



Characterizing the mating behaviours of the Tamesí molly, *Poecilia latipunctata*, a sailfin with shortfin morphology

MARGARET B. PTACEK, MICHAEL J. CHILDRESS & MICHELE M. KITTELL

Department of Biological Sciences, Clemson University

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The Tamesí molly is an unusual molly species because it has features of both shortfin and sailfin mollies. Morphologically, it is classified as a shortfin species, yet phylogenetic evidence and observations of male courtship behaviour indicate that it should be classified as a sailfin molly. We characterized the mating behavioural profiles of dark and silver male *P. latipunctata* by varying the competitive environment (presence or absence of male competitors) and the degree of female receptivity (unreceptive versus receptive females), both factors known to increase rates of courtship displays in sailfin species. We measured rates of three mating behaviours: courtship displays, gonoporal nibbles and gonopodial thrusts (forced insemination attempts) under both types of competitive and female receptivity conditions. Rates of courtship displays were strongly influenced by female receptive state; both dark and silver males elevated their rates of courtship displays in the presence of receptive females in either competitive environment. In addition, the influence of competition for mates (presence of a competitor) had a stronger effect on the courtship display rates of dark males, but only in the presence of receptive females; courtship display rates were almost eight times higher for dark males when at least one female in the social group was receptive (≤ 24 h postpartum). These results suggest that dark males actively compete for receptive females by increasing their reliance on courtship, a behavioural response clearly characteristic of other sailfin molly species.

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Poeciliid fish provide an interesting system in which to study the evolution of courtship behaviours for several reasons. First, courtship is lacking in most poeciliid species (Rosen & Tucker 1961; Farr 1989), suggesting that it is not a necessary prerequisite for successful mating to occur. Instead, males of all poeciliid species observed to date perform a type of forced insemination attempt termed a gonopodial thrust, where the male orients himself behind a female, brings the gonopodium (fused anal fin that serves as an intromittent organ for internal fertilization) to a forward position, and swimming forward, attempts to insert the tip into the female's gonopore for sperm transfer. This behaviour has no signal function and there are no countersignals from the female, so it cannot be considered a form of display (Farr 1989). Second, courtship displays do occur in a subset of poeciliid species, having evolved independently a number of times in different species in the genera *Poecilia* and *Xiphophorus* (reviewed in Farr 1989). Thus, lack of courtship appears to

be ancestral in poeciliids and courtship displays seem to be multiply, independently derived behaviours.

Certain features of courtship displays are shared among courting species of poeciliids. All poeciliid courtship displays are primarily visual and involve a combination of stereotyped swimming motions and fin postures performed by males towards females in an attempt to elicit female cooperation in mating. In many species, female acceptance postures precede copulation by gonopodial insertion (Rosen & Tucker 1961; Houde 1997), and sperm transfer is more often successfully accomplished when females cooperate with gonopodial thrusts by males (Pilastro et al. 2002). Hence, Farr (1989) suggested that the influence of courtship displays in eliciting female cooperation in mating was a primary mechanism by which such courtship behaviours may have evolved in poeciliid fish.

Mollies (genus *Poecilia*, subgenus *Mollienesia*) mimic the pattern of evolution of courtship behaviours seen at the family level. Molly species are divided into two evolutionary lineages (Hubbs 1933; Miller 1983; Ptacek & Breden 1998): 'shortfin' mollies in the *P. sphenops* species complex and 'sailfin' mollies in the *P. latipinna* species complex. Sailfin mollies are derived from ancestral shortfin lineages (Ptacek & Breden 1998; Fig. 1) where reliance on forced

Correspondence: M. B. Ptacek, Department of Biological Sciences, 132 Long Hall, Clemson University, Clemson, SC 29634-0326, U.S.A. (email: mptacek@clemson.edu).

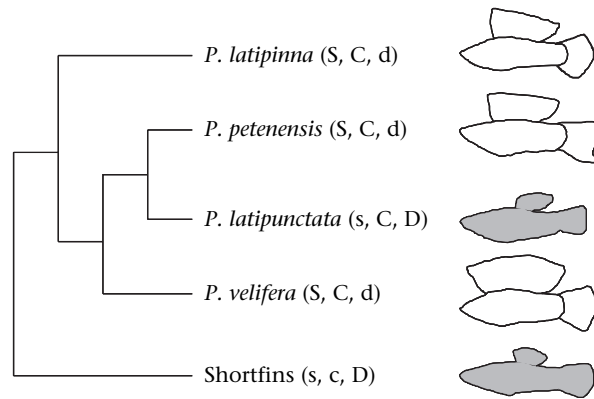


Figure 1. A phylogenetic comparison of dorsal fin morphology (S: sailfin present, s: sailfin absent), male courtship behaviours (C: courtship present, c: courtship absent), and male coloration (D: dark coloration present, d: dark coloration absent) for the monophyletic sailfin molly clade. The tree topology is after Ptacek & Breden (1998).

inseminations is the primary mating strategy. Thus, courtship displays, which are characteristic of all species of sailfin mollies (Farr et al. 1986; Farr 1989; Parzefall 1989; Ptacek & Travis 1996; Niemeitz et al. 2002), are the derived behavioural state in this lineage.

The marked distinction between sailfins and shortfins in their reliance on courtship versus forced inseminations, respectively, has led to divergence between the two lineages in morphological traits and characteristics of their mating behavioural repertoires. Most sailfin species are characterized by a sexual dimorphism in which males have a greatly enlarged dorsal fin (Regan 1913; Hubbs 1933; Parzefall 1969) that is erected and presented to the female in a courtship display (Parzefall 1969, 1979; Farr et al. 1986). Receptive females respond to this display by remaining stationary, folding the median fins and twisting the abdomen to accept copulation (Parzefall 1969). Males of the sailfin species *P. latipinna* show low levels of intermale aggression, both in the field and laboratory (Travis 1994), and are not known to form permanent male dominance hierarchies (Farr 1989). Thus, reproductive success in sailfin species appears to be more a function of female choice than of male–male competition.

In sharp contrast, males of shortfin molly species do not show sexual dimorphism in fin morphology and in four species (*P. sphenops*, *P. chica*, *P. orri* and *P. mexicana*) in which mating behaviours have been characterized (Parzefall 1969, 1979; Brett & Grosse 1982; Balsano et al. 1985; Woodhead & Armstrong 1985; Ptacek 1998, 2002), gonopodial thrusting appears to be the primary mating strategy (male *P. mexicana* perform a low level of courtship displays; the remaining species show no courtship behaviours). Thus, shortfin species do not appear to rely on female cooperation for mating; reproductive success in shortfin species is determined primarily by a social structure based on male–male aggression (Farr 1989). Based on field observations, males of several shortfin species (*P. chica*: Miller 1975; *P. sphenops*: Parzefall 1969; *P. mexicana*: Balsano et al. 1985) form dominance hierarchies, with dominant males assuming coloration (darkening of dorsal

and caudal fins and body to black) that is strikingly different from that of females or subordinate males in mixed groups. Similar changes in coloration by more aggressive, dominant males have been observed in aquarium settings as well (*P. sphenops*: Woodhead & Armstrong 1985; *P. mexicana*: Balsano et al. 1985; personal observation; *P. orri*: Ptacek 1998; personal observation). Dark males aggressively chase and nip at other males, preventing them from gaining access to females, and females are forcibly inseminated by these males through gonopodial thrusting (Farr 1989). Thus, in shortfin molly species, male–male competition appears to be a more prominent factor in determining male reproductive success than is female choice.

In this study, we examined the mating behaviours of the Tamesí molly. This species is unusual for several reasons. Although taxonomically classified as a shortfin species based upon morphology of the dorsal fin (Miller 1983; Rauchenberger 1989), subsequent phylogenetic studies using both nuclear and mitochondrial DNA markers have demonstrated its position within the sailfin molly lineage (Schartl et al. 1995; Ptacek & Breden 1998; Fig. 1). In addition to genetic evidence for its sailfin status, behavioural observations of a natural population in northern Mexico have identified the existence of male courtship behaviours that are characteristic of those performed by other sailfin species (Niemeitz et al. 2002). Niemeitz et al. also reported that during courtship, ‘courting males changed their colour from olivaceous to almost black’ (page 48), a characteristic previously only described for shortfin molly species (Farr 1989). Thus males of *P. latipunctata* appear to have retained courtship behaviour, a derived feature of the sailfin lineage, while having lost the enlarged sailfin, a potential secondary loss of this sexually selected trait (Fig. 1). Males of this species also have the shortfin characteristic that certain males become extremely dark during mating encounters. Because of this unusual combination of sailfin and shortfin morphological and behavioural features, quantifying the role of coloration and male–male competition in mating behaviours shown by male *P. latipunctata* may provide insight into the evolutionary pattern of behavioural divergence between sailfin and shortfin mollies.

Our study extended an earlier qualitative description of courtship behaviour in *P. latipunctata* (Niemeitz et al. 2002) to include quantifying the behavioural repertoire of males in a controlled laboratory setting. We asked whether male behavioural repertoires varied in response to female receptivity (gravid or postpartum) and in the absence or presence of male competitors. We chose these two factors because both influence mating behavioural rates in both courting and noncourting species of poeciliids. For example, within a species, both rates and types of male mating behaviours vary in response to changes in density (e.g. Farr & Herrnkind 1974; Cade & Cade 1992; Jirotkul 1999a), social status (e.g. Farr 1980a; Travis & Woodward 1989; Chase et al. 1994; Hsu & Wolf 1999) and female reproductive condition (e.g. Farr 1980b; Brett & Grosse 1982; Farr & Travis 1986; Sumner et al. 1994). For courting species in particular, increased male competition for mates results in elevated rates of courtship displays by larger, more highly ornamented males, but

smaller, drabber males often increase rates of gonopodial thrusting (Farr 1980a, b; Travis & Woodward 1989; Jirotkul 1999a, b). The presence of receptive females increases rates of both courtship displays and gonopodial thrusts in species with courtship (Crow & Liley 1979; Farr 1980b; Farr & Travis 1986; Sumner et al. 1994) and increases levels of aggression and agonistic behaviours in addition to elevating gonopodial thrusting rates in noncourting species (Brett & Grosse 1982; Woodhead & Armstrong 1985). Thus, we predicted that, if male *P. latipunctata* behave as do other sailfin molly species, they should increase rates of courtship displays in response to receptive females and in the presence of male competitors. Alternatively, if male *P. latipunctata* behave as do other shortfin molly species, they should increase rates of gonopodial thrusting and aggressive behaviours, such as chases and nips, in the presence of receptive females and male competitors. Finally, we compared mating behavioural responses to varying female receptivity and male competition between dark and silver males, predicting that dark males would become more aggressive in response to receptive females and male competitors as do their dark male counterparts in shortfin molly species (Balsano et al. 1985; Woodhead & Armstrong 1985).

MATERIALS AND METHODS

Origin and Maintenance of Subjects

A stock population of 40 males and 40 females was established in the laboratory of M.B. Ptacek at Clemson University in 2002 from wild-caught individuals collected from a spring in the Río Tamesí drainage in Tamaulipas, Mexico in March 2002 (Mexican Collecting Permit No. 01.01.02.613.03). A second collection of an additional 80 individuals was made from this same site in March 2003 (Mexican Collecting Permit No. 240103.613-03) and individuals were shipped to Clemson University to augment the existing stock population. Individual fish were collected by repeatedly towing a 2.8×1.2 -m seine along the shoreline of the spring, where high densities of *P. latipunctata* were found in shallow water near emergent vegetation.

In the laboratory we maintained the fish in a large Rubbermaid stock tank (600 litres; Fairlawn, Ohio, U.S.A.) in calcium chloride-enriched freshwater at a pH of 7.0–7.4 and a temperature of 25°C. Lights were provided by Sylvania Gro-lux fluorescent bulbs (20-W, full spectrum 350–750 nm, with spectral peaks at 400, 440 and 540 nm; Danvers, Massachusetts, U.S.A.) and kept at a controlled 14:10 h light:dark cycle. Fish were fed once or twice daily with commercial flake food (Ocean Star International Freshwater Flake (60%) with Brine Shrimp Flake (38%) and Spirulina Flake (2%) mixture; Burlingame, California, U.S.A.), dried algae wafers and frozen brine shrimp (*Artemia* sp.). Care and housing conditions followed protocols approved by the Clemson University's Animal Research Committee (Protocol No. 20044). The stock population has maintained a density of approximately 150 breeding adults plus juveniles under these

laboratory conditions for 3 years. Adult males and females used in this study were a mixture of wild-caught and first-generation laboratory-reared individuals (see description of individual experiments below).

Establishment of Social Tanks and Colour Status

Subjects were housed in Clemson University's Godley-Snell Animal Research Facility (Animal Research Committee Protocol No. 01-021). We established and maintained 19 social groups over the course of the study (17 July 2002–31 March 2004). Each social group consisted of three adult males and three adult females housed together in a 38-litre ($51 \times 25 \times 31$ -cm) aquarium. Sexual maturity of males was ascertained by the presence of a fully developed gonopodium; in females, sexual maturity was indicated by the presence of a gravidity spot around the female's gonopore (Medlen 1951; Constantz 1989). The three males differed in size by at least 3 mm, such that each social group consisted of a large (mean = 36.74, range 33–44 mm), an intermediate (mean = 30.74, range 28–34 mm) and a small (mean = 26.11, range 23–29 mm) male. This composition allowed for individual identification of each male, which was required for the paired design of behavioural tests and ensured that at least one male in the social group was likely to become dark. Male *P. latipunctata* formed social hierarchies in group tanks, similar to those described for other shortfin molly species (Balsano et al. 1985; Woodhead & Armstrong 1985), with one ($N = 16$), or sometimes two ($N = 3$), of the males becoming much darker than other males and females. Such dark coloration has been observed in courting males in natural populations as well (Niemeitz et al. 2002; personal observation). Based on coloration of the dorsal fin, caudal fin or body, each male in the tank was classified as either silver (lacking black pigmentation) or dark (with black pigmentation). Although the intensity of this black pigmentation for dark individuals was temporally variable, these individuals were always appreciably darker than silver ones. Thus, we assigned males in each group a colour status along with individual numbers. These social groups were maintained throughout the course of each experimental series, and if a male in one of the group tanks became diseased or died during the course of its experimental series, we eliminated the group tank and tested no other males from this tank.

Behavioural Observations

Unreceptive female trials with wild-caught males

In the first series of trials (conducted between 17 July and 13 December 2002), we examined the behaviour patterns of males in response to changes in competitive environment using unreceptive females. Each male was tested twice on two consecutive days, first alone with a single gravid female, and second with a different gravid female and one small competitor male. We tested 14 wild-caught males in this experimental series, three dark males

and 11 silver males from eight social tanks. The small competitor male was one of two silver males less than 26 mm. These males were housed in separate social tanks from test males used in the behavioural trials, and thus were unfamiliar to the test males.

Unreceptive females with laboratory-reared males

We repeated this series of trials between 8 February and 31 March 2004 to increase our sample size and to test for two additional effects: order of treatment (competitor present or absent) and responses of laboratory-reared males (rearing status). Again, each male was tested twice on consecutive days, but this time half the males ($N = 8$) were first tested with a competitor male present and the other half ($N = 8$) were first tested with the competitor male absent. We tested 16 males, all first-generation laboratory-reared, eight dark males and eight silver males from eight social tanks. The small competitor male was one of two different males, both 24-mm silver males that were unfamiliar to the test males.

Object females used with both groups of males were mature, gravid females that were housed separately from test males and thus were unfamiliar to the male subjects. Females that were used as objects in the first treatment (single male) were 35–45 mm long, and those used in the second treatment (addition of competitor male) were 28–44 mm long; the size differences were not significantly different between the two treatment groups (Student's t test: $t_{58} = 0.881$, $P = 0.382$).

Receptive female trials

In the third series of trials (conducted between 29 September 2002 and 7 May 2003), we examined the behaviour patterns of males in response to changes in competitive environment when tested with receptive females. We tested 21 males in this series (12 black and 9 silver), chosen from 11 social tanks. These males were wild-caught and were different individuals than those used in the unreceptive female experimental series; 11 were from the 2002 collection and 10 were from the 2003 collection.

As before, each male was tested twice, first alone with a single receptive (1–2 days postpartum) female and second with a single receptive female and one small, competitor male. Females that were used as objects in the first trial (single male) were 40–50 mm long, and those used in the second trial (addition of male competitor) were 36–48 mm long; the size differences were not significantly different between the two treatment groups (Student's t test: $t_{40} = 1.760$, $P = 0.086$). Gravid females were housed individually in 20-litre aquaria until they had given birth. These females were then used as object females in trials within 48 h of the birth of their broods. Female mollies become receptive immediately after producing a brood and signal this receptivity to males through pheromonal cues (Parzefall 1973; Brett & Grosse 1982; Farr & Travis 1986; Sumner et al. 1994). Because males could only be tested in either treatment when a female had given birth, the time elapsed between the first trial (single male) and the second trial (with the small male competitor) varied between 1 day and 4 months. Male sailfin mollies generally

maintain characteristic activity levels for long periods in the laboratory (Ptacek & Travis 1996, 1997) and behavioural profiles are independent of age through ages of 270 days (J. Travis, unpublished data).

As in experiment 1, competitor males were small (<27 mm), silver males housed in separate social groups from test males and thus were unfamiliar to the male subjects. Four males were used as competitors in this series because of the death of the first two male competitors (same males as used in experiment 1) during the experiment.

We performed behavioural observations for all three experiments in a 20-litre aquarium. To minimize disturbance to the fish from the presence of the observer, we covered the front side with one-way film. The outside of the other three sides of the tank was covered with black poster board to minimize glare and reflectance of the sides of the observation tank to the test males. The tank was illuminated from above by a single, 20-W Gro-lux fluorescent bulb (same lighting as used for stock and social tanks). We placed either the test male or the test male with a small male competitor into the tank and allowed 15 min for acclimation. We then added the object female to the test tank and allowed an additional 15 min for acclimation. We observed test males for 10 min and scored three measures of sexual behaviour with a laptop event recorder: number of courtship displays, number of gonopodial thrusts and number of gonoporal nibbles. All behavioural observations were made by the same observer (M.B.P.).

Established social hierarchy trials

To assess the influence of female receptivity on male mating behaviour by males of different colour status in a more natural social environment (multiple male competitors), one of us (M.J.C.) videotaped 10-min segments from the eight social tanks that were established using the 24 laboratory-reared males. We filmed the fish with a Sony Handycam DCR-TRV740 digital video camera set-up on a tripod in front of a tank. Social tanks were established as described above with three different-sized adult males and three adult females in each. The social tanks were established on 29 January 2004 and video trials were conducted twice for each tank. The first video trial was conducted for each of the eight social tanks 24 h after their initial establishment (30 January 2004). Females in all eight tanks were gravid and thus unreceptive. The second video trial was conducted within 24 h of the birth of young in each of the tanks (16 February, 3 March, 9 March, 20 March and 31 March) to assess the behavioural profiles of males of each colour status in response to receptive females. Observation hour ($F_{1,6} = 1.013$, $P = 0.353$) and trial date ($F_{1,6} = 0.818$, $P = 0.401$) did not significantly influence the number of mating behaviours performed by dark and silver focal males in social tanks with receptive females.

A different observer (M.B.P.) scored each video trial (16 trials, 8 with unreceptive females, 8 with receptive females) for each of two focal males, one black and one silver, haphazardly chosen from the three males in a tank ($N = 32$ behavioural observations). For each focal male, the number of courtship displays, the number of

gonopodial thrusts and the number of gonoporal nibbles directed towards any female was recorded. The observer also recorded the number of chases (focal male swims at an accelerated speed swimming within a single body length or less, often darting at the other individual) directed towards any other male in the tank. Using this design, the observer was blind with respect to the receptive state of females when scoring all video trials.

Statistical Analyses

All statistical analyses were performed with SYSTAT Version 10 (Point Richmond, California, U.S.A.). We first square-root-transformed rates of each behaviour to meet the assumptions of the homogeneity of variances. Both square-root-transformed measures and natural log-transformed measures resulted in well-behaved residuals with similarly normal distributions. However, we chose to analyse the square-root-transformed measures for the behavioural counts, which occasionally included zero sum cells that could not be natural log-transformed.

We first tested for treatment presentation order (male competitor treatment first or second) for the 16 laboratory-reared males tested with unreceptive females and found no significant effects of treatment presentation order for any behaviour (Student's t test: $t_{14} = 0.038$ – 1.962 , $P = 0.070$ – 0.929). We then conducted an analysis of covariance (ANCOVA) to examine the effects of female receptivity (unreceptive versus receptive) and male coloration (silver versus dark) on the behavioural rates for each of the three mating behaviours (displays, nibbles and thrusts). Male size (mm standard length) and male rearing condition (wild-caught versus laboratory-reared) were included as the covariates. The same object males were observed both in the absence and presence of a small, unfamiliar silver male, so we analysed these trials in separate ANCOVAs to meet the assumption of independence of observations, thus avoiding pseudoreplication. For the combined data set, each ANCOVA (no competitor and competitor) was based on a set of 51 independent observations of 51 different males (23 dark, 28 silver). Six different unfamiliar silver males were used as competitor males across the three experimental trial series and the number of behaviours performed was not significantly related to the specific male competitor present (trials with laboratory-reared males: $F_{1,12} = 0.819$, $P = 0.383$; trials with wild-caught males: $F_{3,17} = 0.284$, $P = 0.837$).

Last, we conducted an ANOVA to examine the effects of social tank and male coloration (silver versus dark) on the behavioural rates for the three mating behaviours (displays, nibbles and thrusts) and focal male chases in the eight social tanks. We used social tank as a block variable to control for nonindependence of observations of silver and dark males from the same social tank. Separate ANOVAs were run for observations taken before any female became receptive (unreceptive treatment) and observations taken within 24 h of a female giving birth in the social tank (receptive treatment) to avoid pseudoreplication from observing the same male twice ($N = 16$ males, 8 dark and 8 silver).

RESULTS

Mating Behaviours of *P. latipunctata*

The courtship display observed during laboratory trials was similar to that described for males from a natural population in Mexico (Niemeitz et al. 2002) and to that of field observations of males from the spring population used in this study (M. B. Ptacek, personal observation). Male *P. latipunctata* raised and spread the dorsal fin, oriented themselves in front of or beside a female, and fanned the fin towards the female while bending their body in a C- or sigmoid-shape. These displays contained the same elements and sequence of these elements of courtship displays as those observed for other sailfin species (*P. latipinna*: Parzefall 1969, 1979; Farr et al. 1986; Ptacek & Travis 1996; Ptacek 1998; *P. velifera*: Parzefall 1989; personal observation; *P. petenensis*: personal observation).

Among all 19 social tanks, dark males ($N = 22$) were significantly larger (Student's t test: $t_{55} = 6.507$, $P < 0.001$) than silver males ($N = 35$), but there was overlap in the range of sizes of dark and silver males (dark, mean: 35 mm, range 28–44 mm; silver, mean: 29 mm, range 23–40 mm). The design of these social tanks, with one large, one small and one intermediate-sized male (to ensure individual identification), may have biased the size distribution of dark males towards larger sizes than those of dark males in natural populations. Dark males were usually, but not always, the largest male in a social tank. In five of 19 social tanks containing males of three sizes, the intermediate-sized male was dark and, in two of these tanks, the intermediate-sized male was the only dark male in the social group. In the other three tanks, both the intermediate-sized male and the largest male were dark, resulting in two dark males and one silver male. Although the intensity of male coloration varied temporally, once a male became dark, he was always noticeably darker than other males in the social tank throughout the entire time that the social tank was maintained.

Both dark and silver males of all sizes were observed performing all three mating behaviours (courtship displays, gonopodial thrusts and gonoporal nibbles; see <http://www.clemson.edu/~mptacek/research> for video clips of mating interactions for both dark and silver males). There was no influence of male size on the rates of any behaviour except for thrusts performed in the absence of a male competitor, where smaller males performed more thrusts than larger males (Table 1).

Both wild-caught and laboratory-reared males performed similar rates of all three mating behaviours, except that laboratory-reared males performed significantly lower rates of courtship displays in the presence of a male competitor than did wild-caught males (Table 2).

Influence of Female Receptivity and Competitive Environment

In the absence of a small male competitor, both dark and silver males performed significantly more courtship displays towards receptive females than towards

Table 1. Results of an ANCOVA for male mating behaviours in the absence of a male competitor

Behaviour	Source	MS	$F_{1,45}$	P
Displays	Male size (standard length, SL)	0.073	0.041	0.840
	Rearing status	0.249	0.141	0.709
	Colour status	0.244	0.139	0.711
	Female receptivity	70.231	39.841	<0.001
	Colour×receptivity	0.001	0.001	0.985
Nibbles	Male size (SL)	38.767	3.937	0.053
	Rearing status	0.032	0.003	0.955
	Colour status	22.373	2.272	0.139
	Female receptivity	0.209	0.021	0.885
	Colour×receptivity	15.326	1.557	0.219
Thrusts	Male size (SL)	72.507	4.381	0.042
	Rearing status	2.918	0.176	0.677
	Colour status	22.929	1.385	0.245
	Female receptivity	11.053	0.668	0.418
	Colour×receptivity	25.365	1.532	0.222

The number of each behaviour was square-root-transformed.

unreceptive females (Fig. 2, Table 1). The interaction between colour status and female receptive state was not significant. There was no effect of colour status or female receptive state on rates of gonopodal nibbles or gonopodial thrusts and no interaction between these main effects (Fig. 2, Table 1).

In the presence of a small male competitor, both dark and silver males performed significantly more courtship displays towards receptive females than towards unreceptive females (Fig. 3, Table 2). The interaction between male colour status and female receptive state was also significant because dark males averaged more displays towards receptive females (Fig. 3). There was no effect of colour status or female receptive state on rates of gonopodal nibbles or gonopodial thrusts and no interaction between these main effects (Fig. 3, Table 2).

Table 2. Results of an ANCOVA for male mating behaviours in the presence of a male competitor

Behaviour	Source	MS	$F_{1,45}$	P
Displays	Male size (SL)	0.565	0.263	0.611
	Rearing status	9.385	4.359	0.043
	Colour status	2.550	1.185	0.282
	Female receptivity	19.200	8.917	0.005
	Colour×receptivity	13.592	6.312	0.016
Nibbles	Male size (SL)	12.290	0.994	0.324
	Rearing status	5.813	0.470	0.496
	Colour status	3.386	0.274	0.603
	Female receptivity	20.154	1.630	0.208
	Colour×receptivity	27.409	2.217	0.143
Thrusts	Male size (SL)	23.823	1.362	0.249
	Rearing status	11.912	0.681	0.414
	Colour status	1.141	0.065	0.800
	Female receptivity	30.836	1.763	0.191
	Colour×receptivity	34.034	1.946	0.170

The number of each behaviour was square-root-transformed.

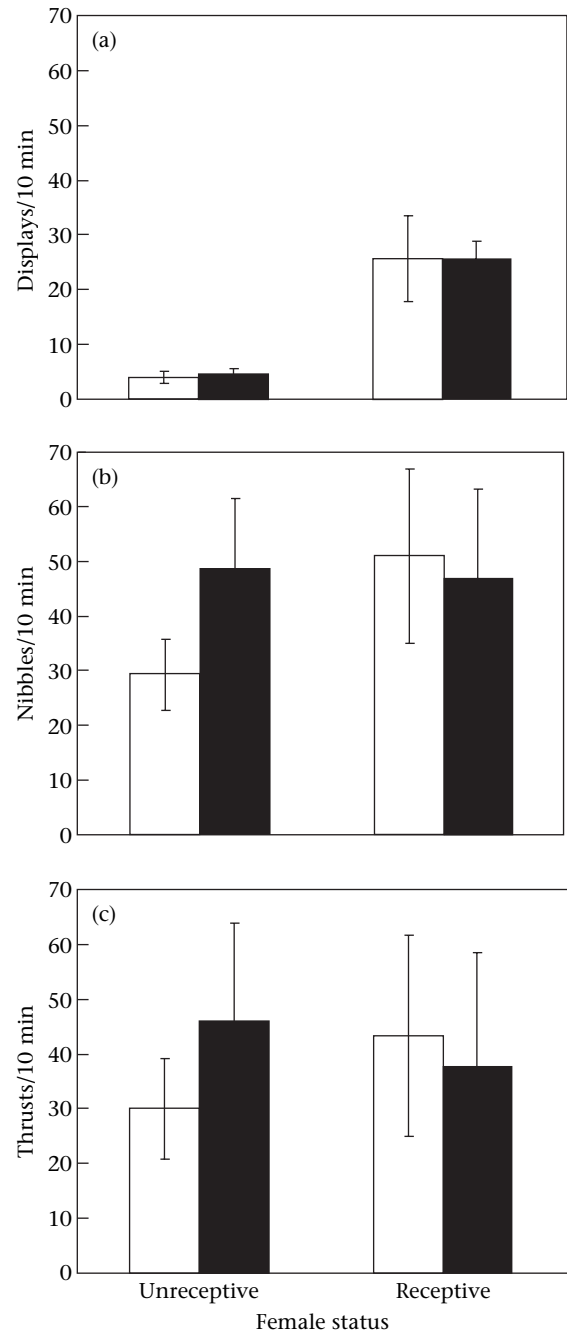


Figure 2. The influence of female receptivity (unreceptive versus receptive) on three mating behaviours of silver (□) and dark (■) males in the absence of a small male competitor. (a) Courtship displays, (b) gonopodal nibbles, and (c) gonopodial thrusts are presented as the mean ± SE number per 10-min observation.

Influence of Female Receptivity in Social Groups

Males of both colours performed extremely low rates of mating behaviours during the first observation of social tanks, when all females were unreceptive (Table 3, Fig. 4). The only significant effect was that of colour status on rates of gonopodal nibbling, with silver males showing

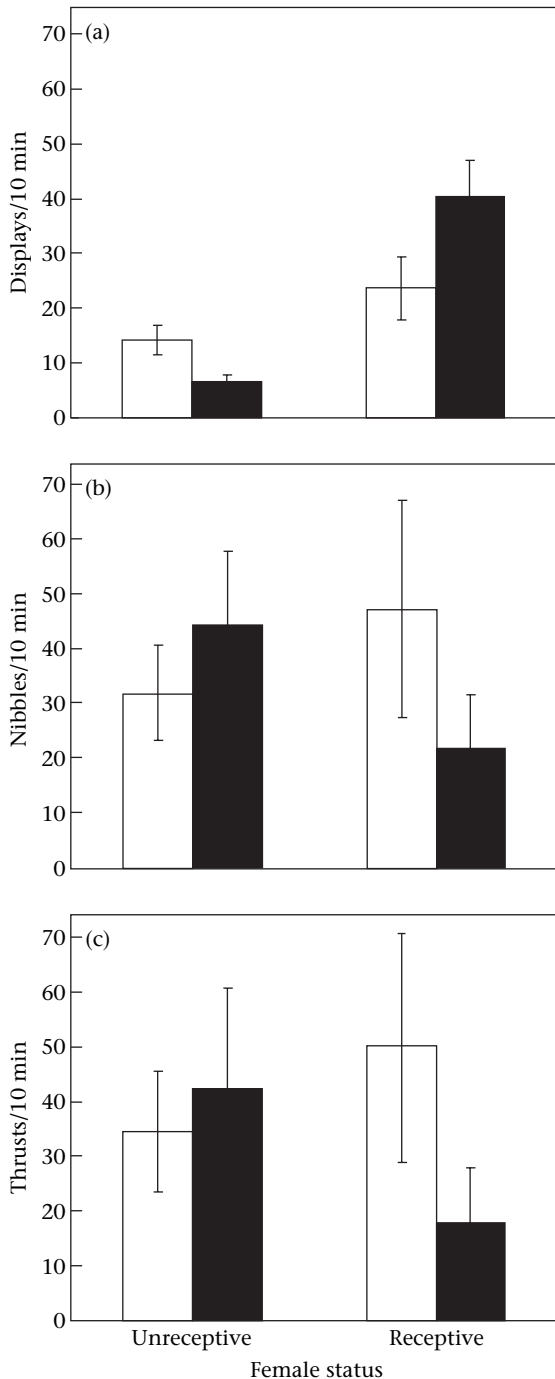


Figure 3. The influence of female receptivity (unreceptive versus receptive) on three mating behaviours of silver (□) and dark (■) males in the presence of a small male competitor. (a) Courtship displays, (b) gonoporal nibbles, and (c) gonopodial thrusts are presented as the mean \pm SE number per 10-min observation.

significantly higher rates than dark males (Table 3). Female receptivity, as in the first two experimental series, markedly influenced courtship display rates for dark males, with an eight-fold increase in display rates observed when receptive females were present in the social tank (Fig. 4). This increase resulted in a highly significant colour effect for display rates when receptive females were

Table 3. Results of ANOVAs for male mating behaviours and focal male chases in a social tank with three males and three females

Tank status	Behaviour	Source	df	MS	F	P
Unreceptive	Displays	Tank	7,7	0.136	0.737	0.651
		Colour status	1,7	0.175	0.943	0.364
	Nibbles	Tank	7,7	0.594	1.542	0.291
		Colour status	1,7	3.071	7.972	0.026
	Thrusts	Tank	7,7	0.487	1.000	0.500
		Colour status	1,7	1.093	2.245	0.178
Receptive	Displays	Tank	7,7	1.349	1.095	0.454
		Colour status	1,7	52.993	43.030	<0.001
	Nibbles	Tank	7,7	1.349	0.659	0.702
		Colour status	1,7	0.817	0.399	0.548
	Thrusts	Tank	7,7	1.394	0.774	0.628
		Colour status	1,7	1.260	0.700	0.430
Chases	Tank	7,7	0.413	1.503	0.302	
	Colour status	1,7	8.300	30.229	0.001	

Tanks were observed twice, once before any female had given birth (unreceptive treatment) and once within 24 h after the first female had given birth (receptive treatment). The number of each behaviour was square-root-transformed.

present (Table 3). The only other significant effect was an increase in the number of chases by dark males when receptive females were present (Fig. 4, Table 3). No other effects were significant, including no significant effects of trial date ($F_{1,6} = 0.818$, $P = 0.401$). Thus, the varying durations that males spent with unreceptive females (18–62 days before the birth of the first brood) between tanks did not appear to influence the behavioural response of males to receptive females.

DISCUSSION

Based upon the results of behavioural profiles of male *P. latipunctata* presented in this study, we conclude that the mating behaviours of the Tamesí molly position it as a member of the sailfin molly (*P. latipinna*) species complex. This conclusion corroborates its phylogenetic position as a sailfin molly (Schartl et al. 1995; Ptacek & Breden 1998). Despite certain morphological features shared with shortfin species (lack of an enlarged dorsal fin and the dark coloration in some males), the presence of the courtship display and the influence of female receptivity and male–male competition on increasing rates of courtship displays characterize *P. latipunctata* as a sailfin species. This result suggests that the transition from reliance on forced inseminations and male–male dominance hierarchies to courtship and female mate choice has been an important characteristic of speciation in all species of sailfin mollies.

Female receptivity influences behavioural rates in other poeciliid species that rely on courtship (Farr 1980b; Farr & Travis 1986), and this appears to be true for male

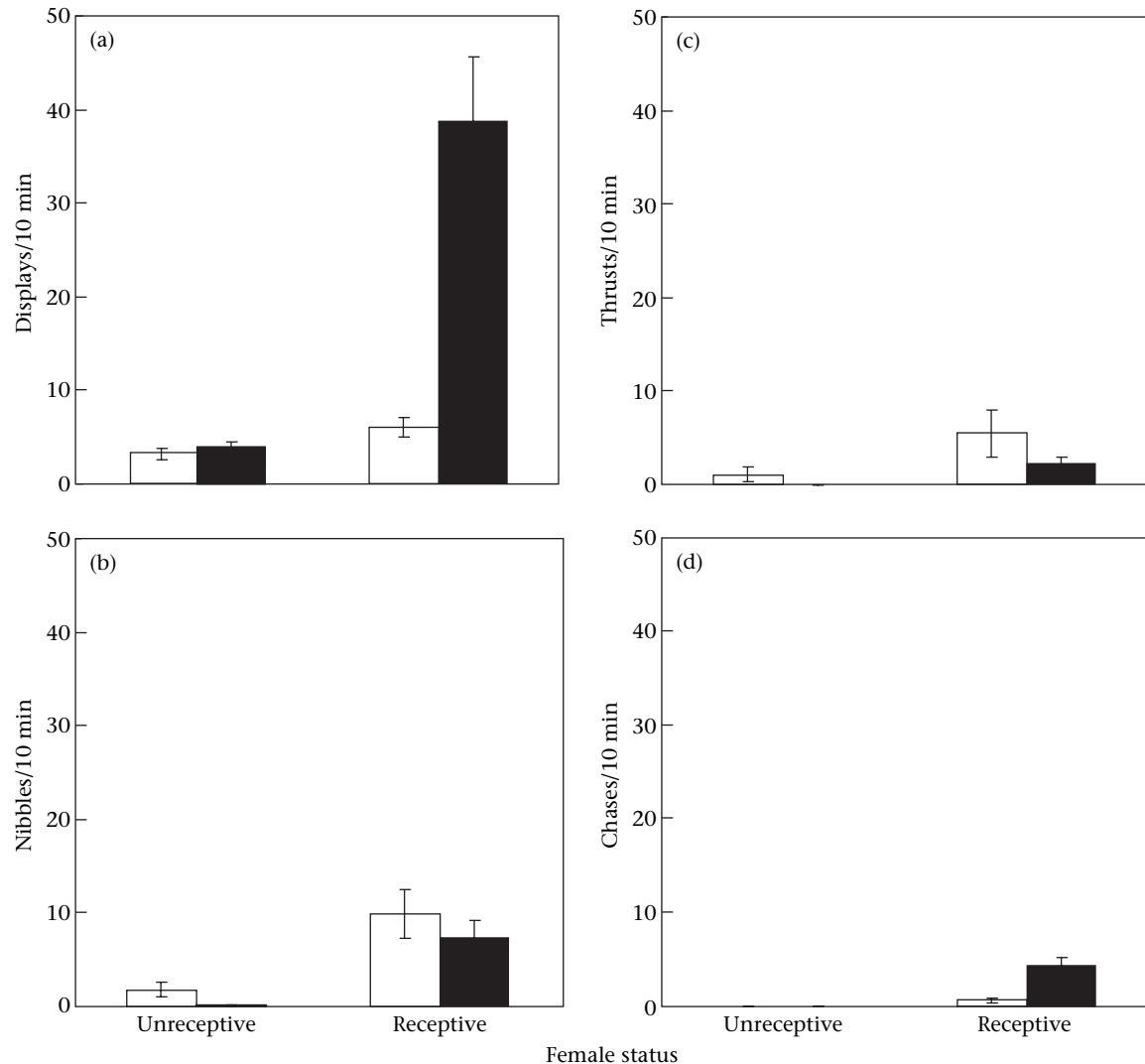


Figure 4. The influence of female receptivity (unreceptive versus receptive) on four behaviours of silver (□) and dark (■) males in an established social tank with three males and three females. (a) Courtship displays, (b) gonoporal nibbles, (c) gonopodial thrusts and (d) focal male chases are presented as the mean \pm SE number per 10-min observation.

P. latipunctata as well. Both dark and silver males increased their rates of courtship displays in response to receptive females. In guppies, males show increased courtship display rates and decreased rates of forced insemination attempts (thrusts) in the presence of receptive females (Farr 1980b). Male sailfin mollies increase the rates of all three mating behaviours in the presence of receptive females (Farr & Travis 1986), but this effect is more pronounced in large males than in small males (Sumner et al. 1994). Males appear to use chemical cues released by receptive females to perceive their receptive state, because elevated responses in behavioural rates towards receptive females have been observed only when males have direct contact with females (*P. reticulata*: Crow & Liley 1979; *P. latipinna*: Sumner et al. 1994) or contact with water containing chemical signals from postpartum females (*P. chicha*: Brett & Grosse 1982). Female *P. latipunctata* appear to release similar pheromones following the birth of young, as evidenced by the elevated courtship display rates observed in males in response to receptive (postpartum) females.

The influence of competitors on male courtship behaviours has been observed in other poeciliids as well. In guppies, *P. reticulata*, males increase their rates of courtship displays in response to the addition of a single, male competitor (Farr & Herrnkind 1974; Farr 1976, 1980a), but when the density of male competitors is high (Jirotkul 1999a) or the operational sex ratio is highly skewed in favour of males (Jirotkul 1999b, 2000), male guppies lower their courtship display rates and switch to gonopodial thrusting. In sailfin mollies, males adjust their behavioural rates in the presence of other males, but whether they increase courtship display rates or gonopodial thrusting rates depends upon both absolute male size as well as the size of a male in relation to other males in his group (Travis & Woodward 1989). Large males perform more displays and fewer thrusts; small males show the opposite pattern. Both dark and silver male *P. latipunctata* in the present study showed the strongest response to male competitors when competing for receptive females, by dramatically increasing their courtship display rates. Thus male *P. latipunctata*, like

large males of *P. latipinna*, increase reliance on courtship under increased competition for receptive females.

The courtship display is retained by male *P. latipunctata* despite the apparent loss of the enlarged dorsal 'sailfin'. This exaggerated sailfin is thought to have evolved to enhance the visibility of the courtship display (Regan 1913; Hubbs 1933; Parzefall 1969) and increases a male's overall size when courting a female (MacLaren et al. 2004). The sailfin is a known target of female mating preferences in *P. latipinna* with females preferring males with larger dorsal fins (MacLaren et al. 2004). Thus, the loss of this ornament in *P. latipunctata* is another example of the apparent loss of a sexually selected male trait (reviewed in Wiens 2001) and suggests that courtship displays may be more important in mate choice in this species.

In addition, the ability of some males of *P. latipunctata* to make their body and fins darker during social interactions is an unusual characteristic heretofore only described for shortfin molly species. Dark males have been observed in several shortfin molly species (*P. chica*, *P. sphenops*, *P. mexicana*, *P. orri*) both in natural populations (Parzefall 1969; Miller 1975; Farr 1989) and laboratory studies (Parzefall 1979; Ptacek 1998). The dark coloration in males of shortfin species is temporally variable, and the intensity of dark coloration has been linked to dominance through increases in levels of aggressive behaviours (nips and chases) directed towards subordinate, lighter males (Parzefall 1969, 1979; Balsano et al. 1985; Woodhead & Armstrong 1985). This temporal variability in the intensity of black colour in shortfin males is different from that of genetically based melanistic body colour, which results in black or spotted males of mosquitofish (*Gambusia affinis*: Regan 1961), sailfin mollies (Schröder 1964; Angus 1983), shortfin mollies (*P. sphenops*: Schröder 1964) and green swordtails (*Xiphophorus helleri*: Rosen 1960). Melanistic coloration and spotting is permanent and controlled by alleles that code for expression of aggregations of macromelanophores on various regions of the body. In contrast, temporally variable darkening of male body and fin pigment, such as that in some males of shortfin molly species and *P. latipunctata*, is controlled by micromelanophores, small melanin-containing pigment cells that tend to occur on the edges of scale pockets and form a network pattern of dark coloration (Angus 1989). In the present study, dark coloration in males of *P. latipunctata* was associated with increased rates of chases in the presence of receptive females and male competitors (Table 3, Fig. 4). The level of aggressive behaviours shown by dark males (as measured by chases), however, was much lower for males of *P. latipunctata* than has been reported for males of shortfin molly species (*P. sphenops*: Parzefall 1969, 1979; *P. mexicana*: Balsano et al. 1985; Woodhead & Armstrong 1985; Parzefall 1989). Thus in *P. latipunctata*, dark coloration appears to be associated more with increased levels of courtship than with increased levels of aggressive behaviours.

There are at least two possible evolutionary scenarios for why dark males exist in *P. latipunctata* (Fig. 1). One is that dark coloration is an ancestral trait found in shortfin mollies that has persisted in *P. latipunctata*, although its function in male–male aggressive interactions appears to have been diminished. Alternatively, dark colour expression

may have evolved independently a second time in *P. latipunctata*, and it may function more in mate attraction through its association with increased levels of courtship displays when males compete for receptive females. Future studies that examine the influence of male coloration in female mating preferences in *P. latipunctata* will help to distinguish between these evolutionary explanations.

Finally, male body size does not appear to strongly influence rates of any of the three mating behaviours (courtship displays, gonopodial thrusts, gonoporal nibbles) in *P. latipunctata* (Tables 1, 2), and this lack of association is probably not merely because of the narrow size distribution of mature males (23–44 mm) in the study population. In the congeneric sailfin species *P. latipinna*, populations with similar male size distributions to that of *P. latipunctata* showed strong positive associations between male size and courtship display rate (Ptacek & Travis 1996). However, other species of poeciliid fish lack relationships between male size and behavioural rates (guppies: Farr 1980a, b; *Heterandria formosa*: Bisazza et al. 1996; *P. mexicana*; one population of *P. latipinna*: Ptacek 2002).

Despite its unusual morphology, *P. latipunctata* is clearly a sailfin molly based upon both its genetic and behavioural allegiance with the sailfin molly clade. Future studies of mate choice should focus on the relative influences of courtship displays, dorsal fin size and male coloration on female mating preferences and how changes in such preferences may have influenced the gain and loss of morphological features in this unusual molly species.

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