable mates.

These results do not indicate there has been no evolution of mate recognition and discrimination in the sexual species. The presence of reproductive character displacement in *P. latipinna* males that live in sympatry with *P. formosa* indicates selection has favored the evolution of stronger male mate discrimination (Ryan et al 1996, Gabor & Ryan unpublished.). The increased selectivity of these males may well impose selection on *P. formosa* to evolve more effective mate attraction cues, but, this study did not reveal weaker discrimination of males against gynogenetic *P. formosa* females or weaker preference for conspecific females as would be expected. *Poecilia formosa* and her hosts could still be locked in an evolutionary arms race; an evolutionary response to increased male mate discrimination may have occurred in *Poecilia formosa* but has not been strong enough to overcome the evolutionary capacity of the sexual species. This study cannot determine directly if mate attraction in *P. formosa* has become stronger through evolution. However, the results reported here, clearly indicate a viable and more parsimonious explanation; the characteristics could be a consequence of the hybrid origin of the gynogenetic lineage.

The possible presence of a coevolutionary arms race was investigated by Lima et al. (1996) in a similar unisexual/sexual species complex of poeciliid fishes. *Poeciliopsis monacha-lucida* is a unisexual, hybridogenetic, clonal lineage that arises through hybridization of sexual species (Schultz 1966). As in gynogenetic reproduction, hybridogenesis is sperm-dependent (Schultz 1973). These elements might cause *P. monacha-lucida* to become a Red Queen as suggested for *Poecilia formosa*. However, *P. monacha-lucida* differs from *P. formosa* Amazon mollies in two important ways. First, hybridogens are readily produced in nature and the laboratory through hybridization of *P. monacha* and *P. lucida* creating numerous clonal lineages within a single population (Vrijenhoek et al. 1978)). Second, hybridogenetic females produce haploid eggs that require incorporation of the paternally contributed genes to create a viable diploid embryo and offspring. The paternally derived genes are expressed in the phenotype of hybridogenetic offspring but they are not incorporated into their eggs during oogenesis (Vrijenhoek et al. 1978, Schultz 1961, 1966, 1973). In the *Poeciliopsis* system new clones are generated frequently which would provide more genetic variation upon which selection in a coevolutionary arms race could act. Despite what appear to be more favorable conditions for the evolution of Red Queens, the results of an investigation of morphological characteristics used in conspecific mate recognition do not support the hypothesis of a coevolutionary arms race (Lima et al. 1996). Lima et al. suggest variation in the ability of clonal females to attract sexual males is due to genetic variation among clones that is frozen at the time of hybridization. While a coevolutionary arms race could not be ruled out by their data, it could not be supported either. Their results provided further confirmation of characteristics frozen at the time of hybrid origination.

Are gynogenetic Amazon mollies Red Queens? If the gynogenetic lineage has evolved characters specifically in response to selection imposed by mate discrimination by sexual males, we expect them to differ from hybrid females. A critical assumption of this study is that sexual hybrid females differ genetically from extant *P. formosa* and are analogs of *P. formosa* at the time of origination prior to any evolution. I based this assumption on several pieces of information. All the data to date suggest extant *P. formosa* originated from a single, or very few, hybridization events in the past (Schartl et al. 1996, Avise et al., 1991, Turner et al. 1989, Turner 1982, Abramoff et al. 1968, Kallman 1962). Hybrids created from extant *P. mexicana* and *P. latipinna* would not necessarily have the same mate attraction behaviors as original gynogenetic *P. formosa* because the hybrids would inherit from the extant sexual species any behaviors that have evolved in response to selection pressure imposed by the gynogens. In addition, if gynogens have caused evolution of increased male discrimination in the sexual species, there is no reason to expect this would be automatically translated into characteristics that thwart male discrimination in hybrid females of similar heritage with greater genetic variation (but see Rieseberg et al. 1996). If the hybrids are not true analogs of *P. formosa* from the time or origination, males might have identical responses to both hybrids and *P. formosa*. While this is possible I do not think it negates the results presented here because the goal of the study is to determine if a coevolutionary arms race is necessary to explain the mate attraction abilities of *P formosa*. All of this suggests the hybrids are appropriate analogs of original gynogens and that if gynogens and hybrids differ in behavior it is not necessarily a result of coevolution.

It is entirely possible *P. formosa* and sexual hybrid females differ in characteristics not tested in this study such as olfactory cues associated with receptivity advertisement. It is important to keep in mind that this study is not intended to document the complete absence of behavioral differences, but is intended to examine whether behaviors differ enough to support the idea of an coevolutionary arms race. If differences do exist, they aren't important in male mate choice in the context of this study. Better knowledge of non-visual cues would enhance our understanding of mate discrimination in this complex.

Time of Origination and Coevolutionary Arms Races

Previous studies have suggested the gynogenetic lineage of *P. formosa* has persisted longer than one would predict based on Muller's Ratchet (Schartl et al. 1996, Schlupp et al. 1996, Schlupp et al. 1998, Gabriel et al. 1993, Lynch & Gabriel 1990). Previous literature has estimated the time of origination of this lineage at about 100,000 years ago, or roughly 200,000 generations (Schartl et al. 1996, Avise 1991). The estimate of 100,000 years ago is important in the support of the hypothesis of coevolution between *P. formosa* and their sexual hosts because it suggests there has been enough time for evolution to occur. However, 100,000 years (Schartl et al. 1996) is merely a point estimate based on a change of 3 nucleotide bases in a nuclear proto-oncogene (tyrosine kinase) and a molecular clock using the average rate of change in mitochondrial DNA of most vertebrates. Using these data to calculate a confidence interval around the time of origination, a much less definitive picture emerges.

Observed Number of Substitions $= 3$

Given: 1377 base pairs of data from *P. mexicana* and *P. formosa*

Assume : Vertebrate mtDNA Divergence rate $= 2\% / 1{,}000{,}000$ yrs

Expected Substitions $= 0.2\%/1377$ bps $= 2.754$
Confidence Interval $= 3 \pm (0.2\%)(1377 \text{ bps}) = 0.01786\% \leftrightarrow 0.41786\%$

$(0.01786\%/0.2\%)(100{,}000 \text{ years}) = 8{,}930$ years

$(0.41786\%/0.2\%)(100{,}000 \text{ years}) = 208{,}930$ years

The confidence interval around this estimate is 8,930 years to 208,930 years. Using the mitochondrial DNA data of Avise et al. (1991), a similar calculation procedure yields an even greater confidence interval that includes 0 and 600,000 years. Biogeographical information suggests an origination time of 300,000 to 600,000 years ago (Abramoff et al. 1968). If *P. formosa* has persisted for 600,000 years there has been ample time for evolution to occur and perhaps the lineage has escaped Muller's ratchet. However, an origination time of 0 to 8,930 years ago does not suggest gynogens have persisted longer than expected and also casts serious doubt on the potential for rapid evolutionary change as the result of an arms race. Whether *P. formosa* has persisted longer than predicted is a that requires better estimates of the time of origin and still remains to be answered.

CONCLUSIONS

Poecilia formosa may be a lineage of Red Queens, struggling to keep up with its sexual hosts in a coevolutionary arms race. But is it equally likely that the ability of these gynogenetic females o attract males is a consequency of their hybrid origins. Predictions of the fates of *P. formosa* and her sexual hosts, *P. latipinna* and *P. mexicana*, based on the assumption that a coevolutionary process is driving evolution of mate discrimination may be fortuitious at best and merely auguries at worst. The evolutionary response of *P. formosa* to her hosts is still uncertain and the stability of such a host/parasite relationship an open question.

Chapter 4: The Behavior of Hybrid Male Mollies and Atlantic Mollies and Their Roles in the Persistence of Amazons

INTRODUCTION

Reproductive isolation of species was eloquently discussed and categorized by Mayr (1963) and Dobzhansky (1970). Both of these biologists suggest natural selection will favor the evolution of reproductive isolation between species through mechanisms that prevent or impede hybridization between species, or that cause decreased fitness of hybrid offspring (Dobzhansky 1970, Mayr 1963). Characteristics that allow individuals to discriminate between conspecific and heterospecific potential mates will evolve when selection prevents the production of viable fertile hybrids (Dobzhansky 1970). In the *Poecilia formosa* species complex, *P. latipinna* and *P. mexicana* males males contribute no genes to the gynogenetic offspring. These males are under strong selection to avoid mating with gynogenetic *P. formosa* females. Thus, we expect these males to discriminate between gynogenetic and conspecific females. Consistent with this prediction *P. latipinna* males prefer to mate with conspecific females rather than with gynogenetic *P. formosa* females (Schlupp et al. 1991, Ryan et al. 1996, Hubbs 1964, Gabor & Ryan unpublished). An early study of mate discrimination of *P. mexicana* males suggested a weak preference for conspecific females (Schlupp et al. 1991). More recent studies have shown they do discriminate between gynogenetic and conspecific females and prefer mating with conspecific rather than gynogenetic *P. formosa* females (Ryan et al. 1996, Ptacek 1998). While these two species of males confront the same mate discrimination problem, their responses differ in magnitude. The avoidance of *P. formosa* females by *P. mexicana* males is weaker than that of *P. latipinna*; the average proportion of thrusts directed towards *P. formosa* females by *P. mexicana* males is greater than that of *P. latipinna* males (Ryan et al. 1996). This prompted Ryan et al. (1996) to suggest the asymmetry in male mate discrimination could be related to the historical relationship between *P. latipinna* and *P. mexicana*, and *P. formosa*.

Poecilia formosa is the result of an historical hybridization (Schartl et al. 1996, Abramoff 1968), and an analysis of mitochondrial DNA revealed the maternal parental species was *P. mexicana*; the paternal species was *P. latipinna* (Avise et al. 1991). If *P. formosa* females were to differentially express maternally derived characteristics, they would be phenotypically more similar to *P. mexicana* females than *P. latipinna* females. Such an asymmetrical similarity could cause *P. mexicana* males to mistake gynogenetic *P. formosa* for conspecific females more frequently than do *P. latipinna* males (McLennan unpublished).

Aggressive behavior between sexual and gynogenetic females may also play a role in the apparent weaknes in mate discrimination of *P. mexicana* males. Behavioral interactions between females can affect the ultimate male mate preference by impeding the access of male access to them (Foran & Ryan 1994, Balsano et al. 1985, Baird 1968). During copulation attempts, the relatively short gonopodia of *P. latipinna* and *P. mexicana* males limits the accuracy of their visual identification of the female (Constantz 1989, Farr 1989); males see little more than the ventral side of a female during copulation attempts (Constantz 1989). It is during copulation attempts that an aggressive female could circumvent male mate preference by swimming between a male and a female and block his access to his chosen mate, or directly attacking the chosen female. Aggressive behavior by *P. formosa* females may be one way they obtain the sperm they require for reproduction in spite of male preference for conspecific females.

It is clear visual, behavioral, and olfactory cues are important in mate recognition of both females and males (Ryan et al. 1996, Foran & Ryan 1994, Schlupp et al. 1991, Farr & Travis 1986, Balsano et al. 1985, Baird 1968), but little is known about which characteristics are used to distinguish between conspecific and heterospecific individuals. The previous chapter (Dries Chapter 3) presented results of a study that provided no evidence that maternally derived characteristics were differentially expressed in female hybrids whose mothers were *P. mexicana* and those whose mothers were *P. latipinna*. This provided no support for the hypothesis that *P. mexicana* males mate with *P. formosa* females because of a asymmetrical similarity to *P. mexicana* females. However, that study did not address the possibility that differential expression of maternally derived characteristics might influence the mate recognition of hybrid males. Perhaps the maternal contribution to *P. mexicana* males has an overriding influence on which females are attractive. One approach to investigating this possibility is to examine the mate recognition behavior of sexual hybrid males. Those whose mothers were

P. mexicana would be expected to prefer *P. mexicana* rather than *P. latipinna* females, while those whose mothers were *P. latipinna* would be expected to prefer *P. latipinna* females. This would provide the first empirical evidence that male mate recognition behaviors can be influenced more strongly by their mothers than their fathers. This would also illustrate that preferences of hybrids are not intermediate, as is generally assumed.

The general purpose of the two experiments that follow is to understand what additional factors affect the probability of insemination of gynogenetic *P. formosa* females by *P. mexicana* and *P. latipinna* males. The first experiment asks if genetic history can affect conspecific mate recognition in male hybrids through differential expression of maternally derived characteristics. The second experiment asks if *P. mexicana* females exhibit the same aggressive behaviors seen in *P. latipinna* and *P. formosa* females.

MATERIALS AND METHODS

Hybrid Male Mate Preference

Seventy hybrid males (35 M_f/L_m: *P. mexicana* mother/*P. latipinna* father, 35 L_f/M_m: *P. latipinna* mother/*P. mexicana* father) were reared at Brackenridge Field Laboratory according to the protocol outlined in the previous chapter. They were brought to the University of Texas at Austin Campus Laboratory for use in behavioral tests in October 1996. All males were maintained in the laboratory during the same time period and under the same conditions of 12 hr.:12 hr. light:dark regime and Tetramin flake food *ad libitum* daily. Twenty-four hybrid males of type M_f/L_m and all but one hybrid male of type died in the laboratory within in 4 months of sexual maturation and were not used in behavioral tests. All of these males remained small in size and always appeared thin and unhealthy. The remaining 11 M_f/L_m hybrid males were used in the behavioral experiments. The single surviving L_f/M_m hybrid male was not tested.

Mate preference tests of eleven hybrid males, (type M_f/L_m), for *P. latipinna* or *P. mexicana* females were conducted according following the protocol outlines in the previous chapter. The testing aquarium was divided into three sections by clear plastic plates that allowed water flow between sections. Males were placed in the center section and a *P. latipinna* and a *P. mexicana* female were each placed in one of the outer sections. Fish were allowed to acclimate for 2 days then the dividers were removed and all fishes were allowed to swim freely for 15 minutes. The number of gonopodial thrusts directed toward each female was counted. Females were matched for receptivity by isolation from males for 60 days prior to use in tests. Female sizes were matched to within 10 mm except in two cases where the differences were 13.4 mm and 12.6 mm. Six of the hybrid males were tested twice over two weeks using a different pair of females matched for size and receptivity for each test. If the *P. latipinna* female was larger in the first test, the *P. mexicana* female was larger in the second. Five of the males fell ill and died before they could be tested again. Two of these were males tested with a pair of females of > 10 mm size difference.

Female Behavior

Behaviors of *P. mexicana* and *P. formosa* females were examined in two types of test. Six tests were conducted with a *P. mexicana* male present and 6 tests without a male. Each pair of females was tested twice, once with a male and once without a male. Each male was used once. Females were isolated from males for 60 days prior to testing to ensure neither female was pregnant and thus standardized for receptivity. Ideally virginal and post-parturition females are most receptive to males and will behave accordingly (Sumner et al. 1994, Farr & Travis 1986). Isolating females for 60 days ensures a vitellogenic cycle could be completed and that females will be receptive to mating rather than rely on stored sperm to fertilize the eggs.

Tests were conducted in a 1.9-liter aquarium divided into three compartments by clear plastic removable walls that allowed circulation of water throughout the aquarium (Fig. 3.1). In tests without a male, a *P. mexicana* and a *P. formosa* female were matched for size and receptivity and each placed in one of the outer compartments. In each test with a male, two size-matched, virginal females were placed in opposite ends of the aquarium and a male was placed in the center compartment. All fish were allowed to acclimate for two days after which the dividers were removed and the fish observed for 15 minutes. The numbers of occurrences of four behaviors were monitored during the tests with males: (1) bites, (2) butts, and (3) chases directed by one female to the other female, and (4) blocks by one female preventing access of the male and the other female to each other. Blocks and gonopodial thrusts were not counted in the tests without males.

Statistical Analyses

The non-parametric Wilcoxon signed-rank test was used to evaluate the results of both male preference and female behavioral tests. Hybrid male preference was evaluated from the results of the first tests of all 11 males. Six of the males were tested twice with a different pair of females. The preferences of these six males were evaluated by comparing the average of responses to each female type across both tests. The number of gonopodial thrusts toward each female in each test for a single male were summed and divided by two. These averaged responses were used in a statistical analysis separate from that of all 11 males. In addition the relationship between female size and male preference was evaluated using an analysis of covariance. Each type of female behavior was analyzed separately and the total number of all behaviors pooled for each female were used to evaluate overall behavioral differences. The significance value used in the comparison of overall behaviors was adjusted for multiple comparisons using Bonferroni techniques. The Statview statistical program for the Macintosh was used for all analyses (SAS Institute, Inc. ©1999).

RESULTS

Hybrid Male Mate Preference

Male sexual hybrids of type M_f/L_m tended to prefer *P. mexicana* females as mates in the first set of tests ($z = {}^{-}1.689$, $p = 0.091$)(Fig. 4.1) but this preference was not significant. When the responses of the five males tested only once were analyzed, the preference for ***P. mexicana*** was nearly significant ($z = {}^{-}1.753$, $p = 0.080$). A test of the responses of the other six males from the first test only was not significant ($z = {}^{-}0.524$, $p = 0.600$). When the averaged responses of the six males tested twice were compared, the preference for ***P. mexicana*** females was significant ($z = {}^{-}2.201$, $p = .028$)(Table 4.1). When the averaged responses were combined with the simple responses of the males tested only once, the preference for ***P. mexicana*** females became even more significant ($z = {}^{-}2.845$, $p = .004$). This slightly strange pattern of results may be due to the influence of 2 males. In the first set of tests, six of the eleven males preferred ***P. mexicana*** exclusively and maintained that preference in the second test. Two of the five males preferred ***P. latipinna*** females exclusively in the first test, but switched their preference to ***P. mexicana*** in the second test. In the second set of tests, all six males directed more thrusts to ***P. mexicana*** females. In some tests the difference The relative size of females used in each test had no effect on the proportion of thrusts directed towards either female (ANCOVA: $F_{1,6} = 0.030$. $p = 0.869$);large female size does not explain the preference for ***P. mexicana*** females.

Figure 4.1: *Poecilia* Male Hybrid Mate Discrimination

Results of male mate discrimination tests showing the number of gonopodial thrusts of hybrid males (M_f/L_m) directed towards gynogenetic *P. formosa* females versus sexual *P, mexicana* females. The means and one standard error are shown in each case. Probability values are two-tailed probabilities of Wilcoxon signed ranks tests.

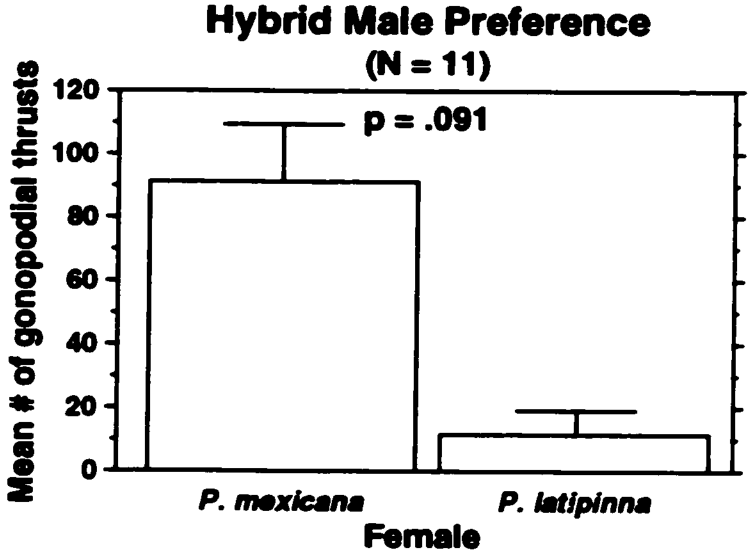

Hybrid Male Preference
(N = 11)
p = .091
Mean # of gonopodial thrusts
120
100
80
60
40
20
0
P. mexicana
P. latipinna
Female

Table 4.1 Descriptive Statistics for Hybrid Male Mate Preference.

This table shows descriptive statistics for the number of thrusts directed by sexual hybrid males, type M_f/L_m, to *P. latipinna* and *P. mexicana* females. Listed are the arithmetic average (Mean), standard deviation (S.D.), standard error (S.E.), and number of tests (N) for the first test of all males and for the average across two repeated test for 6 males used in the first and second tests.

	Hybrid Male Behavior	
Female	Thrusts First test	Thrusts Both tests
P. mexicana		
Mean	76.5	94.9
S.D.	94.6	45.1
S.E.	28.5	18.4
N	11	6
P. latipinna		
Mean	21.6	19.5
S.D.	47.5	31.2
S.E.	14.3	12.7
N	11	6

The preferences of male sexual hybrids of type L_f/M_m are not reported here because only one of the males of type L_f/M_m survived to sexual maturity.

Female Antagonistic Behavior

There was no significant difference in overall antagonistic behavior of *P. mexicana* and *P. formosa* females when males were absent (z = ¯0.405, p = 0.686) (Fig. 4.2). When each behavior was analyzed separately and significance was corrected for multiple comparisons with Bonferroni techniques there were also no significant differences (Bites: z = ¯0.535, p = 0.593; Butts: z = ¯0.524, p = 0.600; Chases: z = ¯0.183, p = 0.855)(Table 4.2, Fig. 4.2). Females also did not differ in behavior when *P. mexicana* males were present (All: z = ¯0.943, p = 0.345; Bites: z = ¯1.604, p = 0.109; Butts: z = ¯1.048, p = 0.295; Chases: z = ¯1.069, p = 0.285; Blocks: z = ¯0.730, p = 0.465)(Table 4.2, Fig. 4.3).

Figure 4.2: *Poecilia formosa* and *P. mexicana* Female Behavior - Male Absent

Results of female behavioral tests showing the number of antagonistic behaviors exhibited by gynogenetic *P. formosa* and *P. mexicana* females when males were absent from the aquarium. The means and one standard error are shown in each case. Probability values are two-tailed probabilities of Wilcoxon signed ranks tests. (a) The total number of antagonistic behaviors exhibited by gynogenetic *P. formosa* females and *P. mexicana* females. (b) The number of each of three types of antagonistic behavior exhibited by gynogenetic *P. formosa* females and *P. mexicana* females. Blocks are not presented because attempts by one female to block mating behavior by a male to another female cannot be counted in the absence of males.

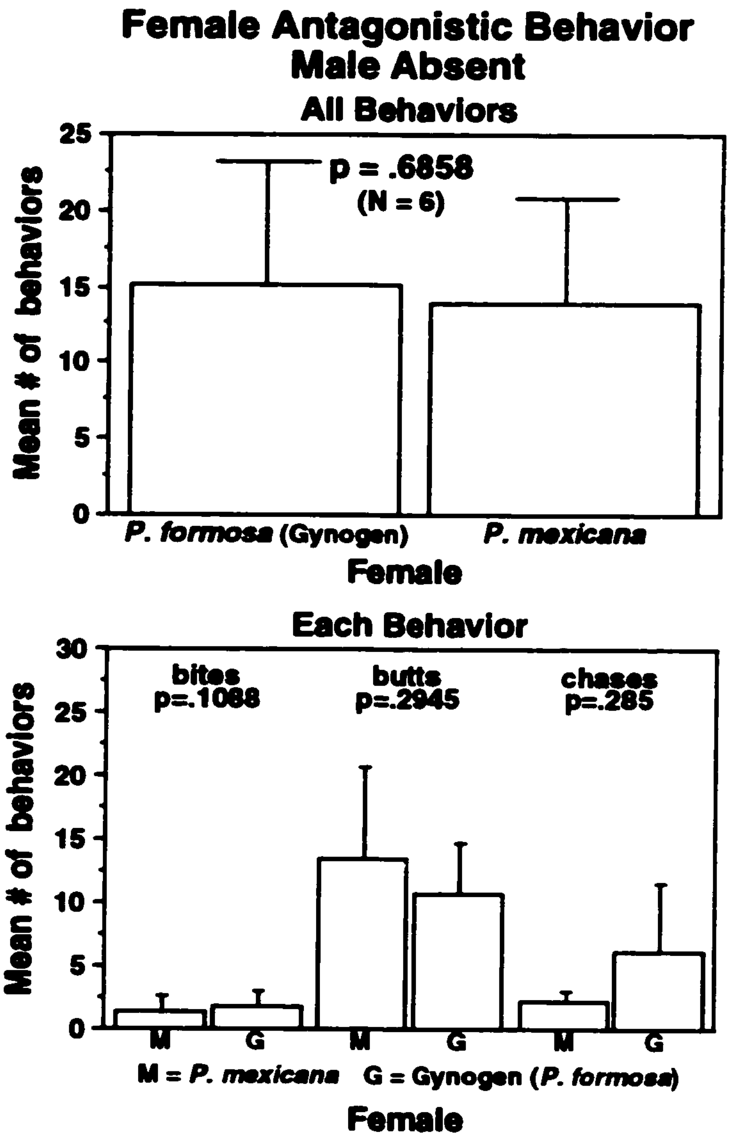

Female Antagonistic Behavior
Male Absent
All Behaviors
Mean # of behaviors
0
5
10
15
20
25
p = .6858
(N = 6)
P. formosa (Gynogen)
P. mexicana
Female
Each Behavior
Mean # of behaviors
0
5
10
15
20
25
30
bites
p=.1088
butts
p=.2945
chases
p=.285
M
G
M
G
M
G
M = P. mexicana G = Gynogen (P. formosa)
Female

Figure 4.3: *Poecilia formosa* and Hybrid Female Behavior - Male Present

Results of female behavioral tests showing the number of antagonistic behaviors exhibited by gynogenetic *P. formosa* and *P. mexicana* when a *P. mexicana* male was present in the aquarium. The means and one standard error are shown in each case. Probability values are two-tailed probabilities of Wilcoxon signed ranks tests. (a) The total number of antagonistic behaviors exhibited by gynogenetic *P. formosa* females and *P. mexicana* females. (b) The number of each of four types of antagonistic behavior exhibited by gynogenetic *P. formosa* females and *P. mexicana* females.

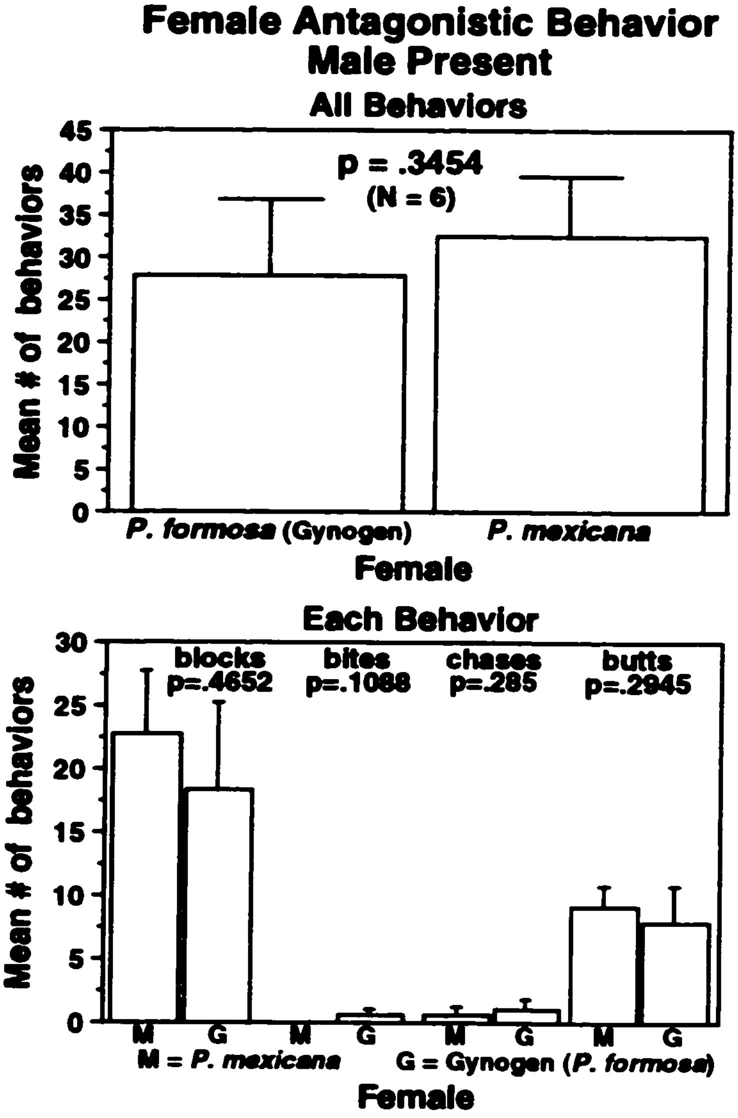

Female Antagonistic Behavior
Male Present
All Behaviors
p = .3454
(N = 6)
Mean # of behaviors
0
5
10
15
20
25
30
35
40
45
P. formosa (Gynogen)
P. mexicana
Female
Each Behavior
blocks p=.4652
bites p=.1088
chases p=.285
butts p=.2945
Mean # of behaviors
0
5
10
15
20
25
30
M
G
M
G
M
G
M
G
M = P. mexicana
G = Gynogen (P. formosa)
Female

Table 4.2 Descriptive Statistics for Gynogenetic and Hybrid Female Behavior

This table shows descriptive statistics for the number of antagonistic behaviors exhibited by *P. mexicana* and *P. formosa* females when *P. mexicana* males were present and absent. Listed are the arithmetic average (Mean), standard deviation (S.D.), and standard error (S.E.) of each type of behavior and all behaviors pooled (All). Sample size in all cases is six (N = 6).

Female Behavior

Female					**Male Present**				
P. mexicana	Bites	Butts	Chases	All	Bites	Butts	Chases	Blocks	All
Mean	1.0	11.7	1.7	13.8	0.0	9.7	0.7	22.7	32.5
S.D.	2.5	14.9	2.1	17.2	0.0	4.3	1.2	12.6	17.5
S.E.	1.0	6.1	0.8	7.0	0.0	1.8	0.5	5.1	7.1
P. formosa	Bites	Butts	Chases	All	Bites	Butts	Chases	Blocks	All
Mean	1.3	8.8	5.0	15.2	0.7	7.8	1.0	18.3	27.8
S.D.	2.8	8.3	11.3	19.5	0.8	7.4	2.0	16.9	22.0
S.E.	1.2	3.4	4.6	8.0	0.3	3.0	0.8	6.9	9.0

DISCUSSION

Hybrid Male Preference

Hybrid males showed a tended to prefer *P. mexicana* females as mates. The unusual pattern of response among the males across tests deserves some discussion. First, these males showed a high level of response and often an exclusive preference for one of the females (Table 4.1, Fig. 4.1). For some males their preference *P. mexicana* females remained during a second test, yet for others, their preference for *P. latipinna* females was reversed to a preference for *P. mexicana* females in the second test.

How can the preferences of hybrid males be explained? Large female size is known to affect the preference of *P. latipinna* and *P. mexicana* males (Bergland et al. 1986), however, size of female had no statistical effect on the preferences of hybrid males. Another possible explanation is that mate preference of hybrid males is influenced by their motivation to mate. The high average number of gonopodial thrusts in all tests indicates the males were very motivated to mate. Since all males had been isolated from females for several weeks, they may have been so highly motivated to mate their mate preferences were relaxed. However, if males relaxed their preference, they would be expected to mate with either female, which did not occur. The hybrid males in this study directed all or nearly all of their attention toward one of the females, not both.

Hybrid male preference may be influenced by experience. This is supported by the pattern of response the males in this study. For two males, the preferred species of female changed between the first and second test, a time period of only two weeks. These males preferred *P. latipinna* females nearly exclusively in the first test, but switched their preference to *P. mexicana* nearly exclusively in the second test. This all suggests that naïve hybrid males may have a weak preference for *P. mexicana* females that is strengthened with experience. Note none of the hybrid males that originally preferred *P. mexicana* in the first test switched their preference to *P. latipinna* in the second test. The general preference for *P. mexicana* females shown by hybrid males may indicate the influence of maternally derived characteristics in mate recognition. This perference could be a mechanism of asymmetrical introgression of *P. latipinna* genes into *P. mexicana* via backcrossing of hybrids.

These results also suggest that when evaluating mate preference at the population level it may be more important than we realize to test many individuals. In addition if we want to understand the variability of mate preference within an individual, it is important to test an individual more than once if we want picture of the repeatability of behavior.

What can the hybrid male preferences tell us about the difference in mate discrimination of *P. mexicana* and *P. latipinna* males? The tendency of hybrid males whose mothers were *P. mexicana* to prefer *P. mexicana* females as mates may indicate differential expression of maternally derived characteristics. If the same is true for *P. formosa*, the proclivity of *P. mexicana* males to mate with them could be related to their attraction for conspecific females. But there are other possible reasons for the weaker discrimination. Triploid *P. formosa* has been found at various frequencies in natural populations of *P. mexicana* and *P. formosa*. Cytogenetic analysis confirms their heritage as one part *P. latipinna* and two parts *P. mexicana*. No triploids of two parts *P. latpinna* have ever been found in natural populations and very few laboratory attempts to synthesize them have been successful. The leaky nature of the genome exclusion in *P. formosa* has been documented in the laboratory and provides a selective reason *P. mexicana* males have not evolved the strong avoidance of *P. formosa* found in *P. latipinna*. If, on occasion, the male genome is incorporated into gynogenetic offspring he will reap a reproductive advantage; his genetic contribution will be passed on untouched by genetic recombination for generations to come (Balsano et al. 1985, Monaco et al. 1984). The selective disadvantage of mating with a gynogenetic female is not as strong for *P. mexicana* as it is for *P. latipinna*. This and the genetic composition of *P. formosa* may underlie the asymmetry in mate discrimination.

Hybrid males in general suffered high mortality. Only 11 of 35 M_m/ L_f and all but one (34 of 35) L_f/M_m hybrid males died before they could be tested. The generally high mortality of all of the hybrid males suggests there is selection on hybrids that would reinforce reproductive isolation between *P. laitpinna* and *P. mexicana*. This striking difference in survival between the hybrid types is of note and suggests the direction of hybridization can have a large effect on survival of hybrid offspring.

Female Antagonistic Behavior

Poecilia mexicana and *P. formosa* females did not differ in antagonistic behavior regardless of the presence of a male. When male was present the majority of interactions observed in this study consisted of blocking access of a male and female to each other. When males were absent butting was the most prevalent behavior in females of both species. The similarity of response in these females suggests that in mixed populations of *P. formosa* and *P. mexicana*, *P. formosa* females are equally aggressive in behavioral interactions. *Poecilia formosa* appears to be able to compete with *P. mexicana* females for the attention of males. The prevalence of blocking behaviors when males were present is similar to the interactions between *P. formosa* and *P. latipinna* females (Ryan & Foran 1994) where gonopodial thrusts were positively correlated with blocking behaviors. Blocking appears to be an effective way to obtain copulations for *P. formosa* in populations with *P. latipinna* and probably has the same effect in populations with *P. mexicana*. The highest levels of aggression occurred when males were absent and were directed toward heterospecific more than conspecific females (Foran & Ryan 1994). This study should be viewed as preliminary because the sample size is only 6 fish of each species and it did not delve into female behavior as extensively as Foran & Ryan (1994). However, the results presented here suggest a more detailed investigation of the interactions between *P. formosa* and *P. mexicana* would be worthwhile.

Chapter 5: Frequency-Dependent Reproductive Success and the Persistence of the Gynogenetic Amazon Molly, *Poecilia formosa*

INTRODUCTION

Gynogenetic lineages are intriguing to evolutionary biologists because not only is their evolutionary persistence dubious, they have the potential to affect the evolutionary fate of the sexual species they parasitize (Kirkendall & Stenseth 1990, Kawecki 1988, Stenseth et al. 1985, Vrijenhoek 1984, Moore & McKay 1971). Numerous theoretical studies have identified several factors that would favor stable coexistence of the gynogenetic parasite and its sexual host, namely, weak mate discrimination, density-dependent mating, and ecological niche-partitioning (Kirkendall & Stenseth 1990, Kawecki 1988, Stenseth et al. 1985, Vrijenhoek 1984, Kiester et al. 1981, Moore & McKay 1971). Empirical demonstration of these factors in natural populations of *Poecilia formosa* and its sexual hosts is scant.

Of the factors identified by the theoretical studies, only ecological niche-partitioning has been between empirically documented as important in reducing the potential risk of extinction (McKay 1971). The model system of this study was all-female hybridogenetic *Poeciliopsis* lineages, which are similar to gynogenetic lineages; clonal reproduction is sperm-dependent. Hybridogenetic clones utilize different resources than their sexual hosts, thereby reducing the threat of extinction caused by competitive exclusion. There is evidence of weak niche-partitioning between *Poecilia formosa* and its *P. mexicana* hosts (Balsano et al. 1985, 1981), and there is no evidence of niche-partitioning between *P. formosa* and its *P. latipinna* host (Balsano et al. 1981). This suggests another factor plays a dominant role in the maintenance of gynogenetic *P. formosa* through evolutionary time.

Weak mate discrimination by sexual males could be a critical factor in maintaining gynogenetic lineages and their sexual host species (Kawecki 1988, Kiester et al. 1981, Moore & McKay 1971), but there is abundant evidence that both *P. latipinna* and *P. mexicana* males discriminate against gynogenetic *P. formosa* females (Ryan et al. 1996, Schlupp et al. 1991, Balsano et al. 1985, Hubbs 1964). On the other hand, the mere presence of gynogenetic lineages indicates that mate discrimination by males is not perfect. The preference of *Poecilia latipinna* males for conspecific females is weaker in populations allopatric from *P. formosa* than populations in sympatry (Ryan et al 1996, Gabor & Ryan unpublished). It has also been suggested mate discrimination may be weakest in smaller or younger males whose access to females is restricted by larger or more dominant males (Balsano et al. 1989, Woodhead and Armstrong 1985). Theoretically males denied free access to all conspecific females would mate with any female they can approach, and sometimes this female is gynogenetic. The importance of this factor alone is doubtful because in natural populations of *P. mexicana* male hierarchies appear to function in defense of resource-based home ranges, not in direct competition for mates (Balsano et al. 1989). Loose dominance hierarchies have been reported among *P. latipinna* males in natural populations but again do not appear to function in the exclusion of some males from access to conspecific females (Baird 1968, Hubbs 1964). Furthermore, dominance hierarchies in *P. mexicana* and *P. latipinna* are not enforced when large numbers of individuals are involved in mating frenzies (Balsano et al. 1985, Baird 1968).

Density-dependent mate discrimination could favor stability of mixed populations of gynogenetic lineages and their sexual host species (Moore & McKay 1971). Discrimination against gynogenetic females would be weaker when males are at high density if intermale competition were dependent on density. This model incorporates the effect of temporal changes in habitat structure on male density and discrimination, and does not require niche-partitioning for coexistence of gynogenetic lineages and sexual species. While this model predicts gynogenetic lineages and their sexual host species can coexist through evolutionary time, some assumptions and features of the model are not likely to apply to *P. formosa* and its hosts. The model assumes there are equal proportions of sexual males and females, and density changes only as a result of an increase in the number of males in the population. Neither this nor previous models incorporate changing population size or the biased sex ratios typical of mixed populations of sexual and gynogenetic *Poecilia*. The models have completely ignored the possible role of female mate preference and competition for mates in population dynamics.

Numerous studies have documented myriad mating and competitive behaviors among *P. latipinna* males and females, and *P. formosa* females. *Poecilia latipinna* and *P. mexicana* males prefer conspecific females. *Poecilia* *latipinna* males prefer larger, receptive females, although males discriminate against gynogenetic females regardless of female size (Ryan et al. 1996, Schlupp et al. 1991, Farr & Travis 1986). There is predictable variation in male mating strategy that is loosely correlated with male size; larger males tend to court females whereas smaller males tend to force insemination (Travis & Woodward 1989, Farr et al. 1986, Luckner 1979). *Poecilia latipinna* females prefer larger males but they will copy the mate choice of both *P. latipinna* and *P. formosa* females. A *P. latipinna* female is attracted to a previously unacceptable mate if she observes that male courting a gynogenetic female (Schlupp et al. 1994). She also changes her preference if an unattractive male, similar in size to her chosen male, is observed courting another conspecific female (Witte & Ryan 1998).

Females compete for mates (Foran and Ryan 1994) and the type and intensity of competition differs dependent upon the other fish present. When males are present, *P. formosa* females attempt to block the access of *P. latipinna* males to *P. latipinna* females and of *P. mexicana* males to *P. mexicana* females (Dries Chapter 4). As a result, *P. formosa* females receive more mating attempts when *P. latipinna* females are present than when only *P. formosa* females are present (Foran & Ryan 1994). Aggressive behaviors between females are observed more often when males are absent than when they are present and aggression is expressed more often between heterospecific females than between conspecific females (Foran & Ryan 1994). These and the mate discrimination studies described here (Chapters 3 & 4) indicate *Poecilia* females play a role in determining the outcome of male mate discrimination. Weakness of mate discrimination may not lie solely with males but could also be a byproduct of the ability of gynogenetic females to deceptively attract them and of the interactions among females.

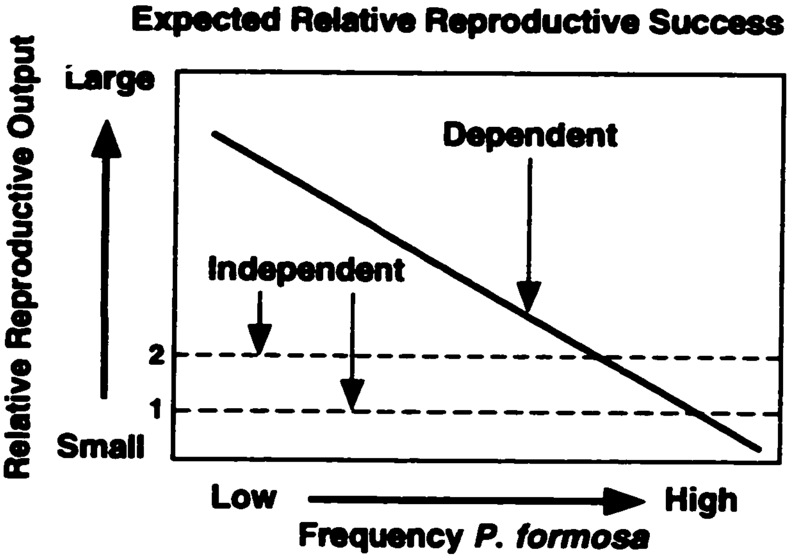
The discovery of many of these behaviors has been a result of studies that concentrate on characterizing one particular aspect of mating behavior at a time. Although these studies have been instrumental in identifying mate discrimination behaviors, most have not empirically addressed the question of how these behaviors operate in natural populations, nor have they directly evaluated their effects on reproductive success (but see Witte & Ryan 1999, Schlupp & Ryan 1996). The outcome of behavioral interactions in natural populations is not easy to predict despite a plethora of behavioral information because molly populations generally consist of large numbers of fish, from twenty to hundreds (Balsano et al. 1985, Hubbs 1964, personal observation). The expression of mating behavior and mate discrimination in natural populations of mollies does not occur between isolated pairs or trios of individuals but rather is expressed in a larger social context that includes both conspecific and heterospecific individuals. Social context can mediate the ultimate behavior expressed by an individual (Pfennig 1998).

One factor that would allow *P. formosa* to coexist with its sexual hosts is frequency-dependent reproductive success (Stenseth et al. 1985). For example, if sexual males are likely to reject gynogenetic females when conspecific females are rare, but less likely to reject them when conspecific females are common, then a stable equilibrium is possible. Likewise stable equilibria are also possible based on frequency-dependent female behavior or mortality. Stenseth et al. (1985) suggested gynogenetic lineages, once established, could be evolutionarily stable if birth rate, mortality, or reproductive success were frequency-dependent. All of the behaviors documented in the *Poecilia formosa* system suggest relative frequencies of fish could affect reproductive success and thus, the evolutionary persistence of gynogenetic *P. formosa*.

It is easy to visualize how the most extreme levels of frequency-dependent reproductive success would result in a stable equilibrium of gynogenetic and sexual fishes. If males mate exclusively with conspecific sexual females when gynogenetic females are common and sexual females are rare, the reproductive success of the gynogens would decrease drastically (Fig. 5.1), and the frequency of gynogens in the population would fall. On the other hand, if males do not discriminate at all between gynogens and conspecific females when gynogens are rare, the reproductive advantage of unisexuality (only producing females) would result in an increase in the relative frequency of gynogens in subsequent generations. Thus, in this extreme case of frequency-dependent mating behavior by the males and the resulting frequency-dependence of reproductive success, gynogens would decrease in frequency when they are common and increase in frequency when they are rare, resulting in a stable equilibrium of sexual and gynogenetic fishes.

Figure 5.1: **Expected Result of Frequency-Dependent Reproductive Success**

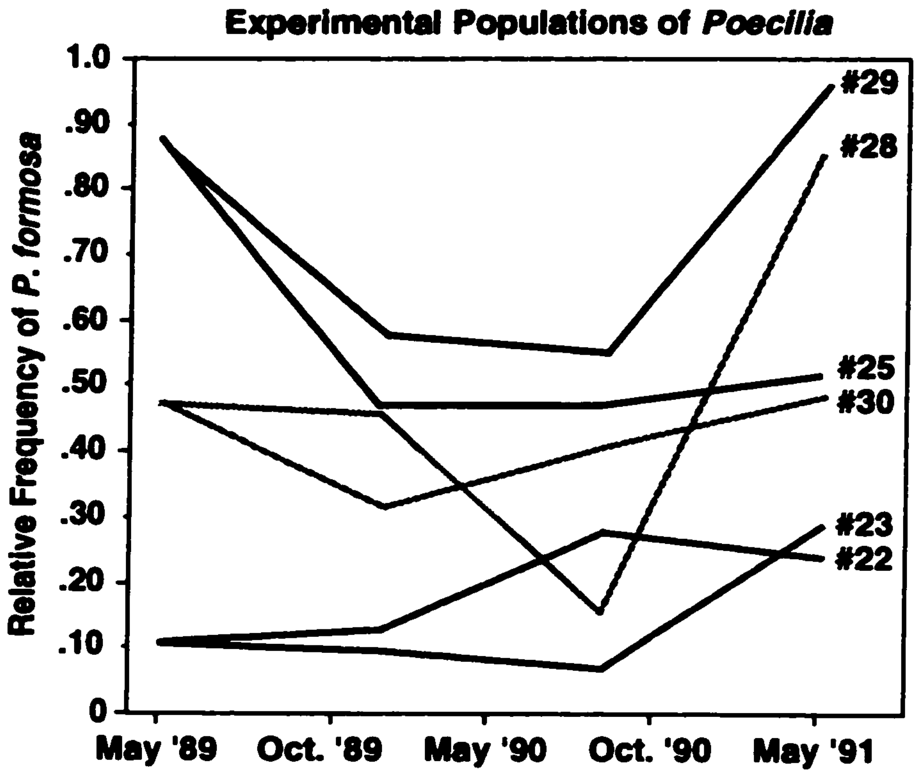
This figure shows the expected change in relative frequency of gynogenetic *Poecilia formosa* if their reproductive success is negatively frequency-dependent. As the frequency of *P. formosa* increases from low to high along the *x*-axis, the number of gynogenetic offspring relative to each sexual offspring (reproductive output) decreases from large to small along the *y*-axis. The line of negative slope, labeled "Dependent", represents this. The lines of slope zero, labeled "Independent", represent the expected relative reproductive output if reproductive success is independent of frequency. The upper independent line (2) indicates the expected reproductive advantage of gynogenetic females as a measure of female offspring: 2 gynogenetic female offspring for every one sexual female offspring. The lower line (1) indicates the expected reproductive advantage as a measure of all offspring: 1 gynogenetic female offspring for each sexual offspring, male or female. Neither of the lines meets the *y*-axis because this represents a frequency of zero; if there are no gynogenetic females in the population, their reproductive success would be zero.


Expected Relative Reproductive Success
Relative Reproductive Output
Large
Small
2
1
Dependent
Independent
Low
High
Frequency *P. formosa*

The possible relationship between frequency and reproductive success was suggested in a preliminary study using experimental mixed populations (Fig. 5.2)(Hillis & Bull unpublished). When the initial relative frequency of *P. formosa* was high, it decreased through time with an attendant increase in the frequency of *P. latipinna*. When the initial frequency of *P. formosa* was low, it increased, and when it was intermediate, it increased in one case and decreased in another. This suggests the relative frequency of gynogenetic *P. formosa* changes through time as if reproductive success were frequency-dependent.

Figure 5.2: Relative Frequency of *Poecilia formosa* in Experimental Ponds with *P. latipinna*: Preliminary Study of Hillis and Bull

This graph shows the frequency of *P. formosa* females relative to *P. latipinna* females in six preliminary experimental populations of Hillis and Bull: two replicates of three different initial conditions. The initial population size was 100 juveniles in different frequencies of each species without regard to gender of *P. latipinna*. The change in frequency over two years is shown and indicates relative frequency changes as would be expected if reproductive success were independent. When the frequency of *P. formosa* was high (0.90) or intermediate (0.50), an initial decrease was followed by an increase (#28 & #29 and #25 & #30); when the frequency was low (0.10) it increased (#23 & #22). These preliminary populations were not under stringent sampling regimes, nor were initial frequencies and sex ratios of adults monitored.


Experimental Populations of *Poecilia*
Relative Frequency of *P. formosa*
1.0
.90
.80
.70
.60
.50
.40
.30
.20
.10
0
May '89
Oct. '89
May '90
Oct. '90
May '91
#29
#28
#25
#30
#23
#22

The abundance of *P. formosa* and *P. latipinna* in Texas provides the opportunity to examine population dynamics in semi-natural experimental ponds and in laboratory aquaria. This chapter describes an investigation of frequency-dependent reproductive success and of its role in the evolutionary persistence of gynogenetic *P. formosa*.

MATERIALS AND METHODS

Fish Collection

Adult *Poecilia latipinna* and *P. formosa* were collected from 3 localities in southern Texas: Brownsville in Cameron County, and Weslaco and Mission in Hidalgo County. One thousand two hundred and fifty-two adults and 109 juveniles were collected on August 23, 1997 from a site known as Airport Ditch. Fish were transported immediately back to Austin and placed in aquaria in the laboratory. Six hundred and ninety-one of these fish died en route (51%). The causes of death were probably related to stress-induced intolerance of water conditions, such as low oxygen content. The water temperature in Airport Ditch was > 32° C; the amount of dissolved oxygen in water of this temperature would have been substantially less than that in cooler water. In addition the water was typical of most south Texas habitats where mollies are found; it was murky, full of floating silt and sediment. Both of these conditions would add to the stress of transportation. All fish that died en route were preserved in 70% ethanol, identified to species and sex, and discarded. The remainder of these fish died in the laboratory before their use in experiments. The causes of death were unclear; a gill parasite was present in some of the bodies and additional causes of death were probably stress and undiagnosed disease.

Adult fish were subsequently collected from the Central Floodway south of Weslaco, Texas on February 26, 1998, and February 1, 1999, and from a slough south of Mission, Texas on three occasions, February 26, 1998, January 30, 1999 and May 30, 1999. The relative frequency of *P. formosa* and their reproductive success relative to female *P. latipinna* were estimated from these samples. All of these fish were transported to Austin the day of collection except those caught on February 26, 1998. I was forced to maintain the fish in coolers in a motel room for one day while the truck was repaired. Maintenance in the motel room consisted of aerating and filtering the water with sponge and carbon filters. We did not attempt to use treated tap water but instead allowed the fish to remain in fresh water from the collecting site. Although water from this site was very murky with floating sediment and silt, a large portion of this material was removed from the water by the sponge filter, which enhanced survival of the fish during subsequent transportation. The temperature of the water during February is considerably lower which also presumably reduced the stress imposed on the fish (and the scientists). Only 11 fish died *en route* or in the first week following transfer to aquaria in the lab (a mere 3% of total fish collected on that date) and dead fish were preserved in Formalin, identified to species, and counted.

Mature females and males were separated and transferred to laboratory aquaria. Females remained isolated in the lab for 60 days to ensure they were not pregnant. The purpose of the isolation was to standardize females for receptivity so that whatever offspring were born during the experiment accurately reflected the mating that occurred during the experiment. Females are most receptive immediately following parturition when they have eggs to be fertilized (Farr & Travis 1986). While females can store sperm, they do not refrain from re-mating after the birth of a brood, and brood size decreases with time when females rely solely on stored sperm (Thibault & Schultz 1978). Based on all of this information, all females were receptive to male attention at the onset of the experiments.

Juvenile fish were collected from the Mission, Texas slough on January 29, 1999. These fish were transported to Brackenridge Field laboratory in Austin, Texas and placed in two small concrete (3768-liter) ponds equipped with net liners. All of the fish were removed from each pond monthly and examined for signs of maturity. All maturing males were removed and placed either in a large, submerged concrete pond at the field laboratory or in aquaria in the laboratory at the University of Texas at Austin. Mature females were brought to the laboratory on campus and placed in aquaria separated from males. All laboratory fish were maintained under 14:10 hrs. day:night lighting regime, and fed Tetramin flake food daily. This diet was supplemented with live brine shrimp and dried spirulina flakes thrice weekly.

Aquarium Experiments

The purpose of the aquarium experiments was to obtain a fine scale measurement of reproductive success and directly observe behavioral interactions among the fishes. Six, 20-gallon (7.6 liter) aquaria were equipped with 4 cups of gravel, a sponge filter, two plastic plants each. They were filled with water from which chlorine and chloramine had removed using AmQuel and NovAqua. The water in the aquarium was aerated and the bacterial fauna was allowed to develop for two weeks prior to the introduction of fish. Mature virgin *P. latipinna* and *P. formosa* females were placed in 6 aquaria under two conditions, high and low frequency gynogens. Males were added to aquaria one week after the introduction of females. The standard length, snout to hypural plate, of each male was measured prior to his introduction to an aquarium. The high frequency condition consisted of 16 *P. formosa*, 3 *P. latipinna* females, and 1 *P. latipinna* male. The low frequency condition consisted of 4 *P. formosa*, 12 *P. latipinna* females, and 4 *P. latipinna* males. The sex ratio within *P. latipinna* was initially identical among all aquaria, 3 sailfin females to 1 male. I chose this sex ratio because it is typical of the sex ratios I observed in natural populations and it has been reported for populations in the Brownsville area (Hubbs 1964). The sex ratios including gynogenetic females were 4 females to 1 male in the low frequency condition and 19 females to 1 male in the high frequency condition. In two aquaria male and female behavior was observed and noted for one hour in the morning or the afternoon. The original intention was to videotape activities in the aquaria for longer periods of time but this was precluded by high mortality of fish.

If a fish died, it was replaced with another of identical species and sex. Thus a constant frequency of gynogens and number of fish were maintained throughout the experiment. In the initial design the number of proposed aquarium experiments was ten at high initial frequency of *P. formosa* and ten at low initial frequency. However, only six were attempted due to biological constraints (see results below). All dead females were dissected and presence of embryos noted. Heart, liver and muscle tissue were removed and frozen at $^{-}80^{\circ}$C.

Aquaria were checked daily for the presence of fry. If present, fry were removed and visually identified to species visually if possible. If visual identification was not possible, fry were frozen to $^{-}80^{\circ}$F and identified using protein gel electrophoresis.

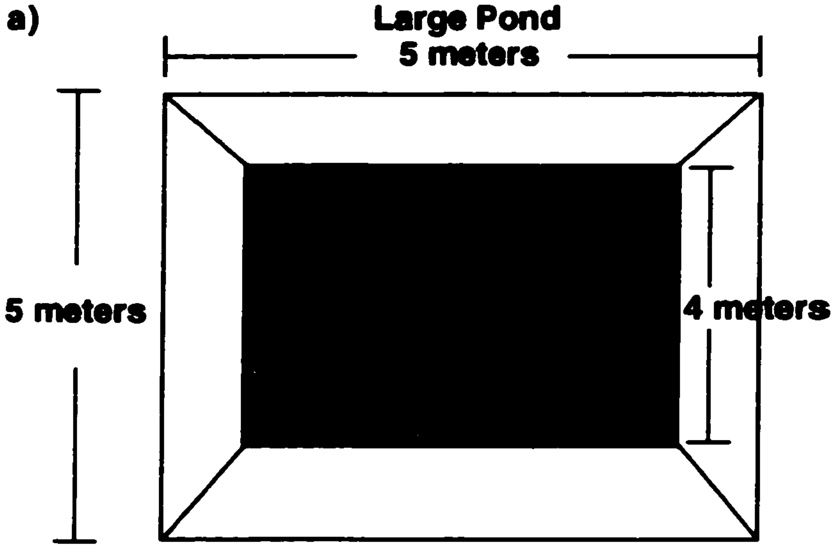
Small Ponds

Thirteen small, circular, concrete ponds (2 meters diameter x 1.2 m deep; 3768 liters) that sit on top of the ground were drained, any fish present removed, and the pond refilled with well water (Fig. 5.3). (These ponds are identical to those in which field-caught fish were maintained prior to experimentation). Each pond was supplied with a constant flow of well water through a faucet; a large, PVC drainpipe embedded in the concrete wall of the pond prevented overflow. Each was equipped with a removable 1/16" mesh, net liner, and equal amounts of plant material were added to all ponds. The ponds were allowed to sit for one month without fish to allow growth of algae and aquatic invertebrate populations.

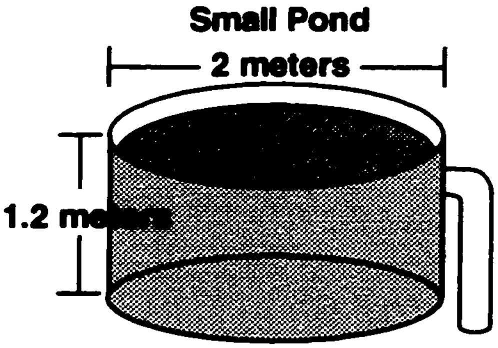
Figure 5.3: Schematic Diagrams of Experimental Ponds

This figure provides schematic diagrams of the concrete experimental ponds used in this study. (a) The dimensions of the large ponds are shown. The 1-meter wide perimeter of the pond sloped gently to a depth of 0.45 meters where the sides dropped vertically to a depth of 10 meters. Plants were placed in the shallow corners of each pond. (b) The dimensions of the small ponds are shown. Each pond was equipped with a vertical pipe on the exterior with a faucet over the top (not shown) through which a constant flow of well water was maintained. A large PVC pipe embedded in the concrete side allowed water to flow out of the pond to prevent overflow.

a)


Large Pond
5 meters
5 meters
4 meters

b)


Small Pond
2 meters
1.2 meters

I used the isolated adult *P. latipinna* and *P. formosa* to stock ponds with a total of 40 fishes at one of two initial frequencies of adult *P. formosa*. Eight ponds were stocked with 32 *P. formosa*, 6 *P. latipinna* females, and 2 *P. latipinna* males representing the high-frequency condition of 0.80. Five ponds were stocked with 8 *P. formosa*, 24 *P. latipinna* females and 8 *P. latipinna* males representing the low-frequency condition of 0.20. These numbers also create an initial sex ratio of 3 female *P. latipinna* to 1 male across all ponds. Two ponds, one high and one low frequency, were stocked December 24, 1997; three high and three low frequency ponds were stocked April 30, 1998. Four high frequency ponds were stocked June 6, 1998, September 10, 1998, October 28, 1998, and March 12, 1999. One low frequency pond was stocked March 27, 1999. Ponds were stocked as fish became available and thus all experiments were not started on the same date. In addition, four ponds were restocked at a frequency of 0.80 *P. formosa* because no reproduction occurred before all sexual fish (*P. latipinna*) died. Two ponds were restocked at frequencies of 0.80 and 0.20 *P. formosa* because no sexual females remained and no sexual juveniles were present. Every attempt was made to stock one pond at low frequency and one pond at high frequency on the same date to minimize any possible affects of initial season on the outcome of the experiments.

During the winter months the water temperature was maintained at 21 ± 2°C with submersible electric heaters. Plastic covers were placed over the surface of the water whenever the air temperature was expected to drop below 0°C. Heaters and covers were removed each spring in March.

Pond populations were monitored monthly, weather permitting. When the air temperature was lower than 10°C ponds were not sampled because exposure of the fish to the air would have caused unnecessary stress. In general, ponds were not sampled during December, January, or February. During sampling, the net liners were removed and all of the fish were identified, counted and returned to the pond. Adult fish were classified according to species and sex and juvenile fish were identified to species. Juveniles too small to identify were classified as fry. In general, it took 30 days for newborn fry to grow large enough to determine their specific identity. The relative frequencies of *P. formosa* and *P. latipinna* adults and juveniles were calculated from these monthly data.

On April 30, 1999, water cabbages were mistakenly introduced to the ponds from a local site. While this had the potential to introduce *Poecilia*, predators and/or pathogens, it does not appear to have affected the results. No fish or large aquatic predators were transferred (Appendix). Any small invertebrate predators introduced would only have supplemented those already present. Since the cabbages were introduced into all of the experimental ponds at the same time and remained for 24 hours, I will assume their presence did not differentially affect any of the ponds.

Large Ponds

Two large, submerged, outdoor ponds of concrete (5 meters x 5m x 8m deep) were drained, the fish removed, and ponds refilled with well water (Fig. 5.3). A constant flow of water into each pond was provided through a hose connected to the underground well via a faucet. Equal amounts of aquatic plants were added to the shallow corners of each pond and the ponds were allowed to sit without fish for one month to allow algae and invertebrate populations to grow. On October 24, 1997 each pond was stocked with a total of 100 isolated adults of *P. formosa* and *P. latipinna*; the initial frequency of *P. formosa* was high in one pond (#3) and low in the second pond (#4). The high frequency pond was stocked with 80 *P. formosa*, 15 *P. latipinna* females, and 5 *P. latipinna* males, and low frequency pond with 20 *P. formosa*, 50 *P. latipinna* females, and 20 *P. latipinna* males. The initial sex ratio of *P. latipinna* was 3:1 as in the small ponds. A small submersible heater was placed in the shallow area of each pond during the winter months to provide a freely accessible warmer refuge for the fish. The entire water body was not heated because the depths of these ponds were below ground level where water temperature is less sensitive to changes in air temperature.

These populations were monitored monthly weather permitting (see Small Ponds above), using a 15 ft, 1/16" mesh seined. Two or three seine hauls were used in different areas of the pond until the total number of fishes caught was at least 200. All fish were identified to species, age class, and gender, and counted prior to returning them to the pond. These monthly data were used to calculate the relative frequencies of *P. formosa* and *P. latipinna* adults and juveniles.

Statistical Analyses

Monthly data from each pond were plotted to evaluate changes in number of all classes of fishes and relative frequency of adult and juvenile *P. formosa*. The relative frequency among the adults was calculated as the proportion of gynogenetic females among all of the adults, including males.

$$\text{Freq. Gynogens}_A = \frac{\text{\# Adult Gynogens}}{\text{Total \# Adults (Gynogens + Sexual females + Sexual Males)}}$$

The relative frequency among the juveniles was the proportion of gynogenetic juveniles among all juveniles.

$$\text{Frequency Juvenile Gynogens}_J = \frac{\text{\# Juvenile gynogens}}{\text{Total \# Juveniles}}$$

The reproductive success of *P. formosa* was determined by dividing the total number of juvenile *P. formosa* in each monthly sample by the number of adult *P. formosa* on the sampling date approximately 90 days earlier. I used this 90-day interval between adults and juveniles for two reasons. One, *P. formosa* and *P. latipinna* females from the Brownsville area have an average interbrood interval of 60 days (Hubbs & Dries in press) so any females inseminated during a given month would not give birth for 60 days. Two, I could not reliably identify newborn fry to species visually because they were too small (≤ 1 cm SL). When juveniles reached or exceeded 2 cm in standard length (approximately 30 additional days of growth) species specific characteristics were easy to distinguish and I could classify juveniles as *P. formosa* or *P. latipinna*. The characteristics I used for classification were the number of dorsal fin rays (≥ 11 = *P. latipinna*), dorsal fin shape (rectangular = *P. latipinna*), presence parallel horizontal rows of black dashes (*P. latipinna*), and absence of gold in anal fin (*P. latipinna*). I used this same procedure to calculate reproductive success of *P. latipinna* females. I used these measures of number of juveniles / female to determine the reproductive success of *P. formosa* relative to *P. latipinna* by constructing a ratio of these reproductive success values.

G = # juveniles/gynogen
S = # juveniles/sexual female
RRS_G = Relative Reproductive Success of gynogens

$$RRS_G = \frac{G}{S}$$

I examined whether reproductive success of gynogenetic females is frequency-dependent by plotting RRS_G as a function of relative frequency of adult *P. formosa* 90-days earlier. I also plotted the average number of juveniles produced by *P. formosa* and *P. latipinna* females as a function of the frequencies of the females.

The errors associated with data points from each pond were analyzed for independence using the Test of Serial Independence (Von Neumann et al. 1941). Raw frequency data and raw relative reproductive success data were transformed using arcsine(square root), and the natural logarithm (ln) respectively, to meet the regression analysis assumption of normally distributed residuals.

I used autocorrelation analysis to determine the independence between successive raw data points at all possible lags between sampling periods of each pond. I examined the correlograms and autocorrelation coefficients to determine if time series modeling was appropriate to evaluate the relationship between adult frequency and relative reproductive success of *P. formosa* (Chatfield 1975). I adjusted transformed data to remove autocorrelation by calculating the average over 180 days of frequency of adult *P. formosa* and relative reproductive success. Finally, I used simple linear and logarithmic regression to evaluate the relationship between transformed raw values and transformed averaged values of relative reproductive success and relative frequency of *P. formosa* adults.

Cumulative mortality of adults was calculated using data from thirteen small ponds. Data were from 7 successful ponds and 6 "failed" small ponds that either reached 100% *P. formosa* or in which reproduction never occurred and which were omitted from analyses of reproductive success. Cumulative adult mortality in the small ponds was calculated for *P. latipinna* males and females, and *P. formosa* females according to the following formula

$$\text{Cum. Mortality} = \frac{1 - \text{Total \# Survivors}}{\text{\# Days Elapsed}} .$$

Mortality values in the seven successful ponds were calculated using only data collected prior to the birth of the first brood of juveniles because I did not mark individuals and therefore could not distinguish mortality from recruitment after this point in time. Cumulative mortality was compared using nonparametric Mann-Whitney U-tests because the sample sizes were small ($n = 13$) and data were not normally distributed.

Student's t-tests, analyses of variance and binomial tests were used to evaluate effects of season and density. Analyses of variance of data from small ponds used n as the denominator of the formulae for variance and standard deviation because all fish were counted; the "sample" was the entire population (N

= n). For the analyses of data from large ponds I used n - 1 as the denominator in the calculation of variances and standard deviations because the data truly were a sample; all individuals were not counted. Seasons were designated as Spring (March, April, May), Summer (June, July, August), Fall (September, October, November), and Winter (December, January, February). These classifications correspond to the breeding (spring, summer, fall) and non-breeding (winter) seasons of *Poecilia* from Texas populations.

RESULTS

Field collections

Relative frequencies of *P. formosa* adults and juveniles were estimated from the field collections. In 1997 *Poecilia formosa* was present in the Brownsville Airport Ditch site and in the Central Ditch site at very low frequencies (Table 5.1). A rough estimate of the relative reproductive success of *P. formosa* in the Airport Ditch population was determined from the number of adult and juvenile *P. formosa* and *P. latipinna* in the samples. It is a rough estimate because any early mortality of juveniles would be undetected and included in reproductive success. The estimated reproductive success of a *P. formosa* female was 2.39 offspring for every offspring of a *P. latipinna* female. Relative reproductive success could not be estimated for the Central Ditch site because juveniles were not identified to species.

In the collections from Weslaco in 1998 and 1999, the relative frequencies of *P. formosa* adults were 0.53 and 0.17, respectively (Table 5.1). The relative frequency of juvenile Amazons in the 1999 collection was 0.33. Based on these data the estimated relative reproductive success for this collection was also 2.39 to 1. The February1998 and 1999 collections from Mission contained *P. formosa* adults in relative frequencies of 0.57 and 0.67, and juveniles in frequencies of 0.31 and 0.63. The estimated relative reproductive successes for these collections were 0.21 and 0.27. The relative frequency of juvenile Amazons from the May 1999 collection was 0.32.

Table 5.1 Relative Frequency of *Poecilia formosa* in Natural Populations

This table lists the number and relative frequencies of adult and juvenile *Poecilia latipinna* and *P. formosa* in the collections taken from Brownsville (CD, AD) Weslaco (WS), and Mission (MS), Texas sites. The estimated relative reproductive success (RRS) of *P. formosa* is included The data represent samples, not a census of the entire population. The data indicate the relative frequency of *P. formosa* varies across site and time in natural populations. Sex ratios of adult *P. latipinna* females to males (Sexuals) and females including *P. formosa* to males (All) are also listed. The gender of juveniles cannot be determined by external characteristics. Data not obtained from a sample is listed as n/a.

Table 5.1 Relative Frequency of *Poecilia formosa* in Natural Populations

Species	**CD**	**AD**	**WS**		**MS**	
Date	Feb. 97	Aug. 97	Feb. 98	Feb. 99	Feb. 98	Feb. 99
Adults:						
P. formosa						
Rel. Freq.	0.12	0.03	0.53	0.17	0.57	0.67
No.	46	43	45	7	74	81
P. latipinna						
Rel. Freq.	0.88	0.97	0.47	0.83	0.43	0.33
No. females	206	916	40	13	34	13
No. males	134	293	n/a	21	21	27
Juveniles:						
P. formosa						
Rel. Freq.	n/a	0.04	n/a	0.33	0.31	0.63
No.	n/a	11	n/a	15	18	49
P. latipinna						
Rel. Freq.	n/a	0.96	n/a	0.67	0.69	0.37
No.	n/a	98	n/a	31	40	29
Sex Ratio:						
Sexuals	1.5:1	3.1:1	n/a		1.6:1	0.19:1
All	1.9:1	3.3:1	n/a		5.1:1	3.5:1
RRS:	n/a	2.39	.207	.899	1.177	.271

Aquaria

Mortality

Two experiments were aborted because fish died faster than they could be replaced and because four gynogenetic females did not appear to be virgin; they gave birth to fry before males were added to the aquarium.

The mortality in these aquaria was unusually high relative to that typical of laboratory maintained fish. In the past in the laboratory, females and males were housed separately or very few males were housed together with a group of females. I replaced fish as they died and ran out of virgin females after maintaining the experiments for nine months. I calculated the total number of fish of each species and sex that died in each experiment. *Poecilia formosa* females were 25% and 20% of the dead fish when they were at a frequency of 0.20, and 76% and 61% of the dead fish when they were at frequency of 0.80. The proportions of dead gynogenetic females were very similar to the proportions of live gynogenetic females under both conditions; suggesting relative mortality is constant across the aquarium experiments. This also confirms that *P. latipinna* and *P. formosa* are competing for some resource other than food (i.e. home ranges) as would be expected under the controlled conditions of the aquaria.

The mortality of males differed between the two experimental conditions. When males were 5 % of the population (a single male in each aquarium), they were 8% and 16% of the deaths. When males were 20% of the population (4 males), they were 40% and 33% of the deaths. Groups of males of similar sizes exhibited territorial behavior and no evidence of disease was subsequently found in the injured or deceased males. I replaced males as they died and deliberately increased the size differences between males in an aquarium, which reduced the incidence of aggression during observations, but it isn't clear how well it reduced injury and mortality.

Visual observations at random time intervals revealed a high level of aggression between both males and females. Aggressive behaviors were evident during all observations, and numerous fish acquired bruises, scrapes and missing scales as a result of these social interactions. The aggressive behaviors and injuries observed in both males and females are consistent with conflict in forming dominance hierarchies and feeding territories. Presumably the aggressive interactions directly or indirectly resulted in the mortality of the majority of the fishes.

Reproduction

Reproduction occurred in only two of the four remaining aquarium experiments. Sixteen fry were born in two experiments in which the relative frequency of *P. formosa* was low, five fry from one aquarium and eleven from another. All fry were visually identified as *P. latipinna*. None of the gynogenetic or sexual females that died were carrying embryos. Courtship behaviors were observed on only two occasions in aquaria in which the relative frequency of *P. formosa* was high. Gonopodial thrusting was observed on one occasion. A single male directed several thrusts toward a gynogenetic female in an aquarium with a high frequency of *P. formosa*.

Small Ponds

Population Extinction

Six small ponds yielded no relative reproductive success data and are referred to below as "extinction" outcomes. In these ponds all *P. latipinna* died before any reproduction occurred (Table 5.2) and, thus all opportunity for *P. formosa* reproduction had likewise ended. All fish were removed from these ponds, and they were restocked with different adult fish for new experiments. Only one of the six extinction ponds was initially stocked with 20% *P. formosa*, the remaining 5 having been stocked with 80% *P. formosa*. The probability of failure across all 13 small ponds was not significantly different than random (Binomial test: $p = 0.462$). There was no significant relationship between initial frequency and extinction (Binomial test $P(1 \text{ low}, 5 \text{ high}) \times P(\text{Failure}) = (0.2188)(0.5) = 0.11$) or between season of initiation of the experiment and extinction ($\chi^2_{[3]} = 3.0$, $p = 0.572$).

Table 5.2 Population Fixation of *Poecilia formosa*

This table lists the small experimental ponds where *P. latipinna* became extinct in the population. The initial (IF) and final frequencies (FF) of *P. formosa* are given, as are the dates of initiation (ID) and termination (TD) of the experiment, and the number days of the experiment (Days). In 4 populations fixation of *P. formosa* occurred prior to any reproduction. However, in 2 populations *P. formosa* produced offspring; *P. latipinna* did not reproduce. Occurrence of reproduction and the number of *P. formosa* offspring are noted (Repro. (#)).

Pond #	IF	FF	Repro. (#)	ID	TD	Days
22a	.80	1.00	no	24Dec. 97	31Mar. 97	97
23a	.80	1.00	no	21Apr. 98	25Jul. 98	95
26a	.80	1.00	no	21Apr. 98	1Jun. 98	41
26b	.80	1.00	no	27Jul. 98	6May. 99	282
28a	.20	1.00	yes (9)	21Apr. 98	1Aug. 98	103
30a	.80	.92	yes (3)	10Sep.98	27Oct. 98	47

Relative Frequencies

In ponds with an initially high frequency of *P. formosa*, their frequency decreased over time (Fig. 5.4a). And in ponds with initially low frequency of *P. formosa*, there was a general increase in their frequency (Fig. 5.4b). These patterns of change suggest that relative frequency of *P. formosa* is correlated with reproductive success.

In ponds with initially high frequency of adult *P. formosa*, relative frequency of juvenile *P. formosa* was initially high, but subsequently decreased (Fig. 5.5a). In populations where the initial frequency of adults was low, the relative frequency of juvenile *P. formosa* among the first broods varied from low (≈ 0.20) to very high (1.00). The relative frequency of juveniles remained high or increased initially and subsequently oscillated (Fig. 5.5b). The prevalence of samples in pond 25, where *P. formosa* comprised 100% of the juveniles, is an extreme example of biased reproductive success but also indicates the asynchrony in reproductive cycles of gynogenetic and sexual females.

Figure 5.4: Relative Frequency of Adult *Poecilia formosa* in Small Ponds

This figure shows the changes in relative frequency of adult *Poecilia formosa* in small experimental ponds. The *x*-axis indicates the sampling day; the -axis indicates the sampling day; the *y*-axis represents the frequency of *P. formosa* adults (gynogens) relative to *P. latipinna* adults. Under both initial conditions, relative frequency exhibits some oscillation around a general trend. (a) This graph shows the general decrease in frequency in all three ponds of initially high frequency *P. formosa*. In pond 22, on sampling day 308, all of the adults in the population were *P. formosa*. However, subsequent maturation of juvenile *P. latipinna* prevented the population from fixation at pure *P. formosa*. (b) In contrast with a), this graph shows the general increase in frequency of *P. formosa* in ponds where the initial frequency was low. In pond 25, on sampling day 506, *P. formosa* females comprised almost 100% of the adults in the population. As in pond 22, there were juvenile *P. latipinna* in the population that subsequently matured, preventing fixation of the population.

Small Ponds
Adults

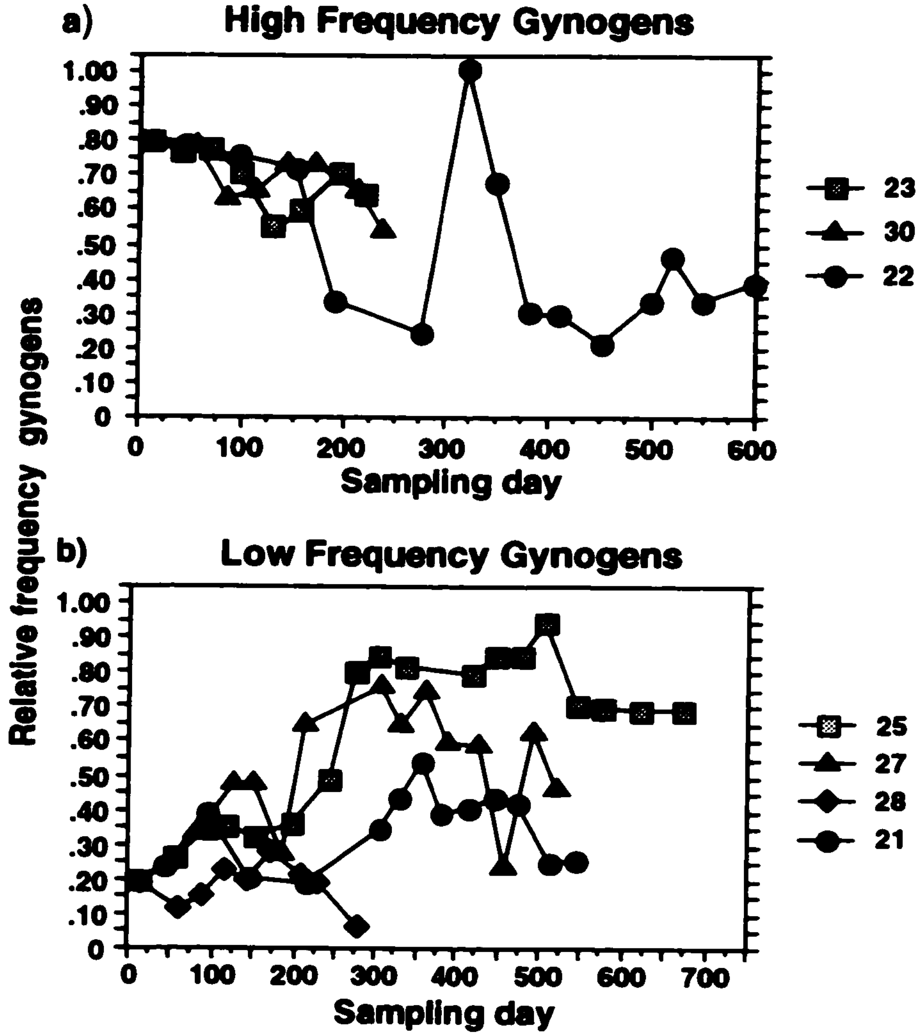
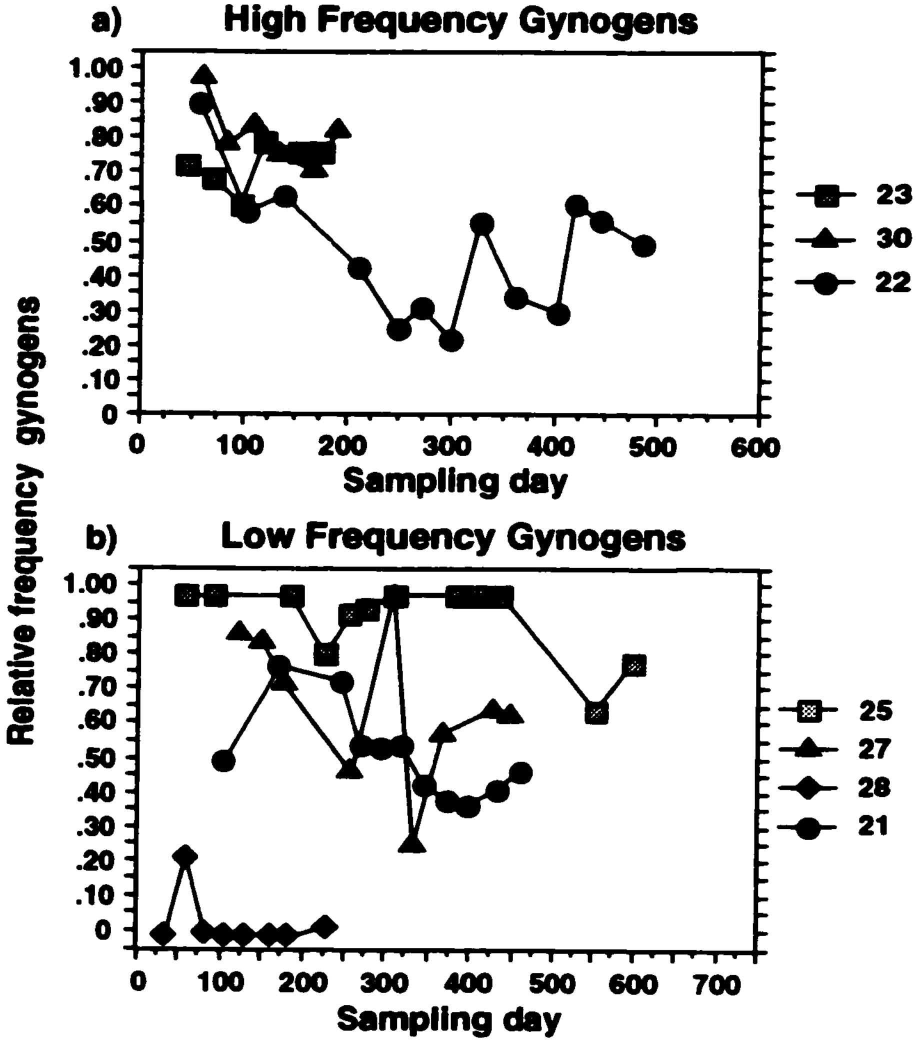


Figure 5.5: Relative Frequency of Juvenile *Poecilia formosa* in Small Ponds

This figure shows the changes in relative frequency of juvenile *Poecilia formosa* in small experimental ponds. The *x*-axis indicates the sampling day; the *y*-axis represents the frequency of *P. formosa* juveniles (gynogens) relative to *P. latipinna* adults. (a) This graph shows the frequency of juvenile *P. formosa* in the ponds of initially high frequency (0.80) adult *P. formosa*. In pond 23 the frequency of juveniles at their first appearance (0.73) was less than that of the adults. In the remaining two ponds (22 & 30) the frequency of juvenile *P. formosa* was initially greater than the adults (0.89 and 0.99) but subsequently dropped below the initial frequency of adults. The general trend is decreasing with oscillation. (b) This graph shows the frequencies of juvenile *P. formosa* in the ponds where adults were initially at low frequency (0.20). The initial frequencies of the juveniles varied among the ponds. In pond 25, all of the juveniles that appeared first were *P. formosa*. In pond 21 half of the juveniles were *P. formosa*. In ponds 27 and 28 *P formosa* were less than 5% of the juveniles. In all of the ponds except 28, the subsequent frequencies of juvenile *P. formosa* increased. In general their frequency remained greater than the initial frequency among adults (0.20). In pond 28, juvenile *P. formosa* never reached a high frequency.

Small Ponds
Juveniles



Visual examination of graphs showing the relative frequency of *P. formosa* juveniles and the relative frequency of their adult *P. formosa* mothers from individual ponds supports a relationship between reproductive success and relative frequency. In ponds where adult *P. formosa* were initially at high frequency, there is a general correspondence between relative frequencies of juveniles and relative frequencies of their mothers (Fig. 5.6, 5.7, and 5.8). When the frequency of *P. formosa* mothers is near 0.80, the frequency of *P. formosa* juveniles is less than 0.80. When the frequency of mothers drops to 0.70 or below, the frequency of juveniles is greater than 0.70 (Fig. 5.6). In the ponds where the initial frequency of adult *P. formosa* was low, the relative frequency of juvenile *P. formosa* was consistently greater than that of their mothers in three of four cases (Figs. 5.7 and 5.8). In pond 28, *P. formosa* juveniles were present only twice and were at very low frequencies suggesting *P. formosa* could have been approaching extinction in this populations.

Figure 5.6: **Relative Frequencies of Juvenile *P. formosa* and Their Mothers in High Frequency Ponds**

The frequencies of juvenile *P. formosa* (gynogens) are plotted with the frequencies of their mothers for all of the ponds adult *P. formosa* were initially at high frequency (0.80). The sampling day on the *x*-axis represents that of the adult *P. formosa*. The sampling day of juveniles is 90 days plus the value on the *x*-axis. This adjustment places the frequency of juveniles directly above that of their mothers. If reproductive success were negatively frequency-dependent, the frequency of juveniles would be low when the frequency of their mothers is high. (a) In pond 22, the initial frequency of juveniles was higher than that of the adults' (0.90), even though the frequency of their mothers was high. Subsequently the frequency of juveniles dropped below that of their mothers and continued to decrease as adult frequency decreased. When adult frequency dropped to 0.30, juvenile frequency increased and exceeded that of the adults. This pattern of lower frequencies of juveniles when their mothers are at higher frequencies is consistent with frequency-dependent reproductive success. (b) In pond 23 the frequency of juvenile *P. formosa* was always higher than that of their mothers until the frequency of adult *P. formosa* decreased to 0.71. After this point, adult frequency continued to decrease and juvenile frequency increased. (c) The initial frequency of juvenile *P. formosa* in pond #30 was high and remained within a range of 0.80 to 0.95. It never dropped below the frequency of the adults. Note the slight oscillation in frequencies of both adults and juveniles in this pond.

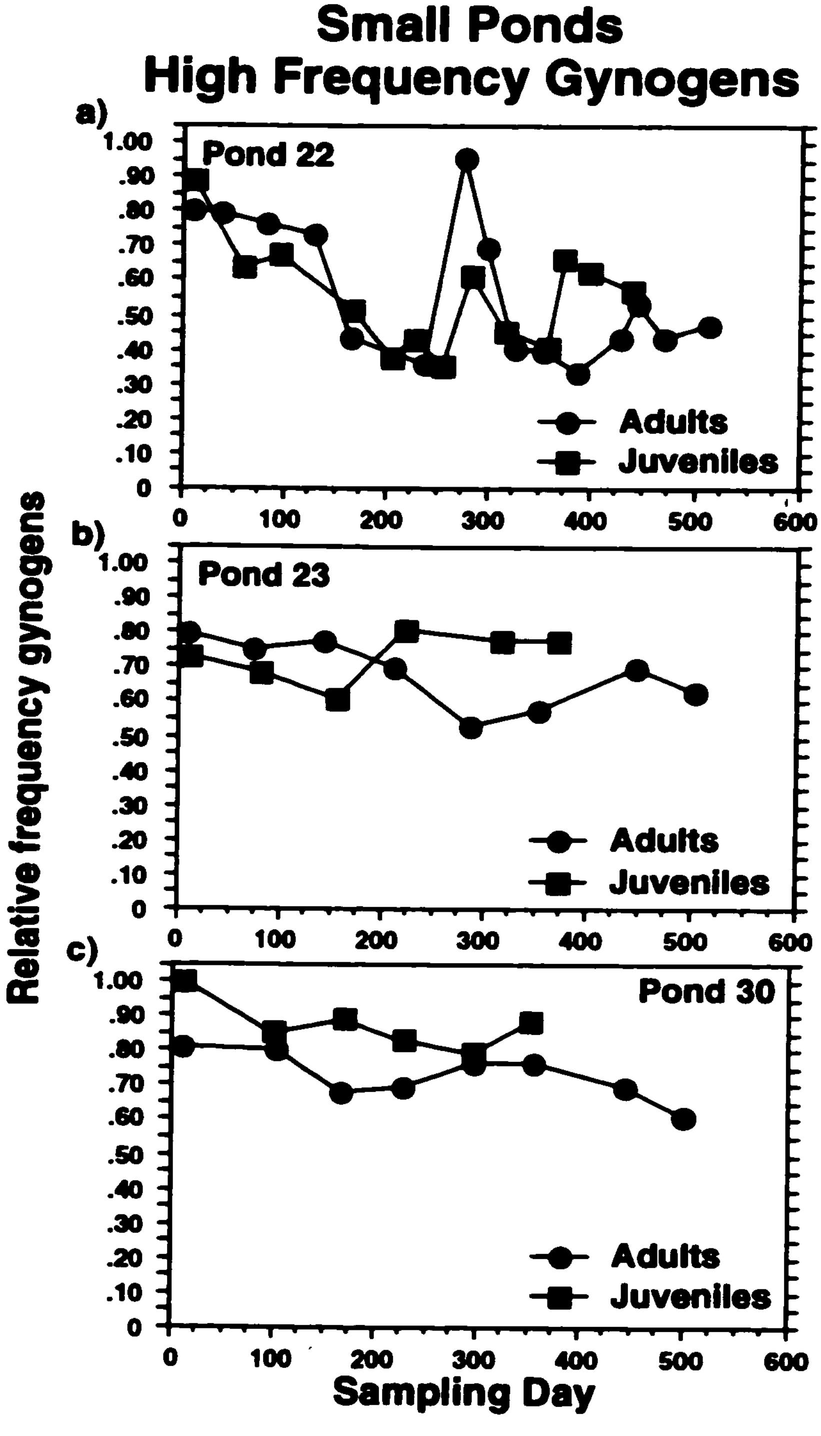

Small Ponds
High Frequency Gynogens
a)
Pond 22
b)
Pond 23
c)
Pond 30
Adults
Juveniles
Relative frequency gynogens
1.00
.90
.80
.70
.60
.50
.40
.30
.20
.10
0
0
100
200
300
400
500
600
Sampling Day

Figure 5.7: **Relative Frequencies of Juvenile *Poecilia formosa* and Their Mothers in Low Frequency Ponds 21 and 25**

The frequencies of juvenile *P. formosa* (gynogens) are plotted directly above the frequencies of their mothers for all of the small ponds with an initially low frequency of adult *P. formosa* (0.20). Sampling day is a reference for adult frequency; sampling day of juvenile frequency is an additional 90 days later. If reproductive success were negatively frequency-dependent, the frequency of juveniles would be high when the frequency of their mothers is low. (a) In pond 21, the frequency of juveniles was initially higher than that of their mothers and continued to increase as the frequency of adult *P. formosa* decreased. The juvenile frequency began to decrease when the adult frequency began to increase. This oscillatory cycle of frequency occurred once more during the experiment. (b) The frequency of juvenile *P. formosa* in pond 25 was initially very high (1.0) and did not decrease until the frequency of adult *P. formosa* had increased to over 0.80. When the adult frequency reached 1.0, no additional *P. formosa* juveniles were born. Only when the adult frequency dropped below 0.70 were new broods of juveniles born. In both ponds frequency of adult *P. formosa* oscillated.

Small Ponds
Low Frequency Gynogens

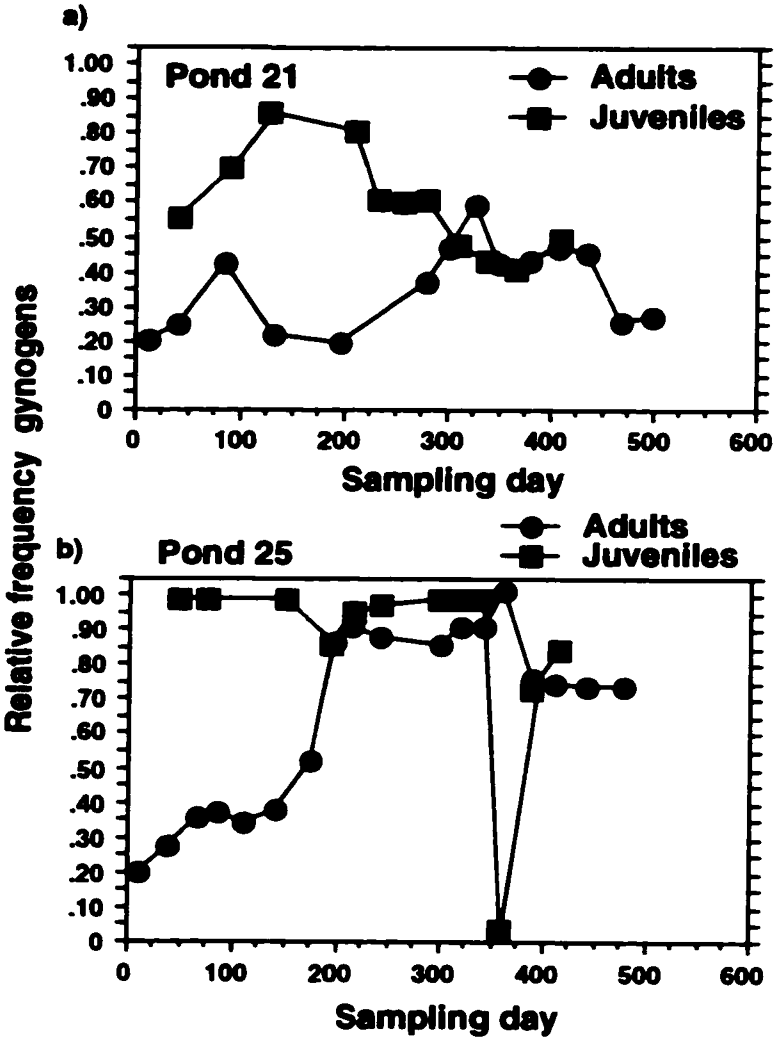
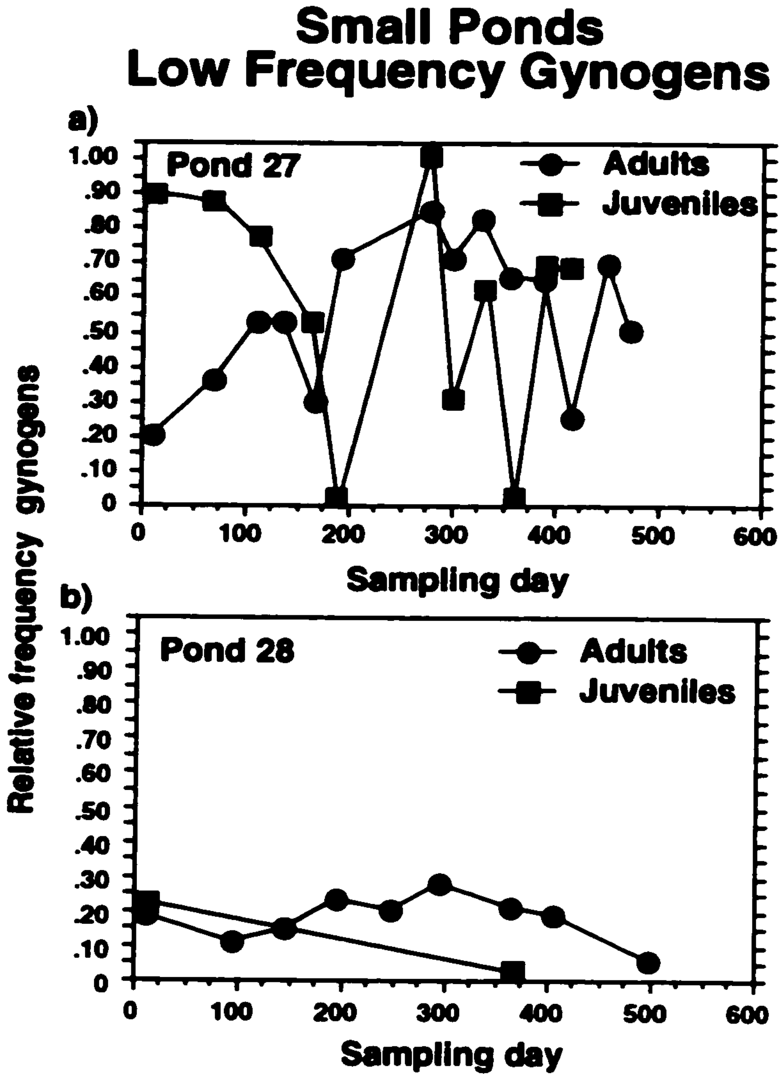


Figure 5.8: **Relative Frequencies of Juvenile *Poecilia formosa* and Their Mothers in Low Frequency Ponds 27 and 28**

The frequencies of juvenile *P. formosa* (gynogens) are plotted directly above the frequencies of their mothers for all of the small ponds with an initially low frequency of adult *P. formosa* (0.20). Sampling day is a reference for adult frequency; sampling day of juvenile frequency is an additional 90 days later. If reproductive success were negatively frequency-dependent, the frequency of juveniles would be high when the frequency of their mothers is low and vice versa. (a) The oscillatory nature of changes in adult and juvenile *P. formosa* frequencies is readily apparent in pond 27. When adult frequencies were low, juvenile frequencies were high; when adult frequencies were high, juvenile frequencies were low. The range of juvenile frequencies included both 0 and 1.0 and corresponded with adult frequencies greater than 0.50. (b) Few juveniles were born in pond 28 and the frequency of adult *P. formosa* never exceeded 0.26. The expected high reproductive success of *P. formosa* relative to *P. latipinna* did not materialize in this pond. This may be related to small population size and the effects of stochasticity.


Small Ponds
Low Frequency Gynogens
a)
Pond 27
Adults
Juveniles
1.00
.90
.80
.70
.60
.50
.40
.30
.20
.10
0
0
100
200
300
400
500
600
Sampling day
Relative frequency gynogens
b)
Pond 28
Adults
Juveniles
1.00
.90
.80
.70
.60
.50
.40
.30
.20
.10
0
0
100
200
300
400
500
600
Sampling day

Relative Reproductive Success

The relationship between relative reproductive success and relative frequency of *P. formosa* is plotted using transformed raw and transformed averaged values (Fig. 5.9). The flat line at 0 represents the natural logarithm of the expected reproductive advantage of *P. formosa* in the absence of frequency-dependence (ln(1)). The negative slopes of both regression lines indicate relative reproductive success decreases as relative frequency increases. However, the relationship is not significant in either case, the slopes of the regression lines are not significantly different than zero, and neither explain a large proportion of the variance (raw data: $F_{1,34} = 1.220$, $p = 0.277$, $R^2 = 0.034$; averaged: $F_{1,10} = 2.123$, $p = 0.141$, $R^2 = 0.186$).

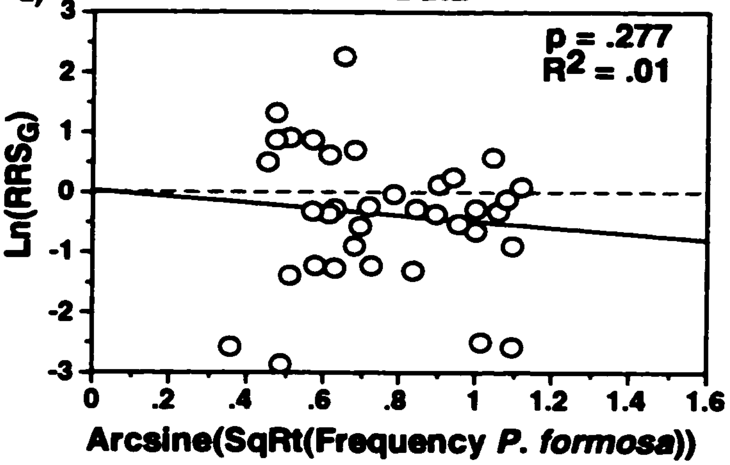
The trend in the relationship between the number of juvenile *P. formosa* and the relative frequency of their mothers is negative but not significant ($F_{1,28} = 1.775$, $p = 0.121$, $R^2 = 0.058$)(Fig. 5.10). The frequency of *P. latipinna* has no effect on the number of *P. latipinna* juveniles ($F_{1,28} = 0.645$, $p = 0.427$, $R^2 = 0.015$)(Fig. 5.10). The distribution of the residuals from this analysis meets the assumptions of regression analysis and therefore the data were not transformed.

Figure 5.9: **Relative Reproductive Success of *Poecilia formosa* in Small Ponds**

The relative frequency of adult *P. formosa* is plotted against their relative reproductive success (*RRS_G*) across all small ponds. The frequency data were transformed by taking the arcsine of the Square Root of relative frequency; values of 0 and 1.6 correspond with frequencies of zero and 1.0, respectively. Relative reproductive success was calculated as the natural logarithm of the number of offspring per *P. formosa* divided by the number of offspring per *P. latipinna* female. The horizontal dashed line represents the expected constant value if there is no relationship between frequency and reproductive success (ln (1) = 0). The expected relationship under negative frequency-dependence is a line of negative slope (Fig. 5.1). The vertical dotted line near zero is an asymptote representing no reproductive success when there are no *P. formosa* in the population (frequency = 0). (a) This graph shows the relationship between frequency of adult *P. formosa* and relative reproductive success is negative but not significant, despite the pattern of change in frequencies of adult and juvenile *P. formosa* illustrated in figures 5.6, 5.7, 5.8. (b) This graph shows the results of linear regression analysis on average relative reproductive success over 180-day time intervals. The averages eliminate serial dependence of data within a pond. The slope of the regression line is negative but not significant. However, a greater proportion of the variance is explained than in a) above. These data do not reject the possibility that reproductive success is independent of frequency.

Relative Reproductive Success
Small Ponds

a) All Data



b) 180 Day Average

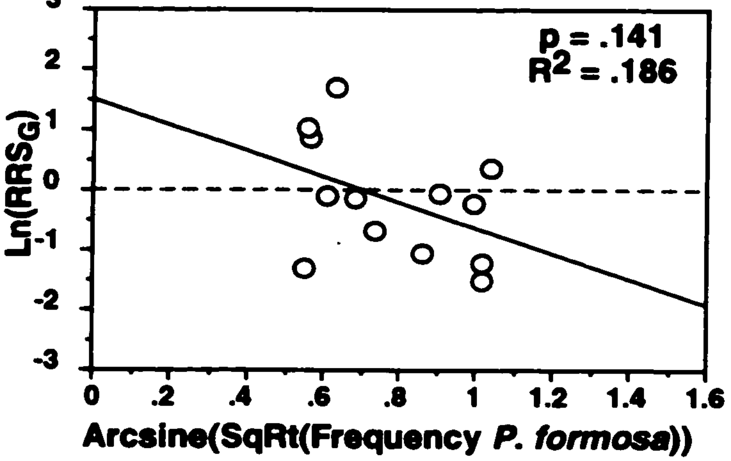
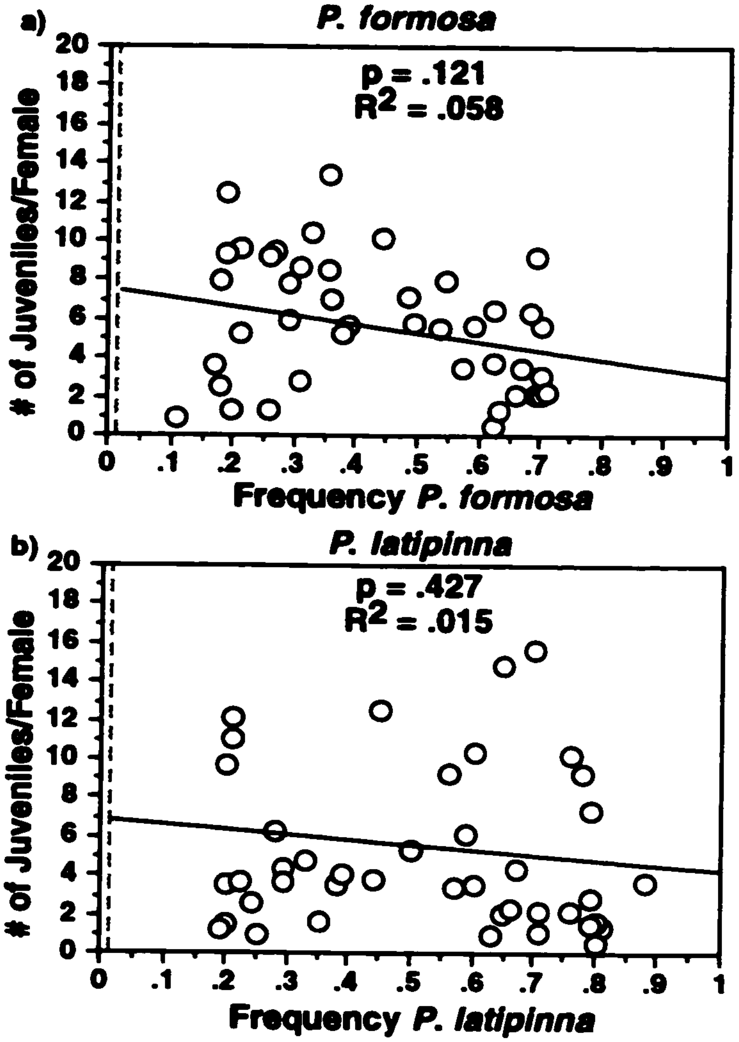


Figure 5.10: Reproductive Success of *Poecilia formosa* and *P. latipinna* females

These graphs show the relationship between number of juveniles per female and relative frequency of *P. formosa* and *P. latipinna*. Gender of juveniles was not determined. (a) The tendency for *P. formosa* females to have fewer offspring as their frequency in the population increases is shown here. The relationship is not significant. (b) The relative frequency of *P. latipinna* does not appear to have a significant effect on the number of juveniles per female.

Female Reproductive Success
Small Ponds



Tests of Serial Independence indicated the errors associated with data points from each pond were not significantly correlated (Table 5.3).

Table 5.3 Statistics of Tests for Serial Independence in Experimental Ponds

Listed below are the values of the test statistics, η, and $|(1-\eta)/2|$, the critical values for $\alpha = 0.05$. All probability values are greater than 0.95 ($p>0.95$). The sample autocorrelation coefficients for Relative Reproductive Success data from lags 1 and 2 are listed for each pond. Coefficients of lags great than 2 approach zero and are not included. These tests indicate all data points at lags of greater than 1 can be considered independent. The sample size (N) for each pond is also provided.

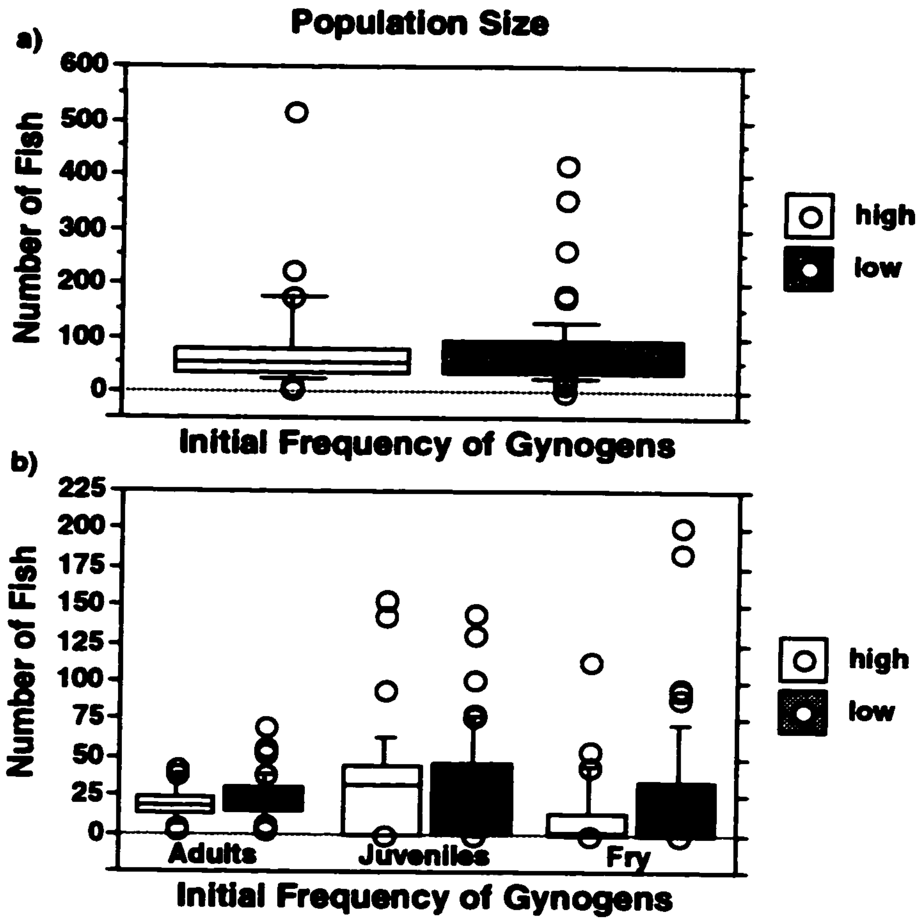
		Serial Independence			Autocorrelation	
Pond	N	η	$\|(1-\eta)/2\|$	$\alpha = 0.05$	Lag 1	Lag 2
Large						
3	10	0.1126	0.443	.0.5444	0.340	$\bar{0}.089$
4	9	0.9129	0.044	0.5650	0.294	0.058
Small						
21	10	0.0978	0.4511	0.5444	0.716	0.423
22	8	0.4371	0.2815	0.5874	0.540	$\bar{0}.032$
23	5	0.0527	0.4736	0.6140	0.083	$\bar{0}.341$
25	4	1.8394	0.4197	0.6161	$\bar{0}.415$	$\bar{0}.156$
27	8	0.9017	0.0491	0.5874	0.004	$\bar{0}.020$
28	4	0.8272	0.0864	0.6161	$\bar{0}.030$	0.236
30	4	0.0569	0.4716	0.6161	0.173	$\bar{0}.199$

Population Size and Density

There were no significant differences in average population size between ponds of initially high frequency adult *P. formosa* and ponds of initially low frequency (U = 941.00, z = $^{-}$0.178, p = 0.859; high: mean±SD = 78.0±88.5, se = 14.9; low: mean±SD = 77.8±76.2, se = 10.3)(Fig. 5.11a). Separate examinations of adults, juveniles and fry also did not reveal any significant differences (adults: U= 701.5, z = $^{-}$1.071, p = 0.284; juveniles: U = 780.5 z = $^{-}$0.332, p = 0.739; fry: U = 746.5, z = $^{-}$0.650 p = 0.516)(Fig. 5.11b). The average density of fishes in high and low frequency ponds was 1 fish / 29.78 gallons (112.7 liters) and 1 fish / 20.9 gallons (79.3 liters), respectively. The average density of fishes in these ponds did not differ between the two initial conditions (U = 810.0, z = $^{-}$0.056, p = 0.955).

Figure 5.11: Population Size in Small Ponds

Box plots illustrate the population sizes in ponds of initially high and low frequency *P. formosa*. The line within a box represents the median, the box indicates the 25th and 75th percentiles, the whiskers represent the 10th and 90th percentiles, and the open circles represent individual values outside 90% of the distribution. The open boxes represent ponds of initially high frequency *P. formosa*; the shaded boxes represent ponds of initially low frequency *P. formosa*. (a) The total number of fish in ponds of initially high and low frequency of *P formosa* did not differ significantly. (b) The total number of adults, juveniles and fry also did not differ between high and low frequency ponds.



Population Size
Small Ponds

Mortality

The changes in numbers of *P. formosa* females and *P. latipinna* males and females, in high and low frequency ponds, showed different patterns (Figs. 5.12 and 5.13). In the ponds where *P. formosa* was initially at high frequency, the number of *P. formosa* females dropped dramatically (Fig. 5.12a) while the numbers of *P. latipinna* females and males did not (Fig. 5.12b,c). However, in ponds where *P. formosa* was initially at low frequency, and therefore *P. latipinna* was at high frequency, the number of *P. latipinna* females dropped (Fig. 5.13b), while the numbers of males (Fig. 5.13c) and *P. formosa* females did not (Fig. 5.13a). The initial decrease in the number of gynogenetic females and sexual females under the two conditions suggests frequency may have an affect on mortality of adult females. In order to compare mortality rate of *P. formosa* and *P. latipinna* adults and avoid inadvertently including recruitment, I used the cumulative mortality of adults before the appearance of offspring in all of the 13 ponds (6 extinction ponds and 7 successful ponds).

Figure 5.12: **Numbers of *Poecilia latipinna* and *P. formosa* Adults in High Frequency Small Ponds**

The numbers of *P. formosa* (gynogens) and *P. latipinna* males and females over time in ponds of initially high frequency *P. formosa* are shown. (a) The number of *P. formosa* adult females decreased dramatically in the first three months of the experiments in all three experimental ponds. (b) The number of *P. latipinna* females decreased slightly in the first few months of the experiments but the change was not as drastic as that of *P. formosa*. (c) The number of *P. latipinna* males did not decrease in all ponds in the first months of the experiment. In general, the number increased over greater lengths of time. These graphs suggest interspecific and intraspecific differences in mortality in the initial stages of the study.

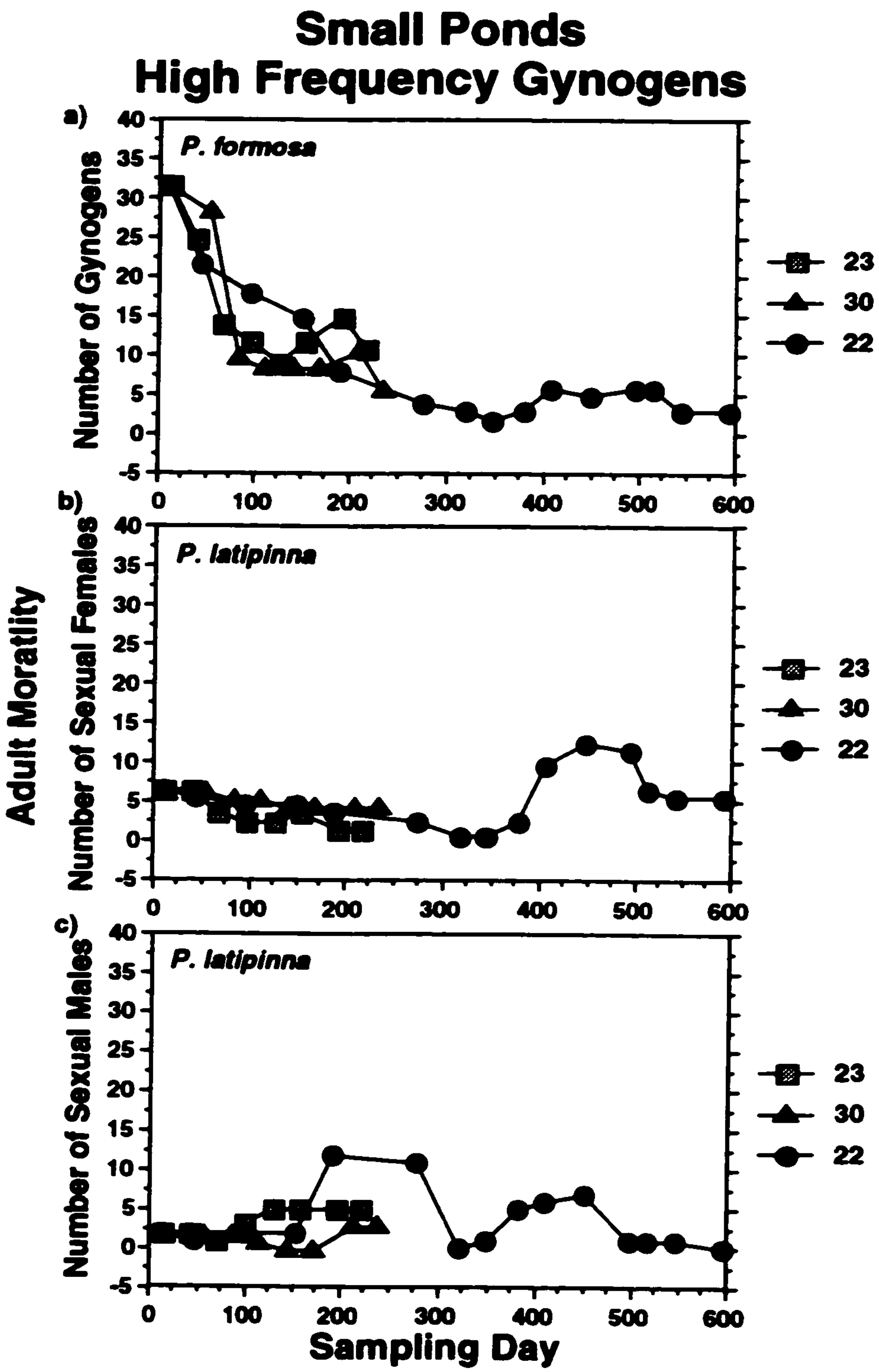
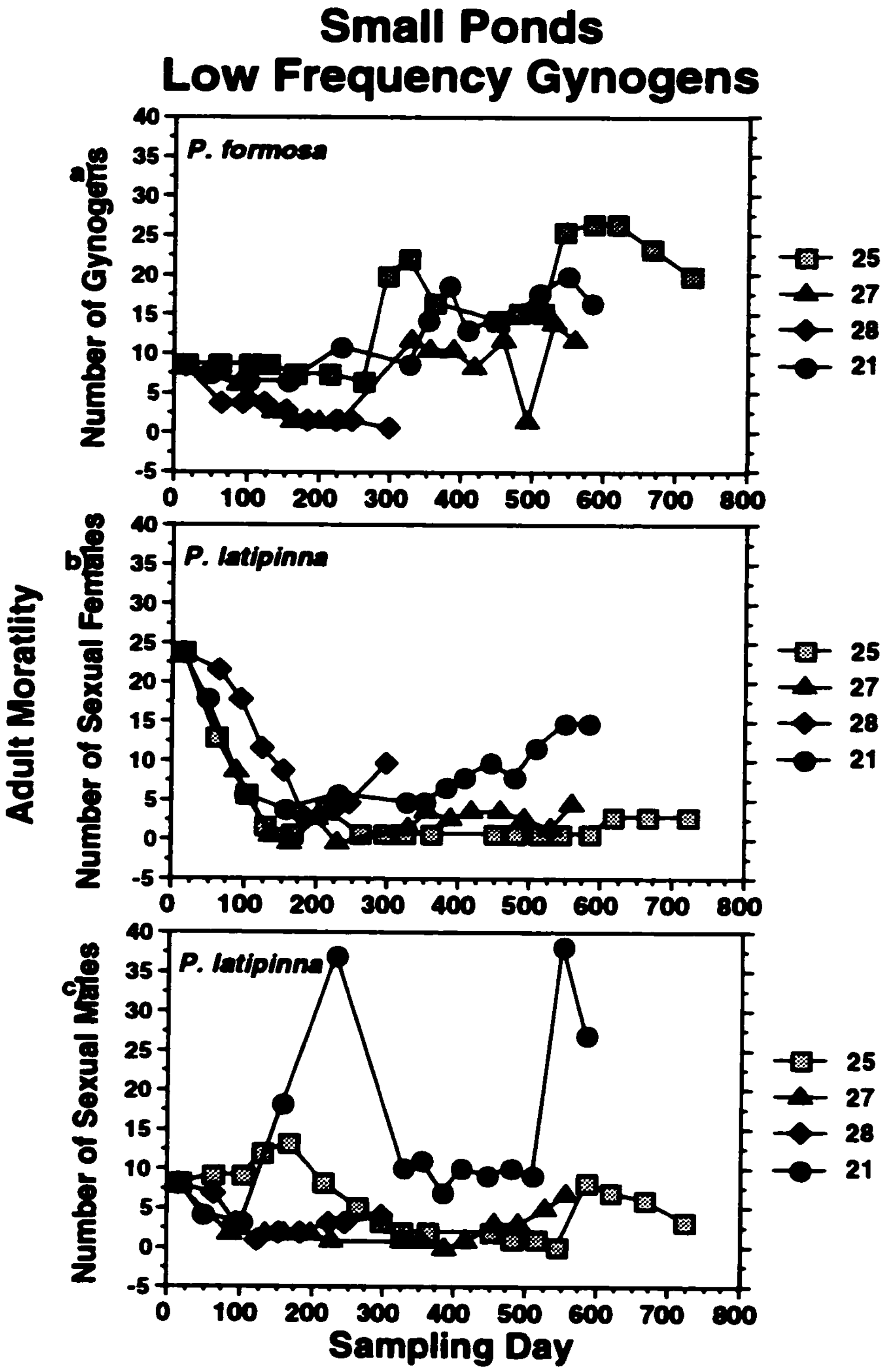

Small Ponds
High Frequency Gynogens
a)
P. formosa
Number of Gynogens
b)
P. latipinna
Number of Sexual Females
c)
P. latipinna
Number of Sexual Males
Adult Moratlity
40
35
30
25
20
15
10
5
0
-5
0
100
200
300
400
500
600
Sampling Day
23
30
22

Figure 5.13: Numbers of *Poecilia latipinna* and *P. formosa* Adults in Low Frequency Small Ponds

The numbers of *P. formosa* and *P. latipinna* males and females over time in ponds of initially low frequency *P. formosa* (gynogens) are shown. (a) The number of *P. formosa* adult females decreased slightly or not at all in the first six months of the experiments in all four experimental ponds. Their number began to increase after 250 days in all but one pond. (b) In contrast with the results in the high frequency ponds, in these low frequency ponds the number of *P. latipinna* females decreased dramatically in the first few months. (c) The number of *P. latipinna* males initially decreased slightly in three of the four ponds in the first three months. Thereafter their numbers increased or remained generally the same throughout the experiment in ponds 25, 27, and 28. Pond 21 was unusual because there were short-term large increases in the numbers of males, even though the frequency of *P. formosa* adults in this pond increased until it reach 1.0 before it dropped. During these periods there were more *P. latipinna* males than *P. latipinna* females, which has never been documented in a field population.


Small Ponds
Low Frequency Gynogens
Adult Moratlity
a
Number of Gynogens
P. formosa
b
Number of Sexual Females
P. latipinna
c
Number of Sexual Males
P. latipinna
40
35
30
25
20
15
10
5
0
-5
0
100
200
300
400
500
600
700
800
Sampling Day
25
27
28
21

I compared the cumulative mortality between and within each species of female under conditions of high and low frequency. In ponds where *P. formosa* was at high frequency, the cumulative mortality of *P. formosa* was significantly higher than that of *P. latipinna* females ($U = 0.00$, $z = {}^{-}3.361$, $p = 0.0008$)(Table 5.4, Fig. 5.14a). The reverse was true in ponds where *P. formosa* was at low frequency, the cumulative mortality of *P. latipinna* females was significantly higher than that of *P. formosa* ($U = 0.00$, $z = {}^{-}2.611$, $p = 0.009$)(Fig. 5.14b).

Cumulative mortality of *P. formosa* was significantly greater at high versus low frequency ($U = 0.0$, $z = {}^{-}2.928$, $p = 0.0034$)(Fig. 5.15a). *Poecilia latipinna* females also experienced significantly greater mortality when at high versus low frequency ($U = 1.00$, $z = {}^{-}2.781$, $p = 0.0054$)(Fig. 5.15b). This corresponds to higher mortality when at higher frequency for both types of females. However, these results do not reflect an interspecific difference between *P. formosa* and *P. latipinna* females because cumulative mortality of females when both were at high frequency or low frequency did not differ (high: $U = 11.00$, $z = {}^{-}1.317$, $p = 0.187$; low: $U = 13.00$, $z = {}^{-}1.025$, $p = 0.306$)(Fig. 5.16). This suggests mortality of both *P. formosa* and *P. latipinna* females is frequency dependent and is not species specific.

Table 5.4 Cumulative Mortality in Experimental Populations

Descriptive statistics of cumulative mortality in small experimental ponds are given below. Cumulative mortality of adult *P. latipinna* males and females, and *P. formosa* females, was calculated as the number of dead individuals divided by the number of days since initiation of the experiment until termination or until the first brood of fry were born. The mean, standard deviation (SD), and standard error (S.E.) are given for each category of adult. The number of ponds in each frequency category is designated by "N". Statistics for ponds with initially high frequency and initially low frequency of *P. formosa* are listed separately. (*Poecilia latipinna* are at low frequency in High Frequency ponds, and at high frequency in Low Frequency ponds.)

	High Frequency (N = 8)			Low Frequency (N = 5)		
Species	Mean	SD	SE	Mean	SD	SE
P. formosa						
females	0.263	0.124	0.044	0.032	0.024	0.011
P. latipinna						
males	0.051	0.030	0.013	0.015	0.008	0.003
females	0.182	0.041	0.018	0.057	0.035	0.012

Figure 5.14: Interspecific Cumulative Mortality of *Poecilia latipinna* and *P. formosa* Females within Small Ponds

The previous graph suggested interspecific and intraspecific differences in mortality in the small experimental ponds. These box plots show the cumulative mortality of *P. formosa* (gynogens) and *P. latipinna* females in the same population prior to the birth of any offspring. The line within a box represents the median, the box indicates the 25th and 75th percentiles, the whiskers represent the 10th and 90th percentiles, and the open circles represent individual values outside 90% of the distribution. (a) The cumulative mortality of *P. formosa* adults is significantly greater than the cumulative mortality of *P. latipinna* females in ponds of initially high frequency *P. formosa*. (b) The cumulative mortality of *P latipinna* females is significantly higher than the cumulative mortality of *P. formosa* in ponds with a low frequency of *P. formosa*. Both graphs indicate negatively frequency-dependent mortality of *P. latipinna* females and *P. formosa*.

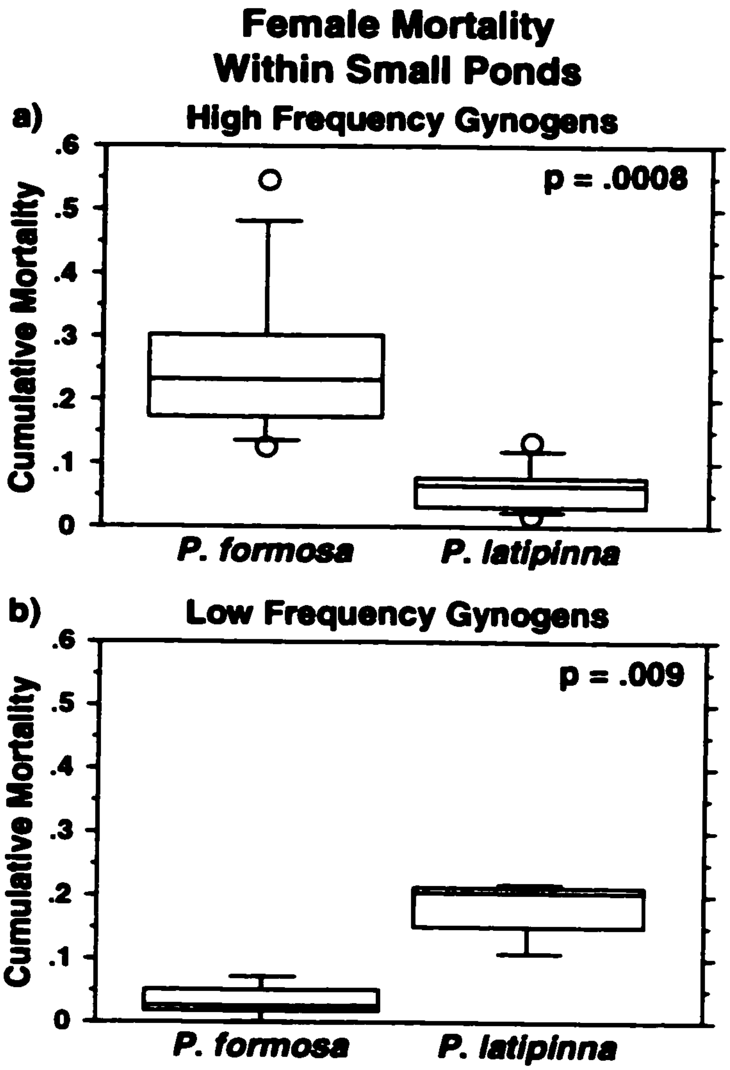

Female Mortality
Within Small Ponds
a)
High Frequency Gynogens
Cumulative Mortality
.6
.5
.4
.3
.2
.1
0
p = .0008
P. formosa
P. latipinna
b)
Low Frequency Gynogens
Cumulative Mortality
.6
.5
.4
.3
.2
.1
0
p = .009
P. formosa
P. latipinna

Figure 5.15: **Intraspecific Cumulative Mortality of *Poecilia latipinna* and *P. formosa* Females within Small Ponds**

These box plots show the cumulative mortality of *P. formosa* (gynogens) and *P. latipinna* females when they are at high and low frequencies prior to the birth of any offspring. The line within a box represents the median, the box indicates the 25th and 75th percentiles, the whiskers represent the 10th and 90th percentiles, and the open circles represent individual values outside 90% of the distribution. (a) The cumulative mortality of *P. formosa* adults is significantly higher when they are at high frequency. (b) The same pattern is seen in *P. latipinna* females. Their cumulative mortality is significantly higher when they are at high frequency. Both graphs indicate negatively frequency-dependent mortality of *P. latipinna* females and *P. formosa* and do not suggest interspecific differences.

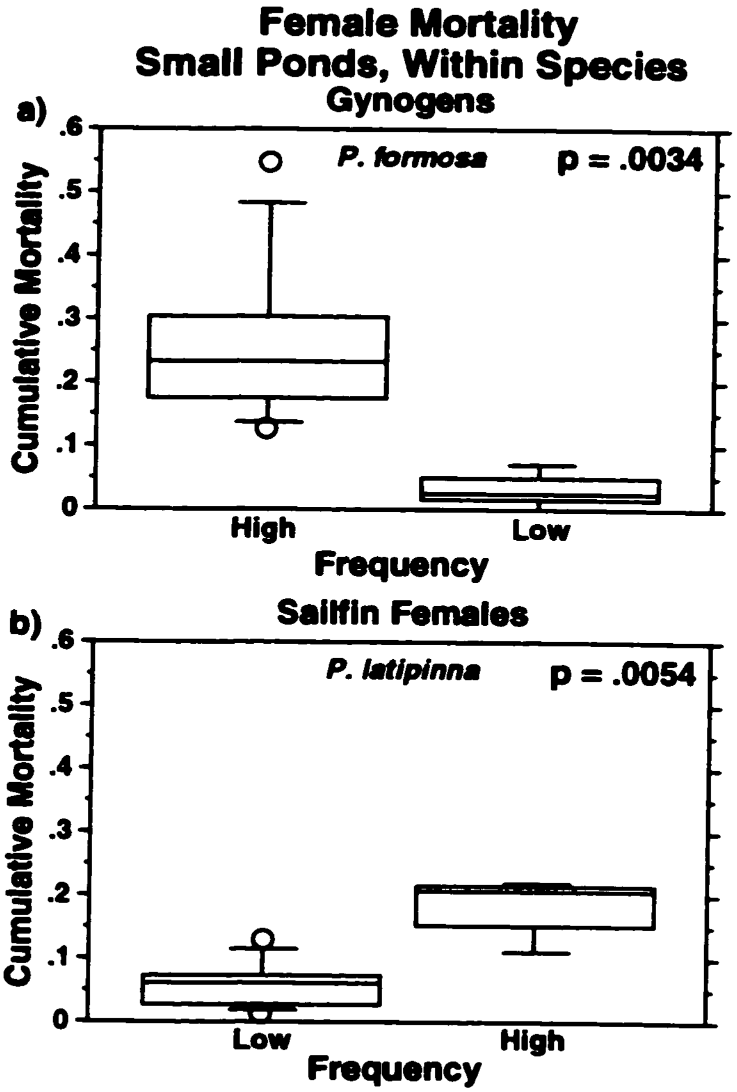
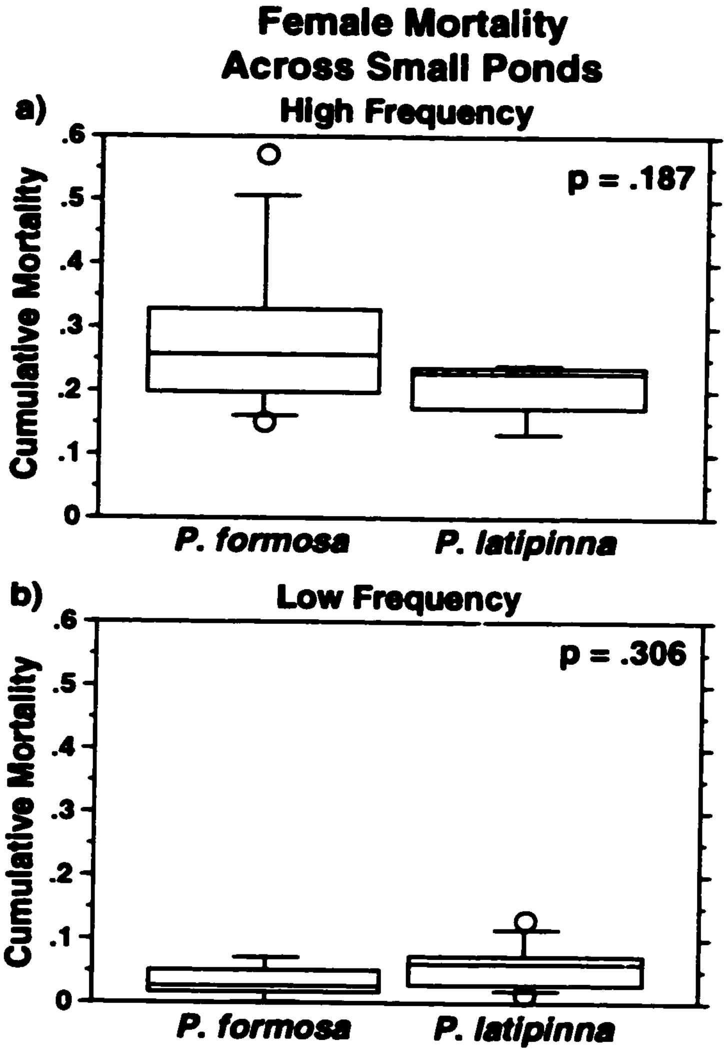

Female Mortality
Small Ponds, Within Species
a)
Gynogens
P. formosa
p = .0034
Cumulative Mortality
.6
.5
.4
.3
.2
.1
0
High
Low
Frequency
b)
Sailfin Females
P. latipinna
p = .0054
Cumulative Mortality
.6
.5
.4
.3
.2
.1
0
Low
High
Frequency

Figure 5.16: Cumulative Mortality of *Poecilia latipinna* and *P. formosa* Females Across Small Ponds

These box plots show the cumulative mortality of *P. formosa* (gynogens) and *P. latipinna* females when both are at high or low frequency prior to the birth of any offspring. *Poecilia formosa* females at high frequency are compared with *P. latipinna* females at high frequency (i.e. from ponds of low frequency *P formosa*). The line within a box represents the median, the box indicates the 25th and 75th percentiles, the whiskers represent the 10th and 90th percentiles, and the open circles represent individual values outside 90% of the distribution. (a) The cumulative mortalities of *P. formosa* and *P. latipinna* females are not significantly different when both are at high frequency. (b) Likewise, the cumulative mortality of *P latipinna* females does not differ significantly from that of *P. formosa* when both are at low frequency. These graphs indicate the difference in mortality with frequency is not species specific.


Female Mortality
Across Small Ponds
a)
High Frequency
Cumulative Mortality
0
.1
.2
.3
.4
.5
.6
p = .187
P. formosa
P. latipinna
b)
Low Frequency
Cumulative Mortality
0
.1
.2
.3
.4
.5
.6
p = .306
P. formosa
P. latipinna

There was a nearly significant difference in cumulative mortality of *P. latipinna* males in ponds of high versus low frequency *P. formosa*; male mortality was greater when *P. latipinna* was at high frequency (U = 7.500, z = ¯1.830, p = 0.066)(Table 5.4, Fig. 5.17). Male mortality was significantly less than *P. latipinna* female mortality when they were both at low frequency (U = 6.500, z =¯2.678, p = 0.0073)(Fig. 5.18a). The same pattern was true when they were both at high frequency U = 0.00, z = ¯2.611, p = 0.009)(Fig. 5.18b). Male mortality was significantly less than *P. formosa* when frequency of *P. formosa* was high (U = 0.00, z = ¯3.361, p = 0.0008)(Fig. 5.19a), but it did not differ from *P. formosa* when frequency of *P. formosa* was low (U = 8.50, z = ¯0.836, p = 0.403)(Fig. 5.19b). It is important to remember that the number of males was one third the number of *P. latipinna* females in both initial conditions. In the ponds where the initial frequency of *P. formosa* was low, the initial number of males equaled the number of *P. formosa*. This may explain why the mortality of these two groups within the low frequency ponds did not differ. In the ponds where the initial frequency of *P. formosa* was high, the initial number of *P. formosa* females was 18 times greater than the number of males (2). This is probably why the difference in mortality was detectable in those ponds but not the ponds of initially low frequency *P. formosa*.

Figure 5.17: Cumulative Mortality of *Poecilia latipinna* Males Across Small Ponds

This box plot shows the cumulative mortality of *P. latipinna* males in ponds of high and low frequency *P. formosa*. The line within a box represents the median, the box indicates the 25th and 75th percentiles, the whiskers represent the 10th and 90th percentiles, and the open circles represent individual values outside 90% of the distribution. Cumulative mortality of *P. latipinna* males is significantly higher when *P. latipinna* is at high frequency: in ponds of low frequency *P. formosa*. This is consistent with the results for *P. latipinna* females.

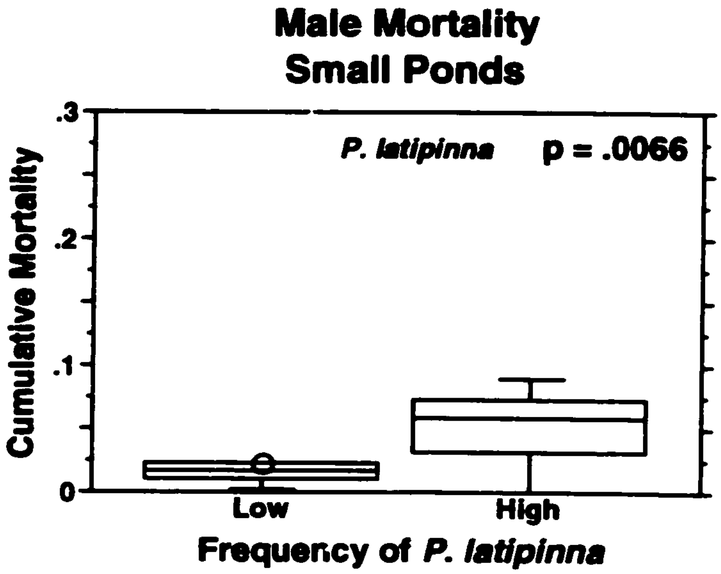
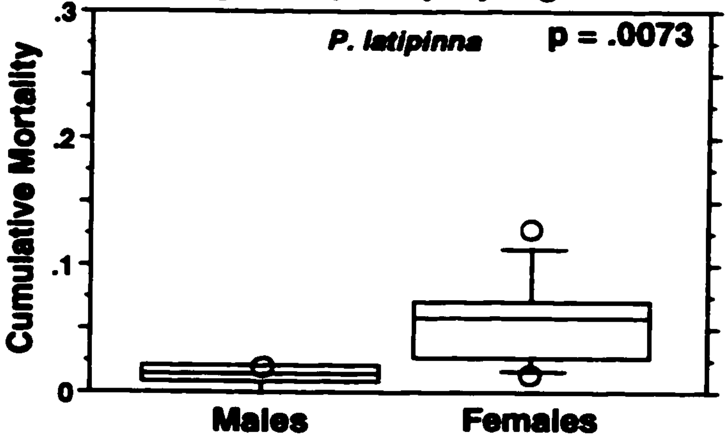

Male Mortality
Small Ponds
P. latipinna p = .0066
Cumulative Mortality
.3
.2
.1
0
Low
High
Frequency of P. latipinna

Figure 5.18: Cumulative Mortality of *Poecilia latipinna* Males and Females Within Small Ponds

This box plot shows the cumulative mortality of *P. latipinna* males and females in ponds of high and low frequency *P. formosa* (gynogens). The line within a box represents the median, the box indicates the 25th and 75th percentiles, the whiskers represent the 10th and 90th percentiles, and the open circles represent individual values outside 90% of the distribution. (a) Cumulative mortality of *P. latipinna* males is significantly lower than *P. latipinna* females when both are at low frequency: in ponds with a high frequency of *P. formosa*. In general there are fewer males than *P. latipinna* females in all ponds. (b) Males also have significantly lower cumulative mortality than *P latipinna* females when both are at high frequency: in ponds with a low frequency of *P. formosa*. Note the greater magnitude of difference in mortality of males and females across ponds.

Adult Mortality
Small Ponds
Males vs. Females

a) High Frequency Gynogens



b) Low Frequency Gynogens

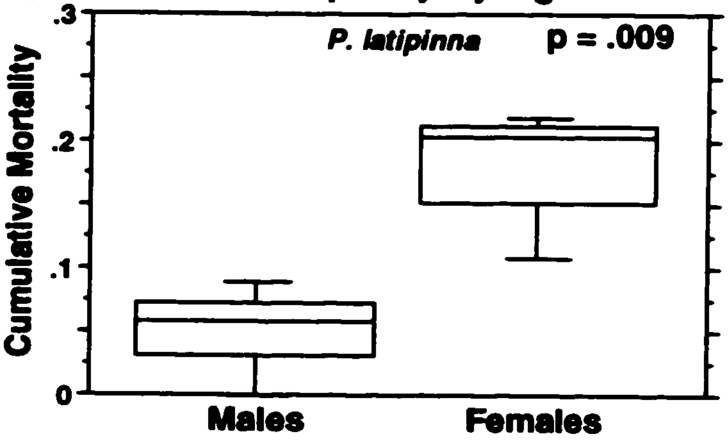
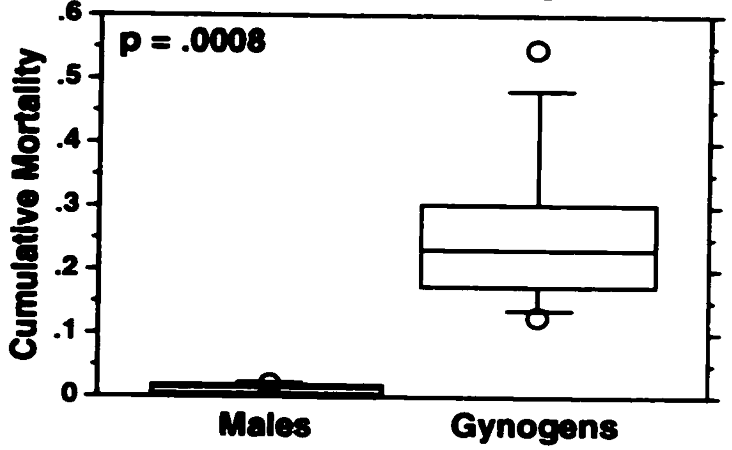


Figure 5.19: Cumulative Mortality of *Poecilia latipinna* Males and *P. formosa* Females Within Small Ponds

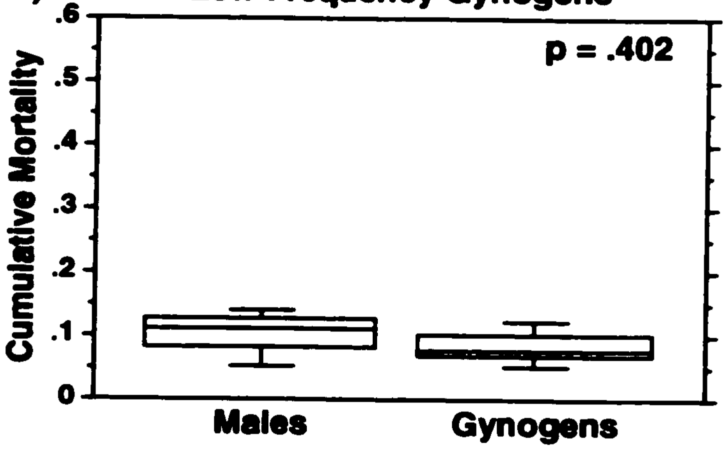
This box plot shows the cumulative mortality of *P. latipinna* males and *P. formosa* females within ponds of high and low frequency *P. formosa* (gynogens). The line within a box represents the median, the box indicates the 25th and 75th percentiles, the whiskers represent the 10th and 90th percentiles, and the open circles represent individual values outside 90% of the distribution. (a) Males have significantly lower cumulative mortality than *P. formosa* when *P. formosa* is at high frequency. This is similar to the effect of frequency on *P. latipinna* females relative to *P. formosa* females in the same ponds. (b) When *P. formosa* is at low frequency, and *P. latipinna* is at high frequency, cumulative mortality of males does not differ significantly from *P. formosa* females. This result differs from the comparison of *P. latipinna* females and *P. formosa* in the same ponds (Fig. 5.15b) and may be due to the small number of males in a pond even when *P. latipinna* is at high frequency.

Adult Mortality
Small Ponds
Males vs. Gynogens

a) High Frequency Gynogens



b) Low Frequency Gynogens



Seasonal effects

Season did not have a significant effect on the total number of adults in small ponds ($F_{3,82}$ = 0.381, p = 0.767)(Table 5.5, Fig. 5.20), nor did the interaction between initial frequency and season ($F_{3,82}$ = 0.941, p = 0.425). The number of adult *P. formosa* did not differ across seasons ($F_{3,82}$ = 1.121, p = 0.345)(Fig. 5.20a), nor did the number of adult *P. latipinna* females and males (females: $F_{3,82}$ = 0.499, p = 0.684; males: $F_{3,82}$ = 1.600, p = 0.196)(Fig. 5.20b,c). The number of *P. latipinna* adults was greater in ponds with a low frequency of *P. formosa* as would be expected; there were more *P. latipinna* adults when they were are higher frequencies.

The total number of juveniles was significantly less in the spring ($F_{3,82}$ = 6.476, p = 0.0005)(Table 5.5). There were significantly fewer *P. formosa* juveniles ($F_{3,82}$ = 6.538, p = 0.0005)(Fig. 5.21a) and *P. latipinna* juveniles ($F_{3,82}$ = 3.344, p = 0.0231)(Fig. 5.212b) in the early spring months of March, April, and May. The effect of season on number of fry was not significant ($F_{3,82}$ = 1.129, p = 0.342).

Table 5.5 Seasonal Effects in Experimental Ponds – Numbers of Fishes

Descriptive statistics for the numbers of adults, juveniles, and fry of *P. latipinna* and *P. formosa* in each of four seasons are summarized in the table below. The mean plus or minus the standard deviation (mean±SD) and the standard error (SE) are listed separately and the relative frequencies of adult and juvenile *P. formosa* are included (RF). Spring includes March, April and May. Summer includes June, July, and August. Fall includes, September. October, and November. Winter includes December, January, and February.

Table 5.5 **Seasonal Effects in Experimental Ponds – Numbers of Fishes**

Species	**Spring**		**Summer**		**Fall**		**Winter**	
Adults:	mean±SD	SE	mean±SD	SE	mean±SD	SE	mean±SD	SE
P. latipinna								
males	4.7±4.1	0.8	3.4±2.9	0.58	6.7±9.0	1.9	9.3±11.9	4.2
females	6.9±7.7	1.5	5.5±4.2	0.8	4.5±4.1	0.9	8.0±7.6	2.7
P. formosa								
females	12.1±8.9	1.7	10.8±7.7	1.4	11.2±7.7	1.6	8.4±5.9	2.1
RF	0.55±0.25	0.1	0.53±0.23	0.1	0.52±0.21	0.1	0.36±0.27	0.1
Juveniles								
P. latipinna								
juveniles	5.0±7.9	1.5	21.2±27.8	5.1	22.2±24.8	5.1	15.3±17.1	6.1
P. formosa								
juveniles	6.1±10.1	1.9	14.9±17.3	3.2	28.1±24.4	4.9	19.7±17.3	6.1
RF	0.37±0.41	0.1	0.35±0.36	0.1	0.58±0.30	0.1	0.57±0.40	0.1
Fry								
number	13.1±21.8	4.1	21.7±34.3	6.3	30.3±53.8	11	1.88±3.5	1.2

Figure 5.20: **Seasonal Effects on Number of Adult Fishes in Small Ponds**

Box plots represent the numbers of *P. formosa* and *P. latipinna* adults in ponds of initially high and low frequency *P. formosa* (gynogens) during four seasons. The line within a box represents the median, the box indicates the 25th and 75th percentiles, the whiskers represent the 10th and 90th percentiles, and the open circles represent individual values outside 90% of the distribution. The open boxes represent ponds of initially high frequency *P. formosa*; the shaded boxes represent ponds of initially low frequency *P. formosa*. Spring represents the months of March through May, Summer the months of June through July, Fall the months of September through October, and Winter the months of December through February. These classifications correspond with the onset and cessation of the breeding season. (a) The number of *P. formosa* adults was significantly lower in low frequency ponds during the winter. (b) There were no significant differences in the number of *P. latipinna* females between the seasons or within a season between high and low frequency ponds. (c) There also were no significant differences in the number of *P. latipinna* males across all seasons and ponds.

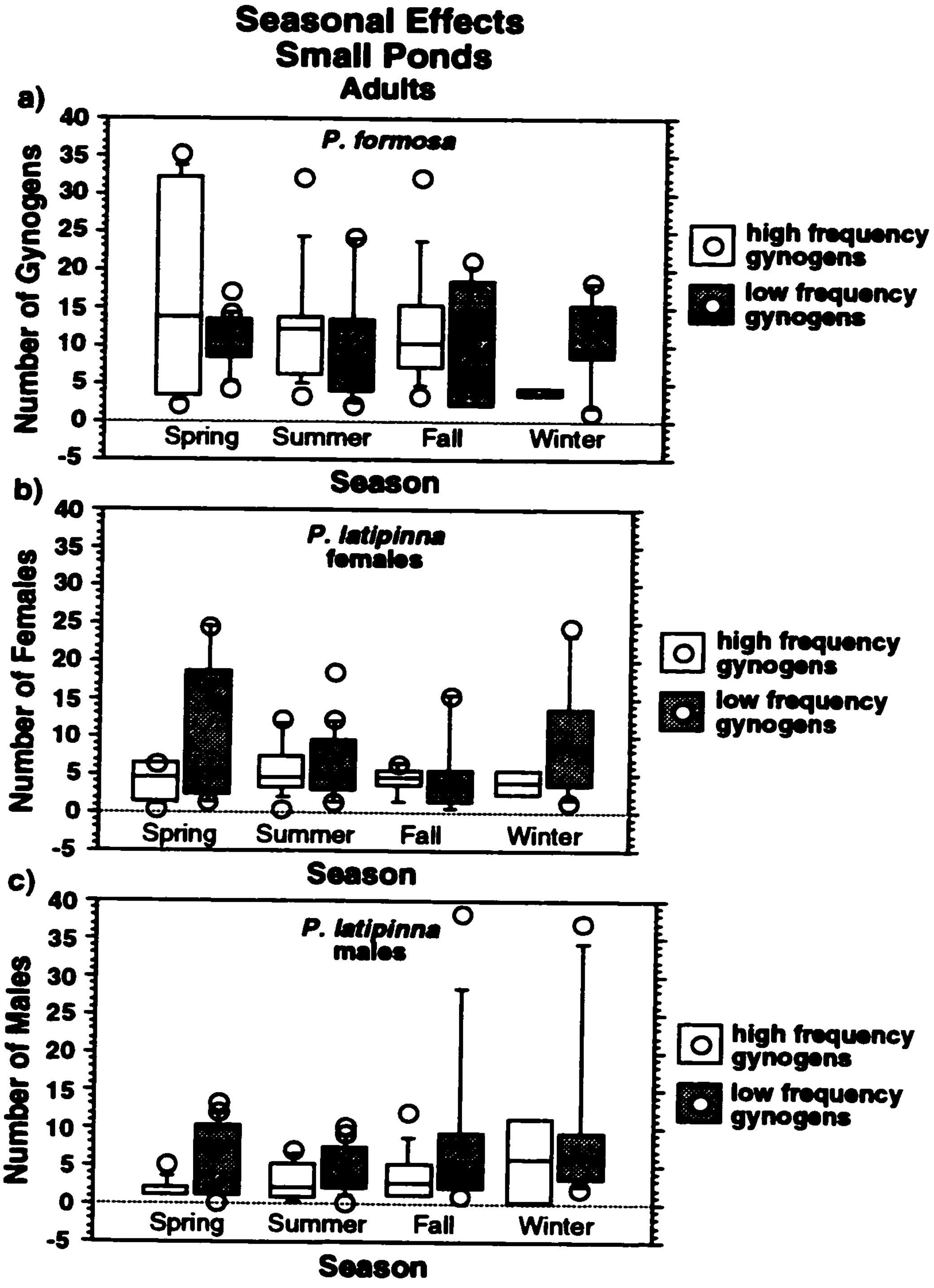
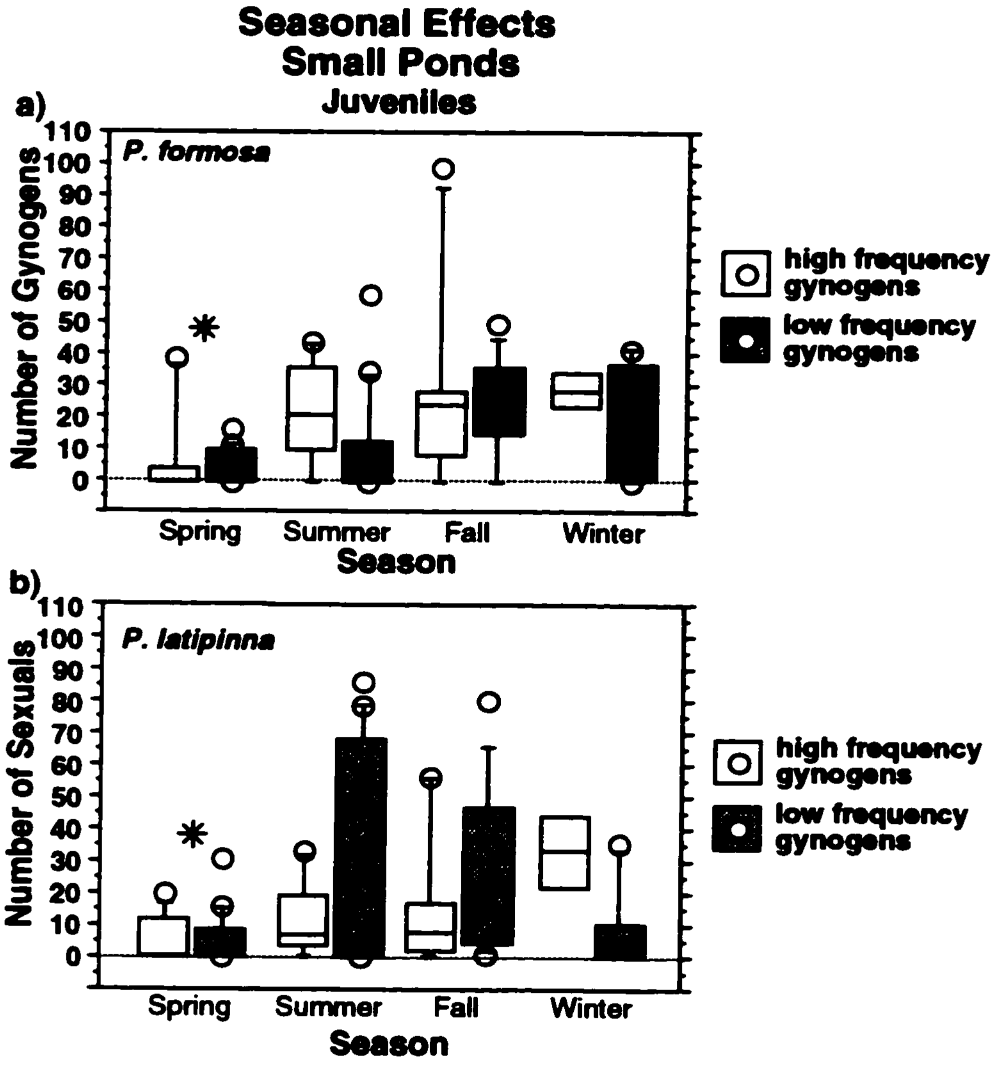

Seasonal Effects
Small Ponds
Adults
a)
P. formosa
Number of Gynogens
40
35
30
25
20
15
10
5
0
-5
Spring
Summer
Fall
Winter
Season
high frequency gynogens
low frequency gynogens
b)
P. latipinna females
Number of Females
40
35
30
25
20
15
10
5
0
-5
Spring
Summer
Fall
Winter
Season
high frequency gynogens
low frequency gynogens
c)
P. latipinna males
Number of Males
40
35
30
25
20
15
10
5
0
-5
Spring
Summer
Fall
Winter
Season
high frequency gynogens
low frequency gynogens

Figure 5.21: **Seasonal Effects on Number of Juvenile Fishes in Small Ponds**

Box plots represent the numbers of *P. formosa* and *P. latipinna* juveniles in ponds of initially high and low frequency *P. formosa* (gynogens) during four seasons. The line within a box represents the median, the box indicates the 25th and 75th percentiles, the whiskers represent the 10th and 90th percentiles, and the open circles represent individual values outside 90% of the distribution. An asterisk (*) indicates a statistically significant difference. The open boxes represent ponds of initially high frequency *P. formosa*; the shaded boxes represent ponds of initially low frequency *P. formosa*. Spring represents the months of March through May, Summer the months of June through July, Fall the months of September through October, and Winter the months of December through February. These classifications correspond with the onset and cessation of the breeding season. (a) There were significantly fewer *P. formosa* juveniles overall during the spring, but no significant differences between high and low frequency ponds during any season. (b) There also were significantly fewer *P. latipinna* juveniles during the spring and no significant differences between high and low frequency ponds. The smaller number of juveniles of both species during the spring reflects juvenile mortality and recruitment over the winter.


Seasonal Effects
Small Ponds
a)
Juveniles
P. formosa
Number of Gynogens
110
100
90
80
70
60
50
40
30
20
10
0
*
Spring
Summer
Fall
Winter
Season
high frequency gynogens
low frequency gynogens
b)
P. latipinna
Number of Sexuals
110
100
90
80
70
60
50
40
30
20
10
0
*
Spring
Summer
Fall
Winter
Season
high frequency gynogens
low frequency gynogens

The interaction between season and initial frequency of *P. formosa* adults had a significant effect on the relative frequency of *P. formosa* juveniles ($F_{3,82}$ = 2.949, p = 0.0376), but neither alone had a significant effect (season: $F_{3,82}$ = 2.208, p = 0.0933; initial frequency: $F_{3,82}$ = 0.016, p = 0.898). Consideration of season and initial frequency suggests season exerts the primary influence on relative frequency of *P. formosa* juveniles, but this influence is weak. Although the relative frequency of *P. formosa* adults changed through time, season did not have a significant effect ($F_{3,82}$ = 1.760, p = 0.161) and there was no significant interaction between season and initial frequency of *P. formosa* ($F_{3,82}$ = 0.704, p = 0.553).

Large Ponds

Observations

A large snapping turtle, *Chelydra serpentina*, was removed from pond 3 after draining and prior to refilling with water and introducing fish. In February of 1998 a large snapping turtle was observed at the surface of pond 3 and a similar turtle was observed in pond 4. Although *C. serpentina* is a known fish predator, I did not drain the ponds and start the experiments over for two reasons. One, it would have required additional field collections of adult fish which would have delayed the project at least one year. Two, since the turtle was seen in both ponds, I assumed its effect was equal in both ponds. If the turtle had a preference for one species I assumed this preference was constant with respect to pond. No snapping turtles were observed over the remaining course of the experiment.

Over the winter of 1997 pond 3 sustained heavy casualties, the number of fish obtained in the March 18, 1998 sample was only 12, the relative frequency of *P. formosa* was 0.10, and there were no males. On April 21, 1998, I added 2 *P. formosa* adult females and 3 *P. latipinna* adult males to maintain the observed relative frequency of 0.10 but also to ensure there were males in the pond. However, it is probably best to consider pond 3 a second replicate of the low frequency experimental condition. An initially low frequency of *P. formosa* is analogous to the conditions faced by a gynogenetic lineage at its inception or when it invades a new population of its sexual host. The results of the large ponds are applicable to conditions commonly faced by gynogenetic lineages when colonizing a new population of *P. latipinna.*

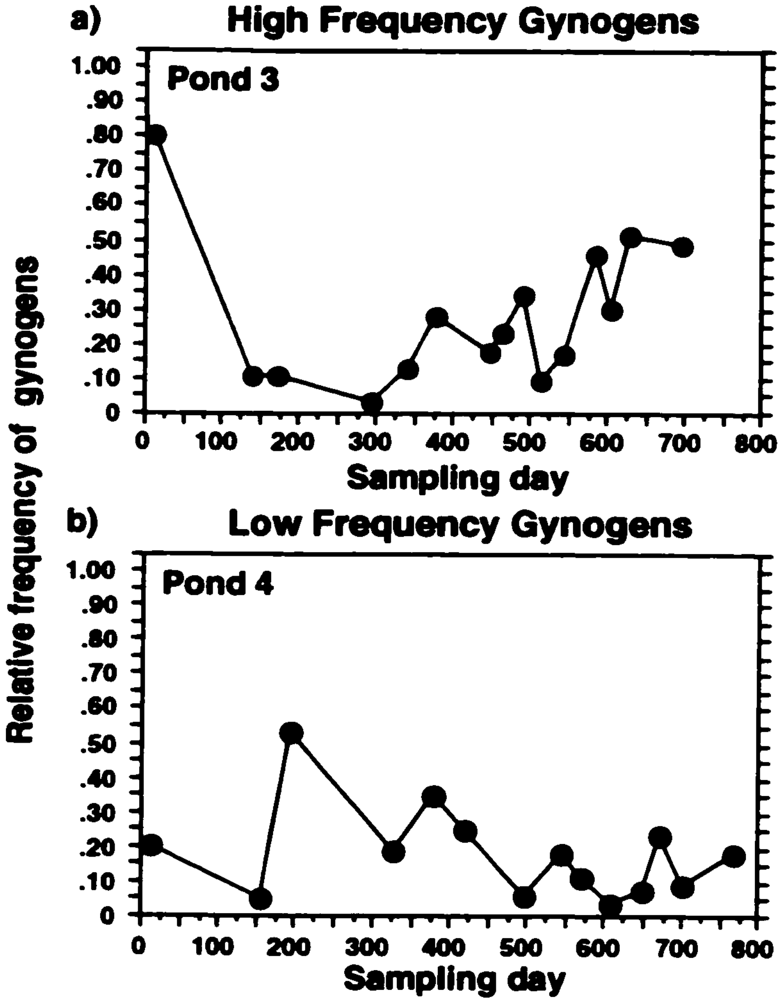
Relative Frequencies

The relative frequency of adult *P. formosa* in both large ponds follow the same general trends seen in the small ponds, relative frequency alternately increases and decreases through time (Fig. 5.22). In the pond of initially high frequency of *P. formosa,* relative frequency initially dropped dramatically and remained low for nearly one year (October 1997 until September 1998). In October 1998, the relative frequency of *P. formosa* began a general increase (Fig. 5.22a). The change in relative frequency of *P. formosa* in the pond of initially low frequency shows a more oscillatory nature but there does not appear to be a trend in a particular direction (Fig. 5.22b). These graphs suggest frequency may oscillate around an equilibrium value perhaps near 0.20.

Figure 5.22: Relative Frequency of Adult *Poecilia formosa* in Large Ponds

This figure shows the changes in relative frequency of adult *Poecilia formosa* (gynogens) in large experimental ponds. The *x*-axis indicates the sampling day; the -axis indicates the sampling day; the *y*-axis represents the frequency of *P. formosa* adults (gynogens) relative to *P. latipinna* adults. Under both initial conditions, relative frequency exhibits some oscillation around a general trend. (a) The relative frequency of *P. formosa* in pond 3 decreased dramatically over the first winter (see text). After this decrease, the relative frequency alternately increased and decreased while following a general trend of increasing over time. (b) In pond 4 the frequency of *P. formosa* also dropped initially, followed by oscillatory changes. There does not appear to be a general increasing trend in the pond. The final frequency (0.18) was nearly equal to the initial frequency (0.20).

Large Ponds
Adults



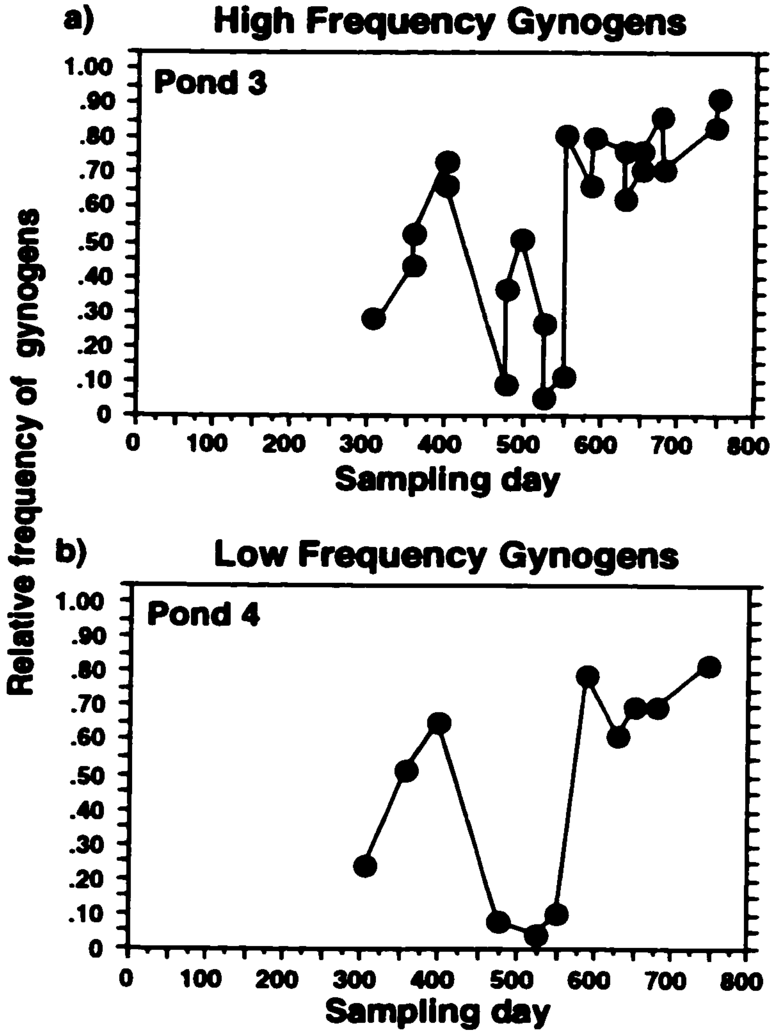
The changes in relative frequency of juvenile *P. formosa* are more extreme than the changes in adult frequency. In both ponds the initial frequency of *P. formosa* juveniles was near 0.20, followed by a short-term increase and oscillation (Fig. 5.23). In the last 300 days of the study the general trend was of increasing relative frequency of juvenile *P. formosa* in both ponds.

Visual inspection of the relative frequency of juveniles and adults in both ponds also reveals a pattern consistent with an effect of adult frequency on juvenile frequency. In general, when the relative frequency of *P. formosa* is high among the adults, it is low among their juvenile offspring, and when the frequency is low among the adults, it is high among the juveniles (Fig. 5.24).

Figure 5.23: Relative Frequency of Juvenile *Poecilia formosa* in Large Ponds

This figure shows the changes in relative frequency of juvenile *Poecilia formosa* (gynogens) in large experimental ponds. The *x*-axis indicates the sampling day; the -axis indicates the sampling day; the *y*-axis represents the frequency of *P. formosa* juveniles (gynogens) relative to *P. latipinna* juveniles. Under both initial conditions, relative frequency exhibits some oscillation around a general trend. (a) In pond 3, the frequency of *P. formosa* juveniles increased initially (to 0.76), then decreased dramatically (0.09). Subsequently there were three large changes in frequency, followed by oscillation around a generally high frequency. (b) The frequency of juvenile *P. formosa* in pond 4 also exhibited large increases and decreases until it reached a high value (0.80). Thereafter frequency showed a slight general increase.

Large Ponds
Juveniles



a) High Frequency Gynogens

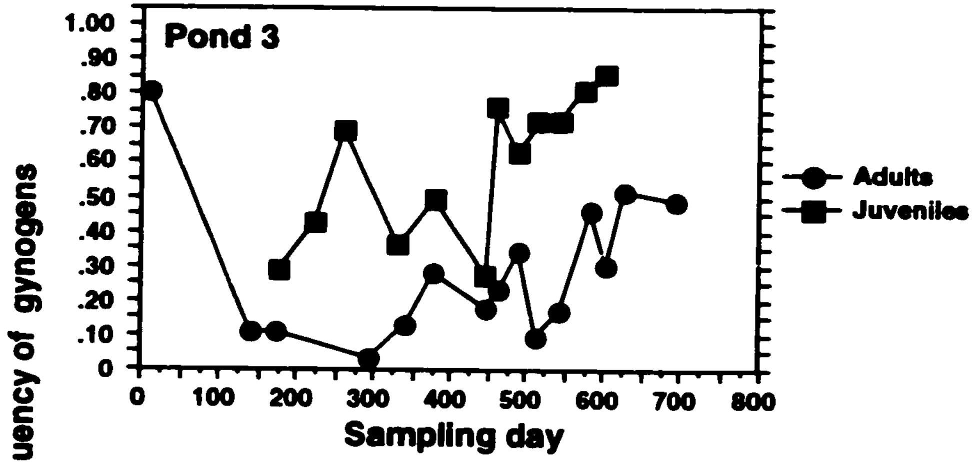
b) Low Frequency Gynogens

Figure 5.24: Relative Frequencies of Juvenile *Poecilia formosa* and Their Mothers in Large Ponds

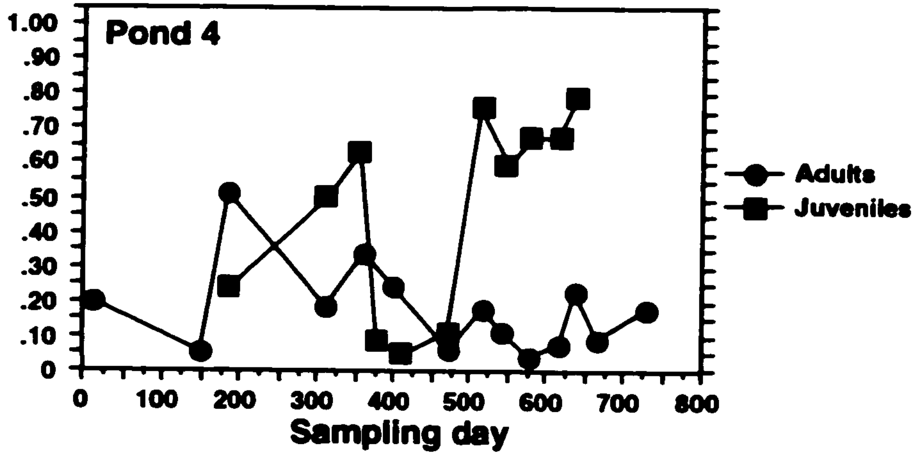
The frequencies of juvenile *P. formosa* (gynogens) are plotted directly above the frequencies of their mothers for the large ponds. Sampling day is a reference for adult frequency; sampling day of juvenile frequency is an additional 90 days later. If reproductive success were negatively frequency-dependent, the frequency of juveniles would be high when the frequency of their mothers is low. (a) In pond 3 the frequency of juvenile *P. formosa* was always greater than the frequency of adult *P. formosa*. The higher juvenile frequencies correspond with lower frequencies of their mothers and the oscillatory nature of the changes is apparent. (b) In pond 4 the frequency of juvenile *P. formosa* was lower when the frequency of their mothers was higher and vice versa. The difference between juvenile and adult frequency is most extreme in that last year of the study when adult frequency was 0.20 or lower.

Large Ponds
Adults & Juveniles

a) High Frequency Gynogens



b) Low Frequency Gynogens



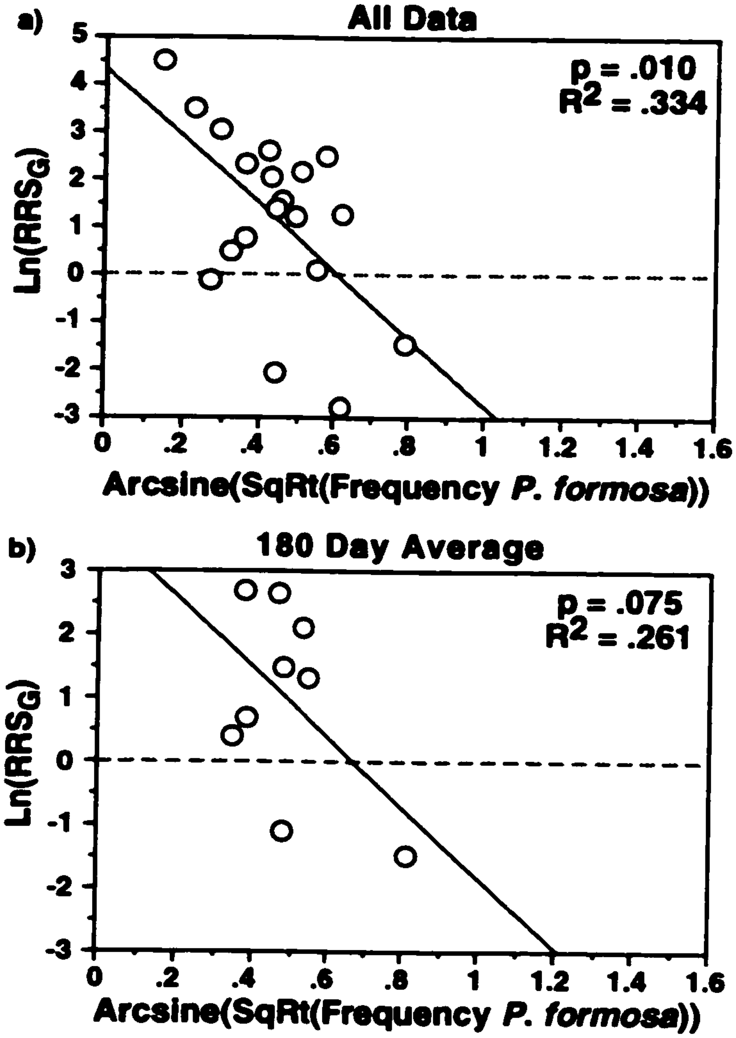
Relative Reproductive Success

The natural log of relative reproductive success (RRS_G) is plotted as a function of the arcsine of the square root of relative frequency of ***P. formosa*** (Fig. 5.25a). The raw values for reproductive success and relative frequency were transformed to remove correlation of the residual error values. A relative frequency of 1.0 corresponds with the transformed value of 1.6. Values of Ln(RRS_G) greater than zero correspond with relative reproductive success greater than 1, values less than zero, correspond with relative reproductive success less than 1. The reproductive success of ***P. formosa*** females relative to ***P. latipinna*** females decreases with increasing frequency of ***P. formosa*** in the population and the negative slope of the regression line is significantly different than zero ($F_{1,17}$ = 8.519, p = 0.010, R^2 = 0.344), and explains one third of the variance. The expected reproductive advantage of ***P. formosa*** adults in the absence of frequency-dependence is 2 gynogenetic females for every 1 sexual female. Since I counted juveniles without respect to gender and assumed the sex ratio of ***P. latipinna*** at birth of 1:1, the expected relative reproductive output is 1 gynogenetic juvenile for each sexual juvenile. This line is shown on the graphs.

Figure 5.25: Relative Reproductive Success of *Poecilia formosa* in Large Ponds

The relative frequency of adult *P. formosa* is plotted against their relative reproductive success (*RRS_G*) for both large ponds. The relative frequency data were (arcsine(square root)) transformed; values of 0 and 1.6 correspond with frequencies of zero and 1.0, respectively. Relative reproductive success was transformed using the natural logarithm of the number of offspring per *P. formosa* divided by the number of offspring per *P. latipinna* female. The dashed line represents the expected value if there is no relationship between frequency and reproductive success (ln (1) = 0). The expected relationship under negative frequency-dependence is a line of negative slope (Fig. 5.1). The vertical dotted line near zero is an asymptote representing no reproductive success when there are no *P. formosa* in the population (frequency = 0). (a) This graph shows the statistically significant relationship between frequency of adult *P. formosa* and their relative reproductive success. As relative frequency of adult *P. formosa* increases, their relative reproductive success decreases. The regression line explains 1/3rd of the variance in this relationship. (b) This graph shows the results of linear regression analysis on average relative reproductive success over 180-day time intervals. The averages eliminate serial dependence of data within a pond. The slope of the regression line is negative but its significance is marginal, and the variance explained by the relationship is slightly less than in a) above. This may be partially due to the small number of data points (N = 8).

Relative Reproductive Success
Large Ponds



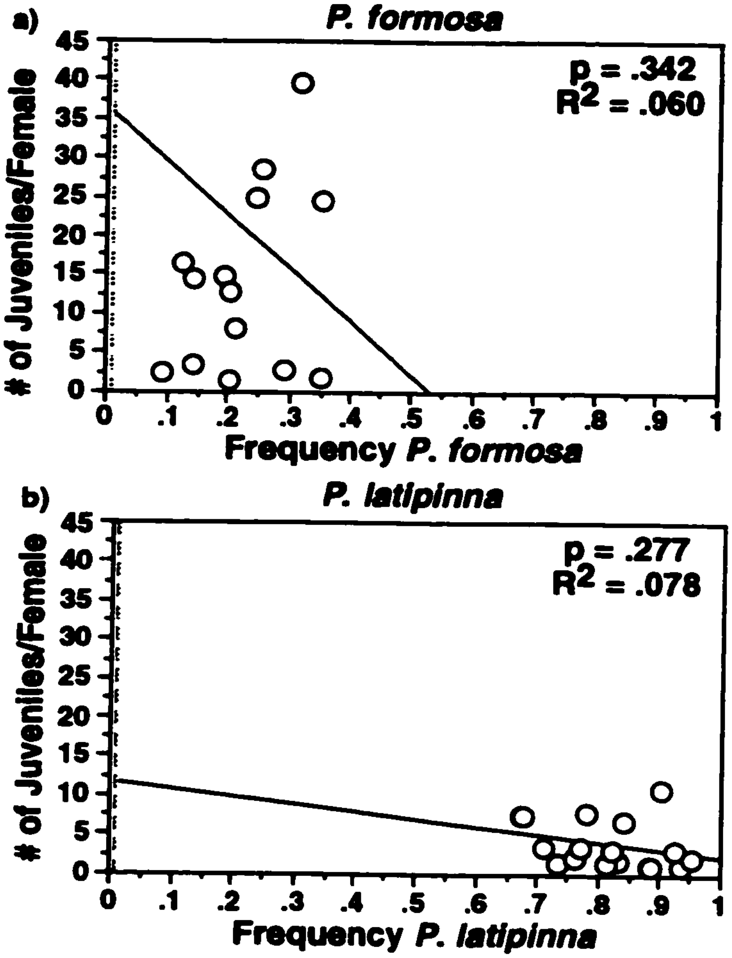
Serial Correlation Tests did not indicate significant correlation between error values and autocorrelation analysis suggested a weak correlation between raw values at a lag of 1 (Table 5.3). This indicated two successive data points might not be entirely independent, so data points within a 180-day period were averaged. This reduced the number of data points considerably but still resulted in a negative relationship between relative reproductive success of *P. formosa* and their frequency in the population (Fig. 5.25b). The slope of the regression is nearly significant ($F_{1,7} = 2.470$, $p = 0.075$, $R^2 = 0.261$) and explains a reasonable portion of the variance. The magnitude of the loss of significance is small, given the data were reduced by one half. Additional data at future intervals of 180 days are likely to confirm the significant effect of frequency on relative reproductive success.

The raw numbers of offspring born to *P. formosa* appear to be influenced by relative frequency of their mothers ($F_{1,,15} = 0.963$, $p = 0.342$, $R^2 = 0.060$)(Fig. 5.26a). Although there is a negative relationship, it is not significant. The relationship between numbers of offspring born to *P. latipinna* mothers and the frequency of *P. latipinna* in the population is also negative and not significant ($F_{1,15} = 1.275$, $p = 0.277$, $R^2 = 0.078$)(Fig. 5.26b).

Figure 5.26: Reproductive Success of *Poecilia formosa* and *P. latipinna* females

These graphs show the relationship between number of juveniles per female and relative frequency of *P. formosa* and *P. latipinna*. Gender of juveniles was not determined. (a) The tendency for *P. formosa* females to have fewer offspring as their frequency in the population increases is shown here although the relationship is not significant. (b) The relative frequency of *P. latipinna* does not appear to have a significant effect on the number of juveniles per female. These graphs only represent one end of the range of possible frequencies for both species and may not accurately reflect the relationship between number of offspring per female and relative frequency.

Female Reproductive Success
Large Ponds

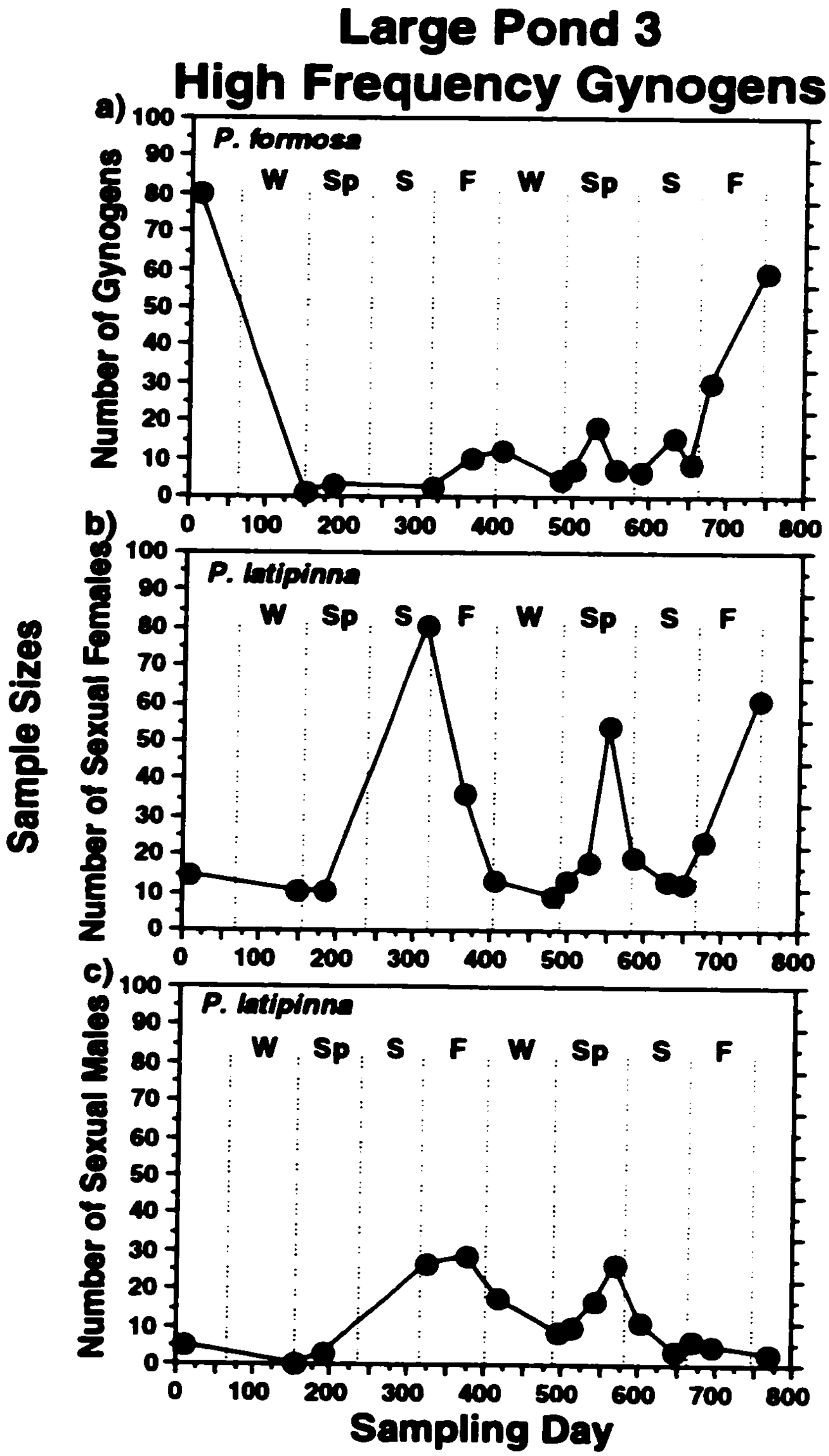


Population Size, Mortality, and Seasonal effects

I did not determine the actual population size after initiation of the large pond experiments. However, the initial population size was 100 fish and the total number of fish in my samples exceeded 200 within 300 days (6 months) and reached 1000 after 600 days (23 months). The number of fish in the samples from pond 3 indicate expected increases in the number of *P. latipinna* males and females in April (approx. sample day 275), but no corresponding increase in *P. formosa* females (Fig. 5.27). In pond 4 there was a slight increase in average numbers of *P. latipinna* males and *P. formosa* females and a much more dramatic increase in *P. latipinna* females (Fig. 5.28). I did not attempt to assess seasonal effects on population size or mortality because I did not completely sample these ponds and no individuals were marked.

Figure 5.27: Numbers of *Poecilia latipinna* and *P. formosa* Adults in Samples From Large Pond 3

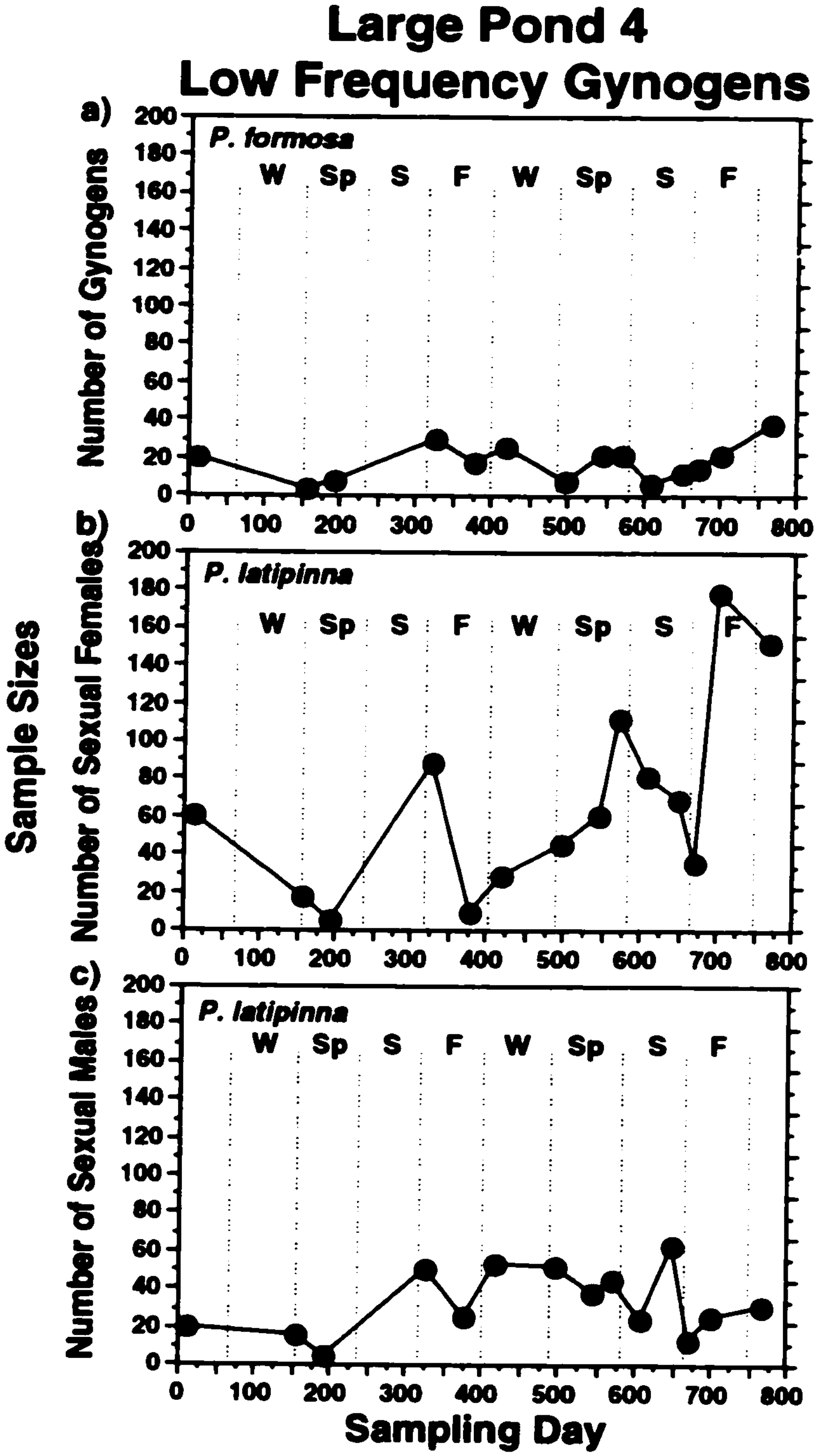
The numbers of *P. formosa* and *P. latipinna* males and females in the samples taken from the large pond of initially high frequency *P. formosa* (gynogens) are shown. (a) The number of *P. formosa* adult females decreased dramatically over the first winter of the study and slowly increased. From the summer of 1999 (sampling day 600) through the early months of winter the increases were larger than during all of the previous months. (b) The number of *P. latipinna* females alternated between large increases and decreases. The increases coincided with spring and summer of 1998 and 1999, and with fall of 1999. Ultimately the number of *P. latipinna* females in the final sample was nearly equal to the number of *P. formosa* females. (c) The number of *P. latipinna* males initially decreased, then alternated between periods of increase and decrease. The increases coincided with summer and fall of 1998, and spring of 1999.



Large Pond 3
High Frequency Gynogens

Figure 5.28: Numbers of *Poecilia latipinna* and *P. formosa* Adults in Samples From Large Pond 4

The numbers of *P. formosa* and *P. latipinna* males and females in the samples taken from the large pond of initially low frequency *P. formosa* (gynogens) are shown. (a) The number of *P. formosa* adult females oscillated around 20 individuals throughout the study. Increases coincided with spring and summer of both years, and included early fall in 1999. (b) The number of *P. latipinna* increased in general over the two years but alternated between increases and decreases annually. The increases were seasonal as for *P. formosa*; they occurred during the spring, summer and early fall. In both large ponds the number of *P. latipinna* females in the final sample was much large than the number in the initial sample. (c) The number of *P. latipinna* males initially decreased slightly, then alternated between periods of increase and decrease that coincided with the seasons. The decreases occurred during the winter months and increases occurred during the rest of the year. Both large ponds show the same seasonal pattern in number of adults in samples.


Large Pond 4
Low Frequency Gynogens
Sample Sizes
a)
Number of Gynogens
P. formosa
W
Sp
S
F
W
Sp
S
F
0
20
40
60
80
100
120
140
160
180
200
0
100
200
300
400
500
600
700
800
b)
Number of Sexual Females
P. latipinna
W
Sp
S
F
W
Sp
S
F
c)
Number of Sexual Males
P. latipinna
W
Sp
S
F
W
Sp
S
F
Sampling Day

Sex Ratio

The ponds were stocked with *P. latipinna* in a sex ratio of 3:1 females to males because it is a common ratio in natural populations. I calculated the sex ratio of *P. latipinna* females and males, and the sex ratio including *P. formosa* females (total sex ratio = *P. latipinna* females + *P. formosa* females/males)(Table 5.6). Both sex ratios varied over the course of the experiment in both ponds (Fig. 5.29) and there was no significant difference in either sex ratio between the two ponds (*P. latipinna*: $U = 76.50$, $z = {}^{-}1.244$, $p = 0.214$; Total: $U = 77.00$, $z = {}^{-}1.222$, $p = 0.223$). There was no significant relationship between relative frequency of adult *P. formosa* and sex ratio (within *P. latipinna*: $F_{1,25} = 0.011$, $p = 0.917$; total: $F_{1,25} = 2.453$, $p = 0.129$).

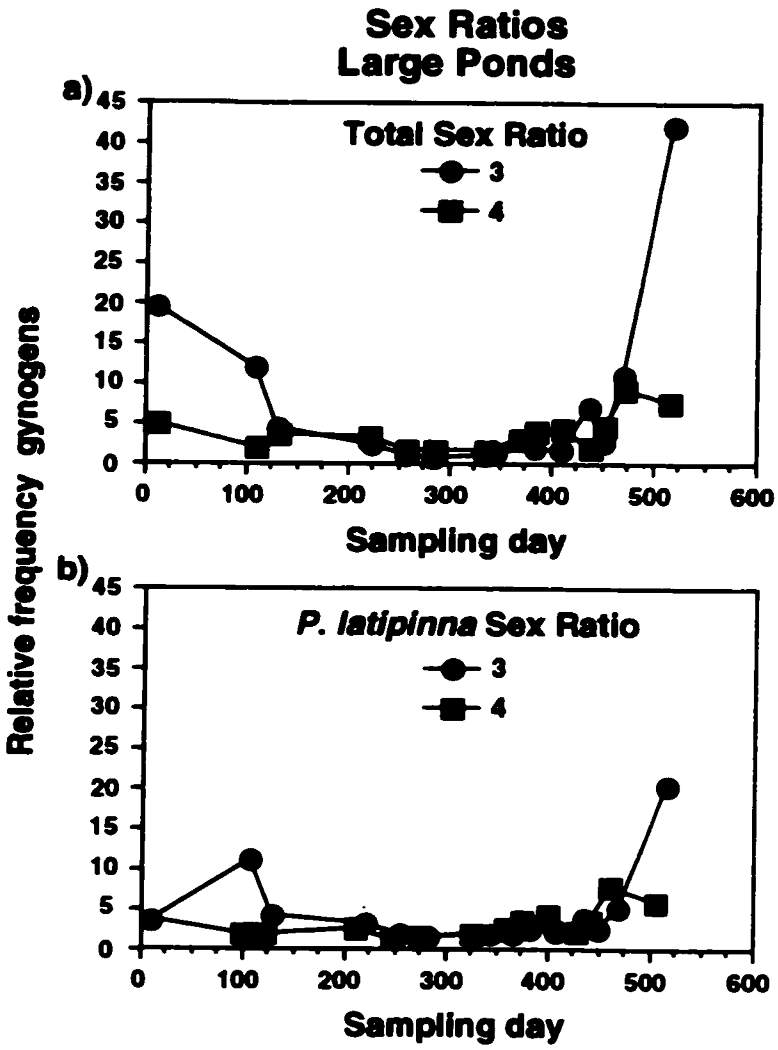
Table 5.6 Sex Ratios of *Poecilia latipinna* and *P. formosa* in Large Ponds

This table provides descriptive statistics of the sex ratios in large ponds 3 and 4. Overall sex ratio is the number of all females (*P. latipinna* females + the number of *P. formosa* females) per male. Sex ratio within the sexual species is the number of *P. latipinna* females per male. The arithmetic average (Mean), standard deviation (S.D.), standard error of the mean (S.E.), minimum (Min.) and maximum (Max.) are listed below for each pond.

	Pond 3	**Pond 4**
Sex Ratio:		
Overall		
Mean	7.5:1	3.0:1
S.D.	10.4:1	2.1:1
S.E.	2.7:1	0.6:1
Min.	1.3:1	1.0:1
Max.	40.3:1	8.0:1
Sexuals		
Mean	4.0:1	2.3:1
S.D.	5.3:1	1.9:1
S.E.	1.4:1	0.5:1
Min.	0.7:1	0.4:1
Max.	20.7:1	7.1:1

Figure 5.29: Sex Ratios in Large Ponds

The sex ratios in samples taken from both ponds throughout the study are shown in these graphs. Shaded circles represent pond 3; black boxes represent pond 4. The initial data points represent the sex ratio chosen for the initial conditions of the study. (a) This graph shows the change in overall sex ratio: the number of *P. latipinna* females + the number of *P. formosa* females per male. If all else were equal, one would expect there to be 2 females (1 *P. latipinna* and 1 *P. formosa*) for every male. The sex ratios typical of natural populations vary from 3:1 to 0.05:1. The average overall sex ratios for ponds 3 & 4 were 7.5:1 and 3.0:1, respectively. In general the overall sex ratio was consistently below 5:1. (b) This graph shows the change in sex ratio within *P. latipinna*: the number of *P. latipinna* females per *P. latipinna* male. The ratios again were consistently below 5:1 and in general were slightly lower than the overall sex ratios. The average sex ratios in ponds 3 & 4 were 4.0:1 and 2.3:1, respectively.

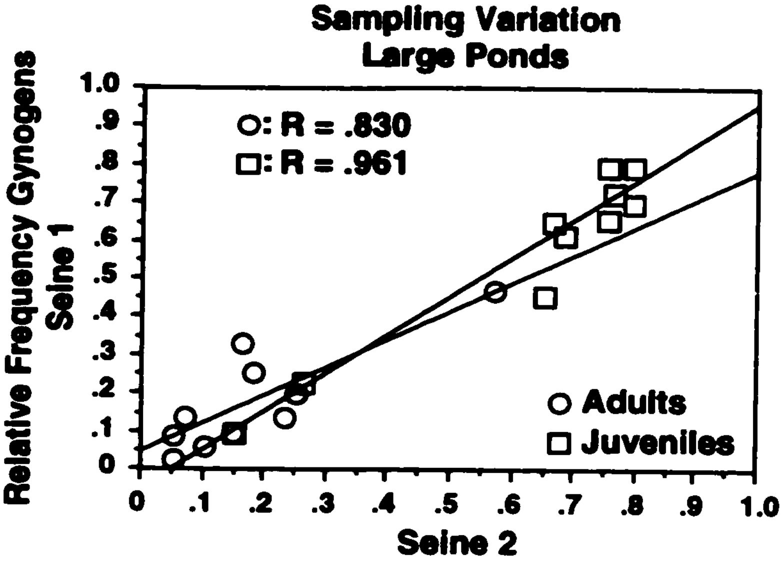

Sex Ratios
Large Ponds
a)
Total Sex Ratio
3
4
Relative frequency gynogens
0
5
10
15
20
25
30
35
40
45
0
100
200
300
400
500
600
Sampling day
b)
P. latipinna Sex Ratio
3
4
0
5
10
15
20
25
30
35
40
45
0
100
200
300
400
500
600
Sampling day

Sampling Variation

A sample from each large pond consisted of all of the fish obtained in two seine hauls unless the sample size was less than 200. In this case a third seine haul was used to obtain the fish. I evaluated whether the relative frequency of adult and juvenile *P. formosa* differed between seine hauls by calculating the correlation between seine haul one with seine haul two (Fig. 5.30). There is a significant positive correlation between the frequencies in seine haul one and those in seine haul two for both adult and juvenile *P. formosa*, and the correlation coefficients are very large and positive.

Figure 5.30: Variation between Sampling Attempts

This graph shows the relationship between the number of adult and juvenile fishes captured in the first and second seine haul on each sampling day. Seine haul 2 is represented on the x-axis; seine haul 1 is represented on the *y*-axis. The correlation values for both adults and juveniles indicate there were no drastic differences in the numbers of fishes between seining attempts. This strongly suggests no particular group of fish was represented more heavily in one seine or the other.


Sampling Variation
Large Ponds
○: R = .830
□: R = .961
○ Adults
□ Juveniles
Relative Frequency Gynogens
Seine 1
Seine 2
0
.1
.2
.3
.4
.5
.6
.7
.8
.9
1.0

DISCUSSION

Aquaria

The effect of relative frequency of *P. formosa* on reproductive success was not revealed by this study because little reproduction occurred. Very few offspring were born, all were *P. latipinna*, and none of the dead females were carrying embryos. This indicates a nearly complete absence of mating behavior. The mortality rates of *P. formosa* and *P. latipinna* females and males were consistent with their proportions in the aquarium populations suggesting mortality affected all fishes equally regardless of sex or species. The common appearance of scrapes and incomplete fins, and the absence of scales, suggests aggressive and competitive behaviors may have prevented successful reproduction. Clearly there is a tradeoff between aggressive competition and mating behavior under these conditions as has been suggested for *P. formosa* and *P. mexicana* in natural populations (Balsano et al. 1981). Even though *P. mexicana* males formed loose dominance hierarchies and defended feeding territories, there was little aggressive exclusion of subordinate males from a territory when receptive females were present. Baird (1968) also noted the higher levels of aggression among *P. latipinna* and *P. formosa* during feeding, particularly between *P. latipinna* and *P. formosa* females. The most important result of the aquarium experiments is that they confirm the logical notion that aggressive and competitive behaviors have negative consequences on reproduction as well as on survival.

Density may have affected the mortality rates in the aquarium populations. The density of fishes in an aquarium in this study was more than 10 times that of fishes in the small ponds. Each fish in a small pond had an average of 25 gallons of water, while each fish in an aquarium had 1 gallon. This may be a major reason mortality was so high, aggression so prevalent, and reproduction nearly absent. *Poecilia latipinna* and *P. formosa* have been successfully maintained in laboratory aquaria at densities comparable to those in this study, except those conditions differ from this study in the composition of the aquarium populations. In the laboratory the two species are often maintained in separate aquaria, there are usually very few or no males in an aquarium of females, and mortality in an aquarium of males is lessened by the presence of one or two females. Further investigation of density and mortality could provide insight into how these two factors interact in natural populations.

Ponds

The data from both large ponds support the hypothesis that reproductive success of *P. formosa* relative to *P. latipinna* is negatively frequency dependent. *P. formosa* females produce progressively fewer offspring as their frequency in the population increases. However, in small ponds the relationship between reproductive success and relative frequency is less clear. The trend for negative frequency-dependence is present but it is not statistically significant. The high density of fishes and the small population sizes, relative to natural populations, would have rendered these populations more susceptible to stochastic effects. The frequency-dependent nature of mortality suggests there may have been niche-partitioning between *P. latipinna* and *P. formosa.*

The proximate cause of a decrease in reproductive advantage of *P. formosa* when they are at high frequencies is still unclear. It is clear *P. formosa* females as a group are producing fewer offspring when they are at high frequency and that it is a phenomenon of *P. formosa* females only. *Poecilia latipinna* females produce the same numbers of offspring regardless of their relative frequency in the population. The reduced reproductive success of *P. formosa* when they are at high frequency may be due to a reduction in the number of embryos per clutch of eggs. All *P. formosa* females would be mated but they would produce fewer offspring than *P. latipinna* or *P. mexicana* females. This could be a strictly constant physiological limitation or a result of reliance on stored sperm that may have lower insemination capacity. This mechanism alone would reduce the reproductive rate advantage of asexuality and possibly prevent *P. formosa* from extinction due to competitive exclusion. Such a constant physiological mechanism does not require any changes in male mate discrimination, and the oscillations in relative frequency are purely a result of characteristics of *P. formosa*. However, there is evidence to refute this hypothesis. *Poecilia latipinna* and *P. formosa* females of the same size have not been found to produce different numbers of offspring (Travis 1994). Although the data of Hubbs (1964) suggests *P. formosa* are more often "partially pregnant", there was no control for female size. Female size is directly related to fecundity so it is unclear if the differences are due to size or specific identity.

An alternative mechanism is that fewer *P. formosa* females produce offspring when they are at high frequency because some *P. formosa* females are uninseminated or relying on stored sperm. Those that are inseminated produce a normal number of offspring per clutch but there are fewer gynogens are pregnant so there are fewer total offspring. This mechanism relies on plasticity in mating behavior of *P. latipinna* males, or plasticity in competitive antagonistic behavior of *P. latipinna* and *P. formosa* females, or both. The mating system of *P. latipinna* is behaviorally complex; there are myriad preferences and behaviors that play roles in the ultimate pairing of male and female mates. The results of this study imply behavioral expression in *Poecilia* may be context-dependent. Contrary to what theory predicts, the evolution of this complexity within the mating system of *P. latipinna* may actually promote the stability of mixed populations and ultimately the evolutionary persistence of the gynogenetic *P. formosa* lineage. Frequency-dependent reproductive success would have difficulty operating under conditions of low mate discrimination.

Population Dynamics

The large experimental ponds harbored mixed populations of *P. formosa* and *P. latipinna* that appeared to be stable over the time period of the study; that is neither species became extinct. Population dynamics in the experimental ponds of *P. latipinna* and *P. formosa* may differ from those observed in natural populations, but there is no empirical evidence to suggest this is so. The frequency-dependence of reproductive success in *P. formosa* was more evident in the large ponds suggesting the effects of niche-partitioning may have enhanced the stability of those populations. In the large ponds there was clearly more room for ecological niche-partitioning than in the small ponds. An analysis of gut contents of fishes from these ponds would more directly and clearly address whether niche-partitioning occurred.

The initially low density of fish in the large ponds may also be a factor in their stability. The populations may not have reached carrying capacity as quickly as the small ponds and competition for resources would not have been as extreme. The reproductive advantage of *P. formosa* would not have had as strong an effect and reduced the necessity for niche-partitioning. Low density could also increase the stringency of conspecific mate preference by reducing the encounter rate of *P. latipinna* males and females. In this case, low density would further enhance the effects of high frequency of *P. formosa* on their reproductive success. I would expect the effects of low density to decrease as population size increased, even if relative frequency remained constant.

Mortality in the small ponds appears to be negatively frequency-dependent for both *P. latipinna* and *P. formosa* females. The mortality of females of either species was statistically significantly higher when they were at high frequency in the population. Although *P. latipinna* males did not suffer the same mortality as *P. latipinna* females, they still experienced greater mortality in high frequency conditions. The difference between males and *P. latipinna* females may be because males were rarely at frequencies greater than 0.50; their relative frequency was closer to that of *P. formosa* than *P. latipinna* females. The mechanism for this sex specific effect is unknown in part because the cause of mortality is unknown. Aggressive interactions could increase mortality in small populations of high density as it does in aquaria. Transmission rates of parasites may be greater within a species than across species boundaries (Pfennig 1999); one strain or species of parasite may be transferred more easily between individuals of the same species. When either female is at high frequency they may be more susceptible to infection causing increased mortality for the group.

Change in numbers of fishes in large ponds suggests *P. formosa* have difficulty getting established in a population of *P. latipinna*. When introduced at high frequency, their proportional representation plummeted before it began to increase. Their frequency did not begin to climb until nearly 730 days (2 years) later. However, this drop reflects the very high mortality over the first winter that may have been affected by a snapping turtle predator. When introduced at low frequency the proportion of *P. formosa* fluctuated without much change in average frequency until almost two years had passed. If we ignore the initial mortality in pond 3 and consider both ponds replicates of initially low frequency conditions, the results suggest *P. formosa* is more likely to persist for longer periods of time if a small number of individuals invade an existing *P. latipinna* population. This possibility is apparent in the pattern of extinction of *P. latipinna* in small ponds; five of the six "failed" experimental populations were initiated with a high frequency of *P. formosa*. Although the probability of extinction was not significantly different based on initially frequency of *P. formosa*, the small sample size was only six ponds. There may be an effect that went undetected in this study.

High frequencies of *P. formosa* in natural populations may be indicators of small, relatively recent invasions of *P. latipinna* populations. We might expect these populations to disappear sooner than those with lower frequencies of *P. formosa*. The wide range of frequencies observed in Brownsville, Texas populations during this study and that of McNeely (unpublished data) suggests *P. formosa* may go through cycles of invasion and extinction if the initial frequency of founding individuals is high relative to *P. latipinna*. It is interesting to note that the "Airport Ditch" site contained thousands *Poecilia* in 1997 but was virtually devoid of both species in 1999. All of this evidence suggests a more expansive study might empirically test the theoretical conditions for successful invasion of a *P. latipinna* population presented by Stenseth et al. (1985).

In the experimental ponds the general changes in relative frequency of adult and juvenile *P. formosa*, and their oscillatory nature, are consistent with the hypothesis that reproductive success is negatively frequency dependent. Although the relationship between relative reproductive success of *P. formosa* and their frequency in the small populations was not significant, this relationship in the large populations is strongly significant. The difference in results of large and small ponds strongly suggests the reproductive advantage is not independent of population composition. Although the sample sizes of the two initial frequency conditions in the small ponds were small and the time spanned by some experiments was relatively short (less than one year), there were numerous data points within each small pond and nearly all populations experienced fluctuations in frequency of *P. formosa*. Small population size may preclude or obscure stabilizing effects of frequency-dependent reproductive success.

There was a high probability of population fixation at 100% *P. formosa* among all 13 small ponds; nearly half of the experiments provided no data on reproduction. Populations with initially high frequencies of *P. formosa* comprised 5 of the 6 populations that experienced extinction of *P. latipinna* with *P. formosa* to follow. This result is consistent with theoretical predictions of extinction of gynogenetic lineages following competitive exclusion of their hosts. There were a total of 8 populations initiated with 80% *P. formosa*, over half of which experienced extinction. It may be when *P. formosa* reaches very high frequencies in small, isolated populations, their fate is determined by random ecological forces, rather than by the deleterious nature of their genome. It is interesting that the density of fish in these ponds is positively related to a high frequency of *P. formosa*. This also supports the conclusion that these populations were small enough to be strongly affected by stochasticity. It also presents a possible factor that interacts with reproductive success. When *P. formosa* invades a small population of pure *P. latipinna*, they may reap the benefits of a reproductive advantage due to their unisexuality and their rare female advantage. The subsequent rapid increase in frequency could cause the density of the now mixed population to increase more quickly than it would have in the pure *P. latipinna* population, pushing it beyond carrying capacity before it can recover.

Seasonal effects on the small pond populations were consistent with previously published studies of the dynamics of natural populations of *P.*

latipinna and *P. formosa* (Hubbs 1964). If anything the effects of season were diminished; females appeared to reproduce throughout most of the year, juveniles were rare at the end of the winter. This corresponds with the onset of breeding season and reflects not only overwinter juvenile mortality but also a limitation of this study. Fry were not classified as juveniles until they could be visually identified to species, about 90 days after birth. The "juveniles" present during early spring would have been classified as fry.

Fewer fry were present just prior to and during the winter, but there is a paucity of data for the winter months of December, January and February because the ponds could not be sampled without causing undue stress on the fish, especially on fry. Season did not affect population size overall or within any particular group of fishes. There were no interspecific differences in numbers of adults or juveniles.

The only seasonal effect of note is the weak effect of an interaction between season and initial frequency of *P. formosa* on the relative frequency of *P. formosa* juveniles. I believe this is a spurious effect because neither season nor initial frequency alone had a significant effect, and the initial frequency of adult *P formosa* does not reflect actual frequency. Current relative frequency of adult *P. formosa* probably exerts a stronger effect than initial frequency or season on the relative frequency of their offspring although I did not test this statistically.

In general, the sex ratios in the large ponds were not unusually high or low. The mean overall sex ratio in pond 4 was consistent with sex ratios observed by Hubbs (1964) in natural populations of *P. latipinna* and *P formosa*. In pond 3 the mean overall sex ratio was slightly higher which may reflect the difference in initial conditions. *Poecilia formosa* was initially at low frequency in pond 4 and at high frequency in pond 3. The sex ratios within *P. latipinna* in both ponds were consistent with female biased ratios among adults in natural populations (Hubbs 1964, but see Snelson & Wetherington 1980). There were some striking outliers in the data sets of both large ponds. Sex ratio within *P. latipinna* in Pond 3 had two samples at 14:1 and 22:1. Pond 4 also had one sample that was not nearly as extreme but it was unusual nonetheless (9:1). Sex ratios in both ponds also reached values as low as 0.4:1. Assuming sex determination is genotypic (Travis 1994, Angus 1989) and *P. latipinna* females bear equal numbers of male and female offspring (Snelson & Wetherington 1980), the sex ratios in these ponds support a hypothesis that males experience greater mortality. Variation in sex ratio in natural populations may be much less deterministic than previously thought and can be influenced by relative frequency of *P. formosa* in addition to selective predation or parasitism. Alternatively, the sex ratio of newborn *P. latipinna* may deviate from 1:1, but this has not been found in *P. latipinna* from Florida populations (Snelson & Wetherington 1980) Juvenile sex ratio is not easily determined visually. An investigation using karyotyping or other molecular methods would be a worthwhile endeavor and could more definitively rule out post parturition effects on sex ratio.

SUMMARY AND FINAL CONCLUSIONS

Reproductive success of *P. formosa* in populations with *P. latipinna* hosts is negatively frequency-dependent. This result supports the theoretical prediction that frequency-dependent reproductive success is sufficient for evolutionary persistence of gynogenetic lineages in the absence of ecological niche-partitioning (Stenseth et al. 1985). A negative relationship between reproductive success of *P. formosa* and their relative frequency is clear and significant in the large ponds. This relationship holds whether you consider the raw data or the transformed and averaged data. In the large ponds, the potential for niche-partitioning on a microhabitat scale was possible; in the small ponds it was much less likely unless it occurred on a very fine scale. The negative effect of relative frequency on reproductive success of *P. formosa* in small ponds was apparent but not statistically significant and may indicate niche-partitioning is necessary for stability in small, high density populations. While microhabitat segregation was possible in the large ponds, I never observed such separation of adults, and previous studies do not suggest *P. formosa* and *P. latipinna* exploit different niches (Hubbs 1964). There was no separation of juveniles and fry; mixed groups of both species were always found in the shallows. An examination of stomach contents of adults and juveniles from these ponds could directly determine if niche-partitioning based on food did occur.

Frequency-dependent reproductive success is unlikely to be the sole factor that allows natural populations of *P. formosa* to persist over evolutionary time. The effects of frequency and density on mortality, predation, parasitism, and microhabitat niche-partitioning all probably play roles in the stability of natural populations. How these ecological factors interact with the genetic consequences of clonal reproduction is unknown at this time. We must begin to integrate the abundant information from several disciplines to fully understand the forces that affect the evolutionary persistence of the gynogenetic lineages.

Appendix

Larry

Memo to: Laurie Dries
From : Larry Gilbert, Director, BFL
Subject: Addition of floating plants to experimental fish ponds
cc: John Crutchfield, Resident Manager, BFL, Tony Alexander, Research Assistant, David Hillis, Mike Ryan, Gill Rosenthal

May 3, 1999

This is an official apology to Laurie Dries, and other researchers, John Crutchfield, and Tony Alexander as well as a report to Laurie for her records on her experiment.

It may be that in twenty years of helping take care of BFL I have become too informal with personally tending to what seem to be cosmetic and trivial aspects of the outdoor facilities As John knows, I occasionally do so without first reviewing these activities with him. I want you to know that in the case of my spontaenously adding floating plants for summer shade on the fish tanks on the evening of April 30. 1999, with out proper review, forethought,and consultation, was half baked, short sighted, stupid and potentially costly to the integrity of research that John and I have worked so hard to promote and encourage at BFL. Good intentions are absolutely no excuse for violating important protocol and appropriate chain of command and review. I would like to commend Tony Alexander for his vigilance in this matter.

I am taking this incident as a wake up call for myself and as a stimulus in general for thinking more formally about how to handle BFL research security as we open the area to more students, who embarassingly enough, have apparently have done better than I have in terms of causing concern. One of our big problems when I assume directorship in 1980, were those folks who just did whatever they pleased at BFL without going through the resident manager. It is a bit painful to see myself in that light. I apologize to John for my own lapses in that direction. It is important that he is made aware of and coordinates all activities at BFL. I hereby instruct John to remind me that it is time for me to step down as director of BFL if I ever pull such a stunt again.

As I examined the samples for the follow-up review of possible impact (below) I though over possible measures that might have to be taken if I proved to myself that I had possibly added fry to your tanks. Given the wasteful hassles (of other kinds) I've recently been through in my own work, it made me almost physically sick to think of causing such grief to a colleague over something so unnecessary. I resolved to personally pay for any work required to separate and eliminate fry from adults in your tanks or for genetic reference work on the potential source of contamination. I felt relieved in finding no evidence that fry were transported, but the fact that I could have done it unwittingly certainly is humbling.

Report on potential for impact on your project:
With respect to any impact on your project I have taken additional steps to determine that no genetic contamination occured as a result of adding these plants. I did that Saturday, May 1, 1999, and found no evidence of fry in plants sampled in the same manner (see below). However, because the situation wasn't as clear cut as I first had assumed (i.e. adults were

present), I am providing a full report so that you can decide if further actions need to be taken to insure the integrity of your project. Here is the information:

Source of added plants: The plants in question were originally placed by Dave Hillis in the tanks you use at BFL and used there for summer shade. I later added some of these to my backyard pond. A freeze killed those at BFL. I also stocked my pond with fish from the spillover pond north of your main tanks and with leopard frogs. The drought of '98 killed much of what was in the pond, I assumed the fish were lost but on checking after we met on Friday, I sampled the pond and found adult mollies amoung the tadpoles. I saw no fry but I was concerned enough about my prior sloppy behaviour to do a careful follow-up to check for sure.

Method of removal and transport: The plants I took to BFL were first lifted from the water surface with a garden leaf rake, tossed on the lawn left for about 20 min. then loaded into a plastic garbage bag transported to BFL and 1-3 individuals were added by hand to each tank. Approximately sixty minutes lapsed between loading the plants in the bag and adding the last to the north ponds in the old area.

Method of double checking for fauna associated with plants: After concerns were raised I carried out the identical proceedure 36hrs. after the first collection was made. In this case *I intentionally collected roughly twice the volume of plants* from the same pond (an entire large garbage bage 3/4 full), dumped it on the grass and without a rinse loaded it into a black plastic garbage bag. I then waited 30 minutes and drained all water which had accumulated in bottom of the bag into a clean white enamel metal tray for inspection. I then proceeded to remove handfuls of plants as I had at BFL and placed them into the tray for a thorough rinse of all creatures associated. Finally, I washed the what remained in the bag out into the tray for inspection.

Survey results. Absolutely no tadpoles or fish fry were present in the wash water taken from over 100 floating plants and other associated aquatic plants. In the bag I found larger invertebrates but not fish or tadpoles. In the plant material I found a rich crustacean, and insect fauna, including tiny dragonfly nymphs a few chironomid larvae. So I conclude that each tank received at most an inoculation of healthy aquatic invertebrates which are likely to be already living in the tanks.

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Vita

Laurie Ann Dries was born in Milwaukee, Wisconsin on 9 December 1959, to William C. and Martha L. Dries. She resided with her family in Madison, Wisconsin until her graduation from James Madison Memorial High School in January 1977. She entered the University of Wisconsin in Madison in 1978 and majored in Communication Arts. She left the University in 1979 and moved to Austin, Texas where she was employed as a baker, pastry chef, waitress, and bartender. She attended the American Academy of Dramatic Arts in New York City, New York in 1985 and subsequently worked as an actress in New York City, Austin, and Dallas, Texas. In 1989, She enrolled in the University of Texas at Austin and received her Bachelor of Arts in Zoology in December 1993. She entered the Graduate School at the University of Texas at Austin in January 1994. Her publications include papers on mate discrimination and reproductive cycles in livebearing fishes, heritability of large size in swordtail fish, and numerous educational materials for secondary schoolchildren. Her awards include a National Science Foundation Dissertation Improvement Grant.

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