Geographical variation in reproductive character displacement in mate choice by male sailfin mollies

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Female Amazon mollies, *Poecilia formosa*, are a unisexual species that reproduce by gynogenesis. They must coexist and mate with males of other species (usually the mollies *Poecilia latipinna* or *Poecilia mexicana*) to induce embryogenesis, but inheritance is strictly maternal. We examined the mating preference of the male sailfin molly, *P. latipinna*, for female sailfin mollies versus Amazon mollies, *P. formosa*. We compared the mating preferences of sympatric and allopatric populations collected throughout the Gulf Coast of North America. Male *P. latipinna* from six populations sympatric with Amazon mollies showed a significantly greater strength of preference for conspecific sailfin females than males from five populations that were allopatric with Amazon mollies. These results provide strong evidence for reproductive character displacement of male mate choice in sympatry. Furthermore, the large geographical range of populations that we tested revealed variation among populations within sympatry and allopatry, indicating that it is important to evaluate a large number of populations when examining reproductive character displacement.

Keywords: gynogenetic; mate choice; *Poecilia latipinna*; reproductive character displacement; speciation

1. INTRODUCTION

Reproductive character displacement is the pattern of greater divergence of a reproductively isolating trait between closely related taxa in areas of sympatry than in areas of allopatry (Brown & Wilson 1956; Howard 1993). In this definition, the term trait includes morphological and signalling-system characters as well as an individual species' ability to discriminate. Reinforcement is the process by which natural selection strengthens prezygotic isolating barriers in zones of overlap or reduces hybridization due to selection against hybrids (Dobzhansky 1940; Blair 1955). Reproductive character displacement is one of the potential outcomes of this process.

Empirical evidence for reproductive character displacement has become more common recently (table 1; and see Coyne & Orr 1989, 1997). Some of the studies purporting evidence for reproductive character displacement derive their support from spatial variation in signals (table 1), but often such studies do not show that the signal properties involved in reproductive character displacement are salient in mate choice because they fail to examine the responses of the receiver to signal variation. Reproductive character displacement can also occur with no divergence in signals; only the receivers need to diverge. Thus, Waage (1979) emphasized the importance of examining actual mating preferences. Several studies present data supporting reproductive character displacement in mating preferences (table 1).

Many studies of reproductive character displacement compare a few sample populations from sympatry and allopatry; however, Butlin (1995) suggested that it is critical to examine patterns of mating preference in greater geographical detail for a robust test of reproductive character displacement. Examining larger numbers of populations allows us to examine sufficient allopatric populations to determine whether divergence is a trend

that actually originated in sympatry (Waage 1979; Gerhardt 1994), to describe more accurately the variation in behavioural preferences within zones of allopatry and sympatry (Butlin 1989) and to ensure that gene flow between sympatric and allopatric populations is not obscuring the pattern of similarity in allopatry and divergence in sympatry (Waage 1979).

Whereas some studies that found evidence of reproductive character displacement have examined signal variation in greater geographical detail, analogous studies of geographical variation in mating preferences are less common (table 1). Here, we document male mate preference in 11 populations along a transect through zones of allopatry and sympatry of the sailfin molly, *Poecilia latipinna* (Poeciliidae). We show that reproductive character displacement occurs in sympatry, and that among populations in allopatry males show considerable variation in the strength of preference for conspecific versus heterospecific females.

P. latipinna can be sexually parasitized by the Amazon molly, *Poecilia formosa*. The Amazon molly is a diploid unisexual (all female) species of fish of hybrid origin that reproduces by gynogenesis (Hubbs & Hubbs 1932). Molecular studies have shown that *P. latipinna* and *Poecilia mexicana* are the parental species involved in the hybridization event that gave rise to *P. formosa* (Avise *et al.* 1991; Schartl *et al.* 1995). Gynogens occur in complexes with bisexual species, usually consisting of *P. latipinna* or *P. mexicana*. Females of *P. formosa* must mate with males of either of these species (Hubbs 1964; Darnell & Abramoff 1968) to trigger embryogenesis (Hubbs & Hubbs 1946; Kallman 1962; Darnell *et al.* 1967) but inheritance is strictly maternal. There is no gene flow between species even though the gynogenetic offspring are of hybrid origin. Gynogens that successfully acquire mates produce twice as many female offspring as their parental species and, therefore, are capable of a higher rate of reproduction. Male sailfin mollies, however, prefer conspecific females over the parasitic Amazon molly (Woodhead &

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Table 1. *Summary of prior studies that have found evidence of reproductive character displacement*

(We only cite studies that found evidence of reproductive character displacement in a trait and that tested natural populations from the field by examining sympatric and allopatric populations of a species. 'Reciprocal test' indicates studies that examined both species in sympatry and allopatry. Traits tested were either variation in a mating signal 'or mate choice' by males or females for the other sex. 'Type of experiment' indicates whether the studies compared sympatric behaviour to allopatric behaviour or looked at the overall geographical variation in a mating trait.)

author	number of allopatric populations	number of sympatric populations	reciprocal test	organism studied	mating signal	mate choice	type of experiment
Higgie *et al.* (2000)	5	3		*Drosophila serrata, D. birchii*	×	×	sympatry versus allopatry
Rundle & Schluter (1998)[a]	2	1		*Gasterosteus aculeatus*, benthic versus limnetic		female	sympatry versus allopatry
Sætre *et al.* (1997)	1, 1	1	×	*Ficedula hypoleuca, F. albicollis*		female	sympatry versus allopatry
Márquez & Bosch (1997)	1, 1	1	×	*Alytes obstetricans, A. cisternasii*		female[b]	sympatry versus allopatry
Ryan *et al.* (1996)	2	1		*Poecilia latipinna, P. formosa*		male	sympatry versus allopatry
Noor (1995)	3	3		*Drosophila persimilis, D. pseudoobscura*		female	sympatry versus allopatry
Gerhardt (1994)	2	3		*Hyla chrysoscelis, H. versicolor*		female[b]	sympatry versus allopatry
Loftus-Hills & Littlejohn (1992)[c]	2	5		*Gastrophryne carolinensis, G. olivacea*	×		geographical variation
Benedix & Howard (1991), first transect	4, 4	2	×	*Allonemobius fasciatus[d], A. socius*	×		geographical variation
Benedix & Howard (1991), second transect	4, 4	4	×	*Allonemobius fasciatus[d], A. socius*	×		geographical variation
Otte (1989)[e]	17	3		*Laupala palola, L. nui*	×		geographical variation
McLain & Rai (1986)	4	1		*Aedes albopictus[f], A. pseudalbopictus, A. seatoi*		female	sympatry versus allopatry
Ratcliffe & Grant (1983)	3	3	×	*Geospiza fulginosa[d], G. difficilis*		male	sympatry versus allopatry
Waage (1975)	3	4		*Calopteryx maculata, C. aequabilis*		male	geographical variation
Waage (1979)	2, 2	3	×	*Calopteryx maculata, C. aequabilis*	×		geographical variation
Fouquette (1975)	4, 6	4	×	*Pseudacris nigrita, P. feriarum*	×		geographical variation
Hill *et al.* (1972)[g]	1, 3	3	×	*Teleogryllus commodus, T. oceanicus*	×		geographical variation

[a] Benthics and limnetics are reproductively isolated freshwater ecomorphs recently derived from the marine form of *G. aculeatus*.
[b] Test of preference for a male's call signal.
[c] Data from the population that showed variation in advertisement calls: *G. carolinensis*.
[d] Displacement was not found in all sympatric populations and was only found for one species of the reciprocal test.
[e] Numerous species' interactions (via signal) were tested with different sample sizes, this is the largest study and the clearest evidence.
[f] Tested with two different species in different areas of sympatry (two sites).
[g] Variation in sympatric versus allopatric female choice did not show evidence for reproductive character displacement.

Armstrong 1985; Schlupp *et al.* 1991; Ryan *et al.* 1996). Ryan *et al.* (1996) found that male *P. latipinna*, in both allopatry and sympatry, preferred female *P. latipinna* to female *P. formosa*. Males from one sympatric population demonstrated a stronger conspecific preference than males from two allopatric populations, thus providing some evidence for reproductive character displacement.

Sailfin mollies are found in many habitats and are continuously distributed in the coastal plain of the Gulf of Mexico from northern Mexico to Florida, throughout peninsular Florida and along the Atlantic coast from Florida to northern South Carolina. The Amazon molly occurs naturally in the rivers and coastal lagoons of south-eastern Texas and north-eastern Mexico. In northern
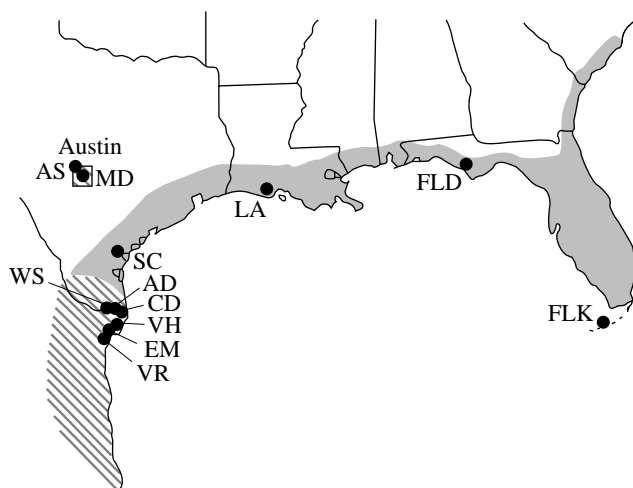
Figure 1. Distribution of sailfin mollies (*Poecilia latipinna*) and Amazon mollies (*Poecilia formosa*). The region of sympatry for sailfin and Amazon mollies is striped, the region of allopatry for sailfin mollies is shaded. The black dots indicate collection sites. The black dot in the hatched square indicates the introduced Martindale, Texas, (MD) control population. The test localities are Villa Real, Mexico (VR); El Moquetito, Mexico (EM); Valle Hermosa, Mexico (VH); Central Ditch, Texas (CD); Airport Ditch, Texas (AD); Weslaco, Texas (WS); Aquarena Springs, Texas (AS); Salt Creek, Texas (SC); Lafayette, Louisiana (LA); Destin, Florida (FLD); Florida Keys, Florida (FLK).

Mexico, south-eastern Texas and isolated introduced populations in central Texas (Martindale and Comal), the sailfin molly and the Amazon molly are sympatric (figure 1). The extensive range of sailfin mollies allowed us to extend the work of Ryan *et al.* (1996) by testing reproductive character displacement of male mate preference in a larger number of allopatric and sympatric populations than was previously studied. Specifically, we examined the distribution of mating preferences of males of *P. latipinna* when paired with females of *P. latipinna* and *P. formosa*. This further enabled us to examine the spatial pattern of variation in preference within sympatry and allopatry.

2. MATERIAL AND METHODS

(a) *Localities*

Fishes were collected from natural populations where *P. latipinna* was either sympatric or allopatric with *P. formosa*. Male and female *P. latipinna* were collected by seine and dip-net from six populations sympatric with *P. formosa* and five allopatric populations (figure 1). Three of the sympatric sites were in the Rio Grande Valley between Texas and Mexico: Weslaco (February 1998), Airport Ditch in Brownsville (February 1998) and Central Ditch, Brownsville (April 1998). The remaining three sympatric populations were in the Tamaulipas region of Mexico, also near the Rio Grande Valley: Valle Hermosa, Villa Real and El Moquetito (June 1998). Two of the allopatric sites were in Florida: Layton in the Florida Keys (September 1998) and Destin (September 1997) in the panhandle. One allopatric site was in Lafayette, Louisiana (September 1997) and two allopatric sites were in Texas: Salt Creek near Port Aransas (June 1998) and Aquarena Springs, San Marcos (July 1998). Aquarena Springs is an introduced allopatric population

(Brown 1953). The females of *P. latipinna* and *P. formosa* used as 'object fishes' (the females with which males were tested) were collected from the San Marcos River, Martindale, Texas (September 1997, October 1998 and March 1999). The Martindale population has *P. latipinna* and *P. formosa*, both of which are introduced (Brown 1953). We used fishes from this introduced population as our object fishes to control for familiarity, so that all object females (unisexual and bisexual) were unfamiliar to all males tested from both allopatric and sympatric populations.

(b) *Study animals*

Fishes were transported to our laboratory in Austin, Texas for use in the behavioural studies. Some fishes were maintained in large outdoor tanks (1800 l) at Brackenridge Field Laboratory in Austin, Texas, prior to use. Either these adults or their offspring were tested. Other fishes were immediately placed in 75-l tanks in the laboratory. Fishes were maintained in the laboratory at 22–28 °C on a 14 L:10 D cycle using 40 W fluorescent lights (General Electric, Cleveland, OH, USA) during daylight hours in addition to natural light. The fishes were fed TetraMin conditioning food daily and supplemented with live brine shrimp nauplii. At least 30 days before testing, males and females from a given population were separated and placed in unisex 75-l tanks to standardize the condition of the fishes. Because sailfin mollies have a 30 day ovarian cycle, most females would have dropped any broods that they may have been harbouring before being tested. All males used were considered mature based on the fusion of the anal fin into the gonopodium. Males display to most females but display is influenced by the female's reproductive cycle (Sumner *et al.* 1994). Secondary sexual characteristics of male *P. latipinna* consist of a large colourful dorsal fin (especially in larger males) and enhanced secondary sexual coloration. Males direct two principal behaviours toward females: courtship display, in which the dorsal fin is erected and presented to the female (often accompanied by a sigmoid curving of the body; Travis & Woodward 1989), and gonopodial thrusts in an attempt to inseminate females.

(c) *Experimental set-up and procedure*

We tested males from each population with three types of females: Amazon mollies, sailfin mollies from the Martindale population and sailfin mollies from the male's own population. Males of all sizes were used in all of the tests (mean ± s.e.m. = 34.12 ± 0.78 mm; range of 18–60 mm). We conducted mate-choice tests in one-half of a 53 l tank (30.0 cm × 30.5 cm × 30.5 cm). The aquarium contained tan gravel and 18 cm of water, which was aerated and filtered, except during testing. A 50 W Gro & Sho plant light (General Electric) was suspended 30 cm above each tank. The tanks were surrounded on all sides with cardboard to prevent the test fishes from being distracted by fishes in other tanks. We covered the fronts of the tanks with one-way film to minimize disturbance of the fishes by our presence. All test animals were fed less than an hour before testing. We haphazardly selected a test male from the test population and placed him under a rectangular box covered by a fine mesh net (17 cm × 12 cm × 13 cm) in the centre of the test aquarium along with an Amazon molly and a female sailfin molly (population of origin depended on the treatment). Paired female test fishes were matched for body size to within 2 mm standard length (SL). The SL of each fish was measured from the tip of the snout to the base of the caudal fin.

Each male was tested in two treatments in random order. In treatment 1, the foreign treatment, males were paired with one

gynogen and one conspecific female from the Martindale population. In treatment 2, the native treatment, the same males were paired with a gynogen from Martindale and a conspecific female from the male's own population. Studies of male mate choice in live-bearing fishes have advantages over analogous studies of female preference. Female association, as measured by time spent near a male, is often used as a bioassay for female preference, but this assay might be less robust (Gabor 1999) than the analogous bioassay in male mate-choice studies. In this study, gonopodial thrusts directed at a female were interpreted as mating attempts. After a 10 min acclimatization period, we carefully lifted the rectangular net box and observed the male's behaviour. We allowed the male 10 min to respond to the treatment. Upon the first gonopodial thrust, we began the 10 min test period and recorded all gonopodial thrusts directed towards either female. If the male thrusted less than five times in the first treatment tested, the male was considered unresponsive and further testing was abandoned; the male was replaced in his holding tank to be re-tested in the future. If the male thrusted at least five times in the first treatment, we removed all of the females from the tank and left the male alone for 10 min. Subsequently, we placed another two females from the opposite treatment in the tank and placed the male under the net divider for another 10 min acclimatization period. Then we tested the male in a second treatment. Again, we gave the male up to 10 min to respond. If he did not, the male was given a score of zero for gonopodial thrusting for the second treatment only. After we completed testing, we measured male SL and body depth with callipers. We conducted trials from 09.00 to 17.00, 12 March 1998 to 4 November 1998 and 7 March 1999 to 1 June 1999.

(d) *Variables scored*

We scored the number of gonopodial thrusts in each test as an indicator of male mate preference. The distribution of the number of gonopodial thrusts across females was not normally distributed. Also, transforming the data did not result in a normal distribution. Thus, all analyses were by non-parametric statistics. We used two-tailed Wilcoxon's signed-ranks tests for paired samples to determine whether there was a significant difference in the numbers of gonopodial thrusts directed at conspecifics and gynogens for each population. Then we estimated the 'strength of preference' as the proportion of the total gonopodial thrusts directed towards sailfin mollies in a given test. We used a Kruskal–Wallis test to examine whether there were differences in the strength of preference within sympatric populations and within allopatric populations for each treatment. We then used a two-tailed Mann–Whitney U-test to compare the pooled strength of preferences between all sympatric and all allopatric populations. A two-tailed Spearman's rank correlation was used to examine the relationship between male size and proportion of thrusts directed at Amazon mollies. Sample sizes varied because not all males responded in both treatments, and the native treatment consisted of only five sympatric populations because native females from Central Ditch, Texas, died prior to testing.

3. RESULTS

In most cases males from both sympatric and allopatric populations thrusted more frequently towards conspecific females than towards Amazon mollies. This was true for both the foreign treatment and the native treatment.
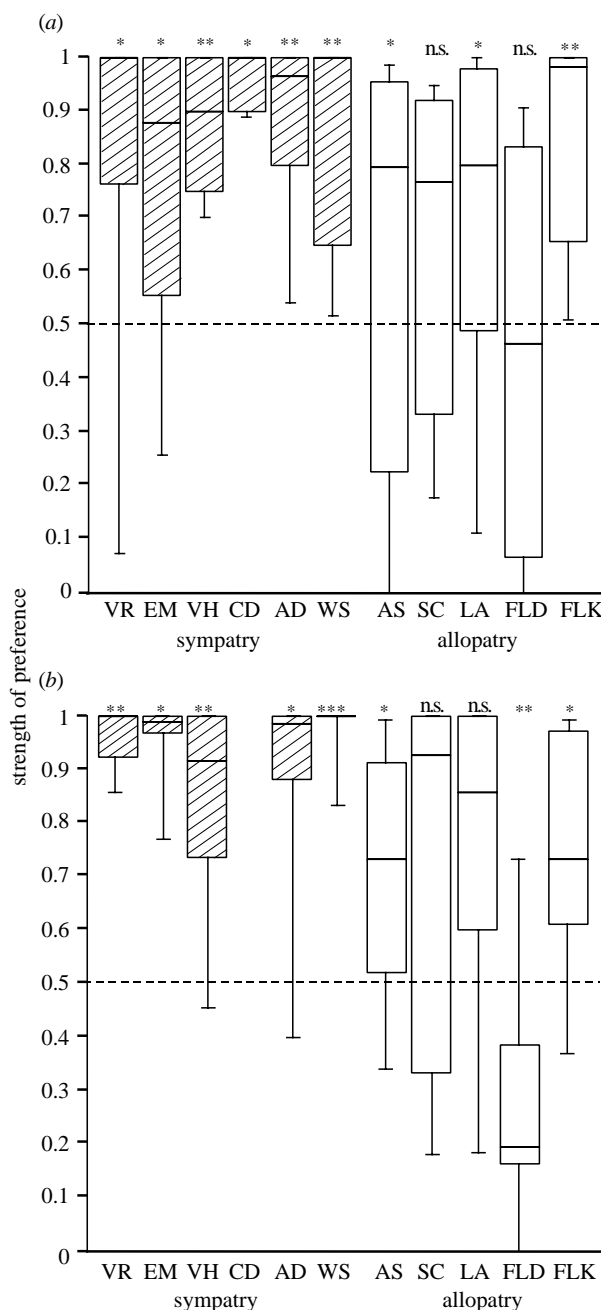
Figure 2. Box plots representing male strength of preference estimated by the number of gonopodial thrusts directed towards *Poecilia latipinna* divided by the total number of gonopodial thrusts. (*a*) Foreign treatment: males were paired with foreign *P. latipinna* and *P. formosa* from Martindale, Texas. (*b*) Native treatment: males were tested with *P. latipinna* from their native population and *P. formosa* from Martindale, Texas. Data above the no-preference line ($y = 0.5$) indicate an increasing strength of preference for sailfin molly females, data below the line indicate an increasing preference for Amazon molly females. The left-most population is the population in 'deepest sympatry'. Populations to the right appear in order of increasing distance from deepest sympatry. The abbreviations of the population names are as in figure 1. The upper and lower horizontal lines of the boxes represent the first and third quartiles and the middle horizontal lines represent the medians (Sokal & Rohlf 1995). The whiskers represent the range. Significance levels are derived from p-values based on Wilcoxon's signed-ranks tests comparing the number of thrusts directed at sailfin molly and Amazon molly females: $^*p < 0.05$; $^{**}p \leqslant 0.01$; $^{***}p \leqslant 0.001$; n.s. $p \geqslant 0.05$ (see table 2 for complete data).

Table 2. *Comparison of gonopodial thrusts between populations of male* Poecilia latipinna *as directed at female* P. latipinna *and* P. formosa

(Wilcoxon's signed-ranks test comparing mean number of thrusts directed at female sailfin mollies, *P. latipinna*, and Amazon mollies, *P. formosa*, for males from each population tested. LA, Louisiana; FL, Florida; TX, Texas; MX, Mexico; sp, sympatric population; ap, allopatric population; *n*, number of individuals that responded to the treatment. The native females of Central Ditch died prior to testing.)

| | foreign treatment | | | | | | native treatment | | | | | |
| | mean ± s.e.m. number of thrusts directed at | | | | | | mean ± s.e.m. number of thrusts directed at | | | | | |
population	*P. latipinna*	*P. formosa*	*n*	*T* +	*z*	*p*	*P. latipinna*	*P. formosa*	*n*	*T* +	*z*	*p*
Villa Real, MX (sp)	40.82 ± 15.42	5.09 ± 4.50	11	57.5	−2.18	0.03	18.82 ± 6.57	0.55 ± 0.37	11	66	−2.94	0.003
El Moquetito, MX (sp)	12.50 ± 3.50	2.70 ± 0.86	10	48.5	−2.14	0.03	27.83 ± 15.31	0.67 ± 0.33	6	21	−2.20	0.03
Valle Hermosa, MX (sp)	35.30 ± 9.68	3.50 ± 1.45	10	55	−2.81	0.005	26.00 ± 4.89	3.80 ± 1.46	10	44	−2.55	0.01
Central Ditch, TX (sp)	35.50 ± 15.58	0.67 ± 0.49	6	21	−2.20	0.03	—	—	—	—	—	—
Airport Ditch, TX (sp)	16.50 ± 7.11	1.00 ± 0.33	10	54	−2.71	0.007	46.67 ± 12.46	4.56 ± 3.13	9	42	−2.31	0.02
Weslaco, TX (sp)	17.08 ± 4.90	4.08 ± 10.56	12	66	−2.94	0.003	47.93 ± 11.18	1.00 ± 0.66	15	117.5	−3.27	0.001
Aquarena Spring, TX (ap)	37.42 ± 9.62	8.50 ± 2.51	12	65	−2.04	0.04	44.33 ± 9.93	12.17 ± 2.72	12	67	−2.20	0.03
Salt Creek, TX (ap)	49.50 ± 13.38	16.00 ± 4.87	14	82	−1.85	0.06	31.36 ± 11.30	3.74 ± 0.79	14	83.5	−1.95	0.05
Lafayette, LA (ap)	25.24 ± 5.22	9.29 ± 2.66	17	125.5	−2.32	0.02	20.14 ± 5.97	8.36 ± 3.87	14	77	−1.54	0.12
Destin, FL (ap)	26.92 ± 9.88	25.38 ± 7.20	12	45	−0.04	0.97	4.17 ± 1.09	16.50 ± 4.01	11	4.5	−2.54	0.01
Florida Keys, FL (ap)	37.20 ± 10.28	4.60 ± 2.63	10	45	−2.67	0.008	36.09 ± 8.95	9.27 ± 3.17	11	59	−2.31	0.02

Males from sympatric populations generally showed a stronger conspecific preference than males from allopatric populations in both the foreign treatment (figure 2*a* and table 2) and the native treatment (figure 2*b* and table 2). There was no significant difference in the strength of male preference between the foreign treatment and the native treatment (Wilcoxon's signed-ranks test, $n = 91$, $T + = 2204$, $z = -0.439$, $p = 0.66$, two-tailed).

There was no significant correlation between the size of the male and the proportion of thrusts directed at Amazon mollies (Spearman's rank correlation, $r = -0.116$, $z = -0.886$, $p = 0.38$ for the sympatric foreign treatment; $r = -0.104$, $z = -0.735$, $p = 0.46$ for the sympatric native treatment; $r = -0.043$, $z = -0.348$, $p = 0.73$ for the allopatric foreign treatment; $r = -0.001$, $z = 0.011$, $p = 0.99$ for the allopatric native treatment).

In both the foreign treatment and the native treatment, males from the pooled sympatric populations showed a significantly greater strength of preference for conspecific females than did males from the pooled allopatric populations (Mann–Whitney *U*-test, $U = 2768$, $z = -4.11$ for the foreign treatment (figure 3*a*) and $U = 2481.5$, $z = -5.12$ for the native treatment (figure 3*b*), two-tailed). There was no significant difference in the strength of preference among the sympatric populations in either treatment (Kruskal–Wallis test, d.f. $= 5$, $H = 2.83$, $p = 0.73$ for the foreign treatment; d.f. $= 4$, $H = 6.90$, $p = 0.14$ for the native treatment). However, the results for the allopatric populations were significantly different, in both treatments (Kruskal–Wallis test, d.f. $= 4$, $H = 10.21$, $p = 0.037$ for the foreign treatment; d.f. $= 4$, $H = 15.63$, $p = 0.003$ for the native treatment). When the results from Destin, Florida, are removed, there is no longer a significant difference in the strength of preference across the other four allopatric populations (Kruskal–Wallis test, d.f. $= 3$, $H = 5.40$, $p = 0.14$ for the foreign treatment; d.f. $= 3$, $H = 0.75$, $p = 0.86$ for the native treatment). However, the inclusion of Destin in the results does not change the significance of the overall comparison of sympatry to allopatry. We did not exclude any populations in our pooled comparisons.

4. DISCUSSION

Our results provide strong evidence for reproductive character displacement in male mate preference. Males of *P. latipinna* from six populations in sympatry showed a significantly greater strength of preference for conspecific females than did males from five populations in allopatry. These results support the earlier findings of Ryan *et al.* (1996) using a few of the same populations of mollies. The large geographical range of populations that we tested
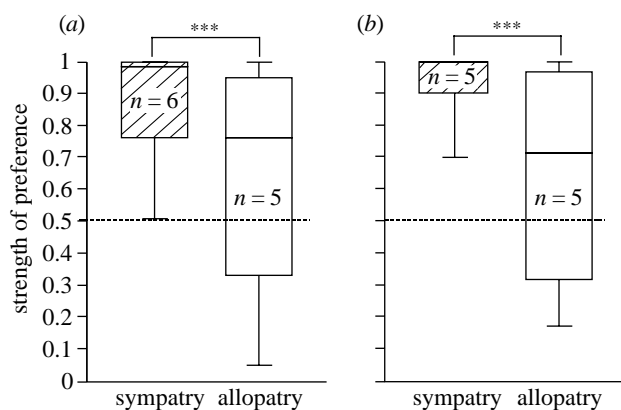
Figure 3. Box plots representing the pooled male strength of preference for (*a*) foreign treatments and (*b*) native treatments. Data above the no-preference line ($y = 0.5$) indicate an increasing strength of preference for sailfin molly females (*Poecilia latipinna*), data below the line indicate increasing preference for Amazon molly females (*P. formosa*); n, number of populations tested; ***$p \leqslant 0.001$, Wilcoxon's signed-ranks test.

reveals how male mate preference can vary along a transect from sympatric to allopatric populations. We found less variation in male behaviour in sympatry than in allopatry. This could occur because there is selection to avoid Amazon mollies in sympatry, but such avoidance in allopatry is probably an incidental consequence of conspecific mate recognition. This is one of only a few studies to examine variation in mating preference, as opposed to merely documenting variation in a signal, across a large number of allopatric and sympatric populations (table 1).

Butlin & Ritchie (1991) examined fine-scale variation in female mate choice in two subspecies of grasshopper, *Chorthippus parallelus parallelus* and *Chorthippus parallelus erythropus*. They examined 16 populations across a narrow hybrid zone extending 25 km north and 35 km south of this zone. They found that selection operates on female preferences in the hybrid zone, based on evidence of an increased homogamic preference on one side of the zone relative to more distant populations. However, their results shed little light on the importance of reinforcement between the incipient species.

Ryan *et al.* (1996) found that male sailfin mollies from a population sympatric with Amazon mollies showed a greater strength of preference for conspecific females over gynogenetic *P. formosa* females than did males from two allopatric populations. They suggested that the male mating preferences of *P. latipinna* were likely to have been influenced by selection for reproductive character displacement. Their results were also consistent with earlier findings by Hubbs (1964) that males from sympatric and allopatric populations preferred females of *P. latipinna* to those of *P. formosa*, and that the strength of preference was greater in sympatric populations. We tested fishes from a population near the sympatric population (Brownsville, Texas) and from populations near the allopatric populations (Galveston, Texas and Florida Keys, Florida) collected by Ryan *et al.* (1996). Our results for these populations are very similar to those of Ryan *et al.* (1996).

Their Florida Keys population of males showed an especially strong preference for conspecifics in the foreign treatment ($p = 0.005$) and a weaker preference in the native treatment ($p = 0.036$) (Ryan *et al.* 1996). Their Galveston and our Salt Creek, Texas, populations showed an intermediate preference for conspecifics ($p = 0.02$) in the foreign treatment. Our data also showed a similar strength of preference for conspecifics ($p = 0.004$) by males to their sympatric populations. This suggests that these behavioural traits remain relatively constant over time and might explain why there is less variation in the strength of male preference in sympatry compared to allopatry. Furthermore, we tested males from an introduced population (Aquarena Springs, Texas) that consisted of fishes introduced from Louisiana and Florida (allopatry) sometime after 1941. In this population we observed the expected outcome for an allopatric population. Thus, the behaviour of males from allopatry was consistent even when their localities were changed.

Ryan *et al.* (1996) proposed that the general preference for conspecifics by both allopatric and sympatric males indicates that this discrimination response is not an evolutionary response to selection to avoid mating with Amazon females. They suggested that discrimination between sailfin and Amazon mollies is an incidental consequence of a more general conspecific preference, but they pointed out that the strengthening of preference for conspecifics in sympatry is indicative of reproductive character displacement. Our results provide the necessary detailed analyses to support this conclusion. Because hybridization does not occur, we did not examine whether or not reinforcement was the process by which our pattern of reproductive character displacement arose.

Our fine-scale analysis supports the hypothesis that gene flow from sympatric populations does not swamp the behavioural differences between males from allopatric populations and those from sympatric populations. Moreover, there is no unique environment in sympatry that created the observed displacement in mating preference. However, it is apparent that there are subtle differences among populations, suggesting that had we selected one or two points in allopatry or sympatry we might not have detected reproductive character displacement (figure 2a,b). Prior to these results it had been implicitly assumed that there was no variation within sympatry or allopatry. This can no longer be assumed based on our data.

Woodhead & Armstrong (1985) suggested that *P. formosa* are more likely to mate with small males. Smaller males are more likely to thrust than to court a female (Farr *et al.* 1986; Ptacek & Travis 1996). Larger males are also more discriminating than smaller males (Ptacek & Travis 1997; Sumner *et al.* 1994; Travis 1994) suggesting that there might be a negative relationship between male size and the proportion of thrusts directed to the Amazon molly. Our results indicate that there is no negative relationship between male size and the proportion of thrusts directed at gynogens. Ryan *et al.* (1996) also rejected this hypothesis.

It is clear that our primary results stem from males in sympatry showing greater strength of preference for conspecifics than males from allopatry. Moreover, additional support for reproductive character displacement

arises from the observed variation in the response of males from sympatric populations to native and foreign females. While we did not directly test male preference for native versus foreign conspecifics in a treatment combination, it is clear that male *P. latipinna* show a stronger preference for native females than for foreign females, based on their higher strength of preference and lower variance in strength of preference in the native treatment than in the foreign treatment (figure 2). This pattern is clearly evident in the pooled comparison (figure 3). However, when we compared the proportions of thrusts directed towards native versus foreign females we did not find a significant difference, nor was there a consistent pattern of higher levels of thrusting directed towards native females than towards foreign females across all sympatric populations (table 2; two populations show the reverse trend and Central Ditch could not be examined). In the three populations that did show higher levels of thrusting directed towards native females, however, the difference in the number of thrusts was 35–45% higher in response to the native females (table 2). This increased strength of preference for native females over foreign females in sympatric male populations is exactly what we would predict if reproductive character displacement in male mate preference was occurring. On the other hand, Ptacek & Travis (1997) did not find that males showed a differential response to native and foreign conspecific females. Both of the populations they examined would be classified as far allopatry in our study and thus there should be no selection pressures on males to refine their mating preferences in these allopatric populations. Ptacek & Travis (1997) indirectly came to the same conclusion when they suggested that selection on males to discriminate between foreign and native females was likely to be extremely weak; whereas they suggested that selection on females should be stronger, and thus females in their study preferred native males to foreign males.

It is not clear why the results from the two allopatric Florida populations were not consistent with the results from our other allopatric populations (the last two populations in allopatry on the right of figure 2*a,b*). It is possible that the behavioural responses of these populations are a result of gene flow from sympatric or allopatric populations. We are in the process of examining the population genetic structure of the 12 populations used in this study to help clarify these results. Our results are consistent with the large inter-population variance found in male behaviour within Florida (Ptacek & Travis 1996). Alternatively, our Florida results might stem from an interaction of female behaviour with male behaviour. For example, the Amazon mollies might be more receptive than sailfin females at all times, and this might have caused the males from Destin, Florida, to preferentially thrust towards the Amazon females. We are in the process of examining some of these issues.

The question that arises from our results is how are gynogens maintained in the system? Our data and previous data (Hubbs 1964; Woodhead & Armstrong 1985; Schlupp *et al.* 1991; Ryan *et al.* 1996) suggest that males of *P. latipinna* (and *P. mexicana*) can discriminate between conspecific females and gynogenetic Amazon mollies. It has been assumed that males obtain no benefits from mating with gynogens. But Schlupp *et al.* (1994)

demonstrated in the laboratory that when males of *P. latipinna* associate with *P. formosa*, conspecific females copy the mate choice of the females of *P. formosa*, thus increasing the males' attractiveness to conspecific females. It is not clear whether this benefit of increased attractiveness outweighs the costs of mating with a heterospecific. In addition to the benefits of mating with gynogens, it is also evident from our research that males continue to attempt to mate with these gynogens even when there is a conspecific female available. Even if mating with a gynogen results from a mistake on the male's part, it may take only one mating to start embryogenesis in the gynogen. Finally, none of the studies so far have ascertained the method by which males differentiate gynogens from conspecifics. Gonoporal nibbling by orally contacting the female's gonopore (the opening of the female genital tract into the vent area) may aid a male in differentiating between females. There may also be morphological differences in body shape that males could use as cues to differentiate the species.

In conclusion, our data provide strong evidence for reproductive character displacement of male mate preference in sympatric populations. Our data verify the change in male mate choice across a large portion of the range of sailfin mollies and demonstrate that gene flow between sympatric and allopatric populations of *Poecilia* is not obscuring the pattern of similarity in allopatry and divergence in sympatry. While the overall pattern of reproductive character displacement is clear, our data indicate that it is necessary to evaluate numerous populations in sympatry and allopatry because variation in behaviour occurs within sympatry and within allopatry; thus, testing only a few populations might obscure the overall pattern.

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