

Melanic body colour and aggressive mating behaviour are correlated traits in male mosquitofish (*Gambusia holbrooki*)

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Correlated traits are important from an evolutionary perspective as natural selection acting on one trait may indirectly affect other traits. Further, the response to selection can be constrained or hastened as a result of correlations. Because mating behaviour and body colour can dramatically affect fitness, a correlation between them can have important fitness ramifications. In this work, melanic (black) male mosquitofishes (*Gambusia holbrooki*) with temperature-sensitive body-colour expression are bred in captivity. Half of the sons of each melanic sire are reared at 19 °C (and express a black body colour) and half are reared at 31 °C (and express a silver body colour). The two colour morphs are placed in the same social setting and monitored for behavioural differences. Mating behaviour and colour are correlated traits. Mating behaviour differs markedly between the two phenotypes, despite high genetic relatedness. Melanic (black) phenotypes are more aggressive towards females, chasing them and attempting more matings than their silver siblings. Females avoid melanic-male mating attempts more than silver-male mating attempts. When males with temperature-sensitive colour expression are melanic and aggressive, they probably experience a very different selective regime in nature from when they are silver and less aggressive. Under some conditions (e.g. predation), melanic coloration and/or aggression is advantageous compared with silver coloration and/or less aggressive behaviour. However, under different conditions (e.g. high-frequency melanism), melanism and/or aggression appears to be disadvantageous and melanic males have reduced survival and reproduction. Selective advantages to each morph under different conditions may enable the long-term persistence of this temperature-sensitive genotype.

Keywords: teleost; live-bearing fishes; melanism; phenotypic plasticity

1. INTRODUCTION

Correlated traits abound in natural organisms (Lande & Arnold 1983; Campbell *et al.* 1994; Brooks & Endler 2001; Kjellberg *et al.* 2001) and, because selection on one trait can indirectly affect another trait, the response to selection may be constrained or hastened as a result of correlations (Pearson 1903; Lande & Arnold 1983). For example, in a pentatomid bug (*Euschistus variolarius*), direct selection to decrease wing length results in indirect selection to decrease thorax width. Indirect selection counteracts the direct effect of selection to increase thorax width (Lande & Arnold 1983). Several important correlations exist in guppies (*Poecilia reticulata*): between female mating preference and male colour pattern (Houde & Endler 1990), and between male attractiveness and mating success (Brooks & Endler 2001). Brooks and Endler (2001) determined that indirect sexual selection on males weakens or opposes direct sexual selection for the elaboration of individual colourful ornamental characters.

The mosquitofish (*Gambusia holbrooki*), a relative of the guppy, harbours a simple male colour polymorphism: the silvery-grey (silver) morph is very abundant in nature (99% of males) and the melanic (black) morph is quite rare (figure 1). In some populations (e.g. Miami, FL, USA; Horth 2001) this stable polymorphism results from the presence of a silver and a melanic genotype. In other

populations (e.g. Newport Springs and St Marks, FL, USA; Horth 2001; Horth & Travis 2002; see also Regan 1961; Martin 1984; Angus 1989), there exists a silver genotype, but in place of the constitutive melanic genotype there exists a temperature-sensitive melanic genotype, for which black body colour is expressed in cold conditions and silver body colour is expressed in warm conditions. The silver phenotype is indistinguishable from that of the silver genotype previously described.

A priori, there is reason to believe that colour and aggression are correlated in mosquitofish. Martin (1975, 1977) noted that melanic males are sometimes more aggressive than silver males. Also, females can be more aggressive towards melanic males (Martin 1977). Finally, melanic males have lower survival (0.47) when present in relatively higher frequency than they do when present in lower frequency (0.81), which may result from aggression between melanic males at high frequency.

In general, fishes (Regan 1961), insects (Majerus 1998), birds (Theron *et al.* 2001) and mammals (e.g. Kijas *et al.* 2001) all harbour melanic morphs. There is a tight biochemical link between dopamine (which affects animal behaviour) and melanin production, which is evolutionarily conserved among taxa (Hadley 1996). Melanin synthesis is initiated when α -melanocyte stimulating hormone (α -MSH) binds to melanocytes (see figure 2; Rawles 1948; Kanetsky *et al.* 2002). If α -MSH is injected into yellow mice, their pelage becomes black (Geschwind *et al.* 1972), and when α -MSH concentrations are increased in frogs (Hadley 1996), their body colour dark-

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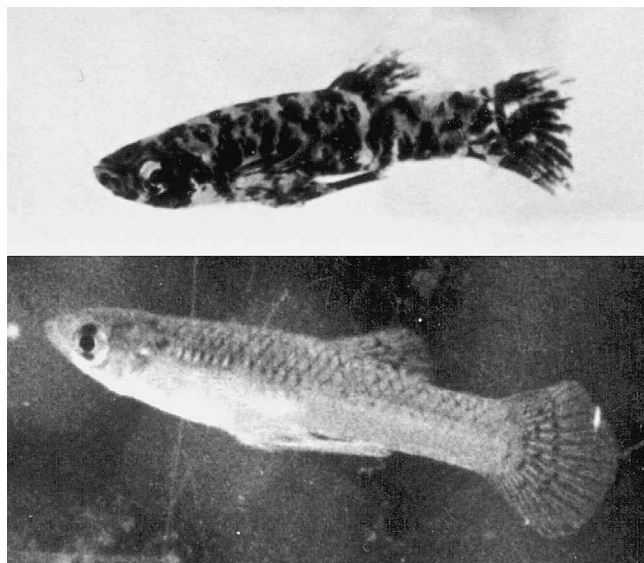


Figure 1. Colour polymorphism in male mosquitofish.

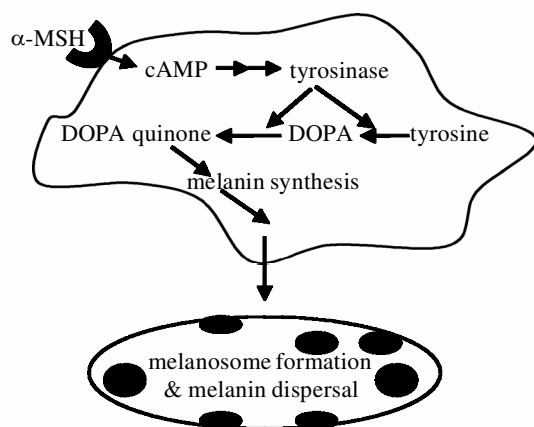


Figure 2. Biochemical pathway producing melanin (after Hadley 1996, with permission).

ens. A link has also been established between α -MSH, its activation of melanin dispersal and its stimulatory effects (though sometimes inhibitory) on behaviour. Namely, increased α -MSH increases sexual activity, aggression, anxiety (Gonzalez *et al.* 1996; but see Tizabi *et al.* 1982), feeding (Gonzalez *et al.* 1998), grooming and locomotion (Sanchez *et al.* 1997) in mice.

Here, an ecological manipulation (see Travis & Reznick 1998) is performed on male mosquitofish (*G. holbrooki*) with the temperature-sensitive genotype. Sibling males are raised at two temperatures. This results in highly related males with different phenotypes (silver or black). These males are monitored in the same social setting to determine whether aggressive mating behaviour and body colour are correlated traits. Important potential fitness ramifications are discussed.

2. MATERIAL AND METHODS

(a) Study system

Melanism in *G. holbrooki* is expressed almost exclusively in males (see Snelson *et al.* (1986) for the rare occurrence in females). Scant data have been collected regarding temperature-sensitive melanin expression, but it is known that fishes with this

genotype are silver until exposed to cold temperatures and once melanin is deposited it is permanent.

(b) Sibling colour morphs

In February 2000, juvenile and adult mosquitofish were collected from St Mark's National Wildlife Refuge (Wakulla County, FL, USA). Juveniles were reared individually in 2 l tubs in the laboratory. As they matured, virgin females were selected. Each was paired with a unique melanic male. Ten F_1 sibships were split into two groups; one group was reared at 31 °C and one at 19 °C. A silver and a melanic sibling were selected from each sibship for use in this study.

One week before experimentation, melanic males were transferred from 19 °C to 31 °C. *Gambusia* have high thermal tolerance and great thermal plasticity (Gerking *et al.* 1975; Heath *et al.* 1993; Lutterschmidt 1997), and there is evidence that small fishes equilibrate to this type of temperature increase within a few days (Chung 1981, 2000; Lutterschmidt 1997; Lutterschmidt & Hutchison 1997). No signs of heat stress were evident in transferred males (e.g. unresponsive motionless movement towards the water surface or sinking to the bottom (Otto 1973), escape attempts, loss of righting, body shudders or opercular quivers (Lutterschmidt & Hutchison 1997)). Typically, males attempt matings within a few minutes of being transferred between these two temperatures (personal observation).

Behavioural observations were conducted at 31 °C as this closely approximates the Florida water temperature during the primary breeding season (July and August). The activity of each colour morph (from each sibship) was observed in the same social setting for 5 days, for 10 min d^{-1} . The experimental arena was a large rectangular wading pool (3 m \times 1.8 m \times 0.5 m), filled with well-water (137 l) and laden with vegetation (elodea, *Egeria densa*). A thin layer of sand from a local lake covered the pool bottom. Light operated on a 14 L : 10 D cycle.

This experiment was purposefully conducted at the lower end of natural mosquitofish densities, to alleviate the artefactual behaviour sometimes encountered at high densities in poeciliids (Farr & Herrnkind 1974). Throw-trap collections (Leips & Travis 1999) demonstrate a mean density in nature of 8.77 (± 7.69 s.e.m.) mosquitofishes per 100 l, and a range of 0–43 fishes per 100 l. Here, 10 fishes were held in 137 l, equalling 7.30 fishes per 100 l.

(c) Social background fishes

Background adults were colour-marked with inert paint (Northwest Marine Technologies, Shaw Island, WA, USA) that was injected with a 27.5 gauge needle. Each fish was marked with one of five randomly assigned colours (yellow, green, blue, red and orange). The standard length (snout to caudal peduncle) (SL) of each fish was measured to the nearest 0.05 mm. Silver fish were used as background fishes because the mean frequency of melanism in nature is low (0.01 ± 0.02 s.e.m.; Horth & Travis 2002), suggesting that the most likely colour for a male randomly encountered in nature is silver.

(d) Experimental design

Each day, a subset of five female and four male background fishes were haphazardly selected from multiple holding tanks and placed in the experimental pool. This allows groups of fishes to have repeated exposure to several individuals but not always to exactly the same individuals. Background fishes were allowed 24 h to acclimatize to the pool, then one focal male was introduced into the group. A dot of temporary water-based paint was

Table 1. Significance of log-odds ratios between male colour and six behavioural traits.

	χ^2	p
focal male behaviour		
total time chasing females (s)	3.67	0.055
number of focal thrusts at females	3.59	0.058
number of rapid chases of females	3.64	0.056
behaviour towards focal		
number of silver chases of focal	4.67	0.030
female tilts and hops	1.55	0.212
female approaches	2.68	0.101

placed on his head, allowing quick assurance of identity, as the abdominal colour marks on the background fishes were not always visible. This focal male acclimatized for 10 min. Then his social interactions were monitored for 10 min from behind a green blind. The focal male was then removed from the pool and replaced by his sibling, whose behavioural interactions were scored. Five out of 10 sibling pairs were tested on one day; the five remaining pairs were tested the next day (10 days in total, five trials per fish). To circumvent bias resulting from testing order, the colour morph tested first for each sibling pair was tested second in the next test period. In addition, the first colour morph used on a given day was reversed the following day.

Fishes were generally healthy during the experiment. However, two unrelated fishes died (aggression during the study was sometimes vicious and occasionally gonopodial hooks caught in fishnets; both events can injure fishes). Two new, substitute, sibling pairs were used for the remainder of the experiment. They came from a family already used in the original test group. Thus, in the final 3 days of the experiment, eight family pairs remained from the original test group.

Behaviours monitored included the major social interactions observed (see Appendix A; Clark *et al.* 1954; Hubbs & Delco 1960; Farr 1976). These include the number of times the focal male chased females, attempted to mate with females and fought with other males. In addition, female behaviour towards each focal colour morph was monitored, including aggressive chases by the female of the focal male, tilts and hops in avoidance of mating attempts, and non-aggressive approaches towards the male. Finally, the amount of time that a male spent chasing a female was monitored with a stopwatch.

(e) Statistical analyses

First, linear logistic regressions between body colour and each of six behavioural traits (total time chasing females by focal male, number of thrusts at females, number of rapid chases of females, number of silver background male chases of focal male, number of female tilts and hops away from focal male, and number of female approaches) were computed for data from eight silver and eight black males (table 1). Next, paired *t*-tests were conducted on the mean differences in behavioural-activity rates between sibling colour morphs (table 2). The average count, for 5 days of observations, was compared for each sibling pair for every behaviour, as was the cumulative amount of time that each colour morph spent pursuing females. Because two males died during the study, all statistical tests were conducted using two partially overlapping datasets: (i) eight sibling pairs (unique families) that survived all five testing days and (ii) 10 sibling pairs (including three pairs from one family) that survived days

3–5. Statistical results for these two datasets were usually similar in direction, though strengthened in magnitude with increasing sample size. For simplicity, only the conservative results (eight unique families) are reported.

Because there is evidence that size differences result in mating-success differences in mosquitofishes (Bisazza & Marin 1995; Bisazza & Pilastro 1997), a comparison of the body-size distributions of sibling colour morphs was made.

3. RESULTS

(a) Focal males

Log-odds ratios between male body colour and each of six behavioural activities were calculated (table 1). In general, melanic males had a higher level of mating activity (chasing, rapidly chasing and attempting to mate with females) than silver males (table 2). Melanic males spent almost twice as much time (cumulative number of seconds) as their silver siblings chasing females ($t_{0.05(2)7} = 2.74$, $p = 0.029$). Melanic males pursued females in rapid chases more often than did silver males ($t_{0.05(2)7} = -2.38$, $p = 0.049$) and thrustured their gonopodiums towards females (attempting mating) more than twice as often as did silver males ($t_{0.05(2)7} = -2.49$, $p = 0.041$). The average number of chases did not differ ($t_{0.05(2)7} = -0.11$, $p = 0.917$). The mean time (average number of seconds per fish for five observation periods) spent chasing females did not differ (see figure 3), but a similar trend to that of the cumulative-time data existed in these data, despite low power.

Male SL did not differ between sibling melanic and silver males (melanic = 21.70 mm, silver = 21.40 mm; $t_{0.05(2)9} = -0.297$, $p = 0.773$). Sibling males were also partitioned into two groups: (i) family used more than once (repeat family), and (ii) other unique families. Then, two additional paired *t*-tests were conducted to assess sibling size differences within groups (i) and (ii). Neither indicated differences in size between colour morphs (mean SL for repeat family: melanic = 20.50 mm, silver = 19.67 mm; $t_{0.05(2)2} = -0.585$, $p = 0.590$; unique families: melanic = 22.00 mm, silver = 21.79 mm; $t_{0.05(2)7} = -0.162$, $p = 0.874$).

(b) Females

Females responded differently to the different male colour morphs (table 3). Females approached or followed silver males in a non-aggressive (no nipping or rapid chasing) fashion about twice as often as they did melanic males (average number of follows of: melanic = 1.23, silver = 2.21; $t_{0.05(2)7} = -4.63$, $p = 0.002$). The number of aggressive chases by females of focal males did not differ by colour (melanic = 3.15, silver = 2.52; $t_{0.05(2)7} = -1.43$, $p = 0.196$). Females did tilt and hop (breaking the water surface) away from melanic males twice as often as they did from silver males (melanic = 4.87, silver = 2.00; $t_{0.05(2)7} = -3.29$, $p = 0.013$). In most observation periods, very aggressive acts (e.g. nips) did not occur; however, they can be quite violent. Thus, periods with non-zero counts are noted here: females nipped melanic males (eight events) more often than they nipped silver males (one event). Because nips can be injurious, it is noteworthy that one melanic male was nipped repeatedly (six times) and died the following day. In addition, two melanic males and one silver male were each nipped once.

Table 2. Focal male colour morphs' behaviour towards females.

focal behaviour	focal male		$t_{0.05(2)7}$	p
	melanic mean	silver mean		
cumulative time (s) spent in chases of females	2038.50	1249.75	2.74	0.029
rapid chases of females ^a	2.97	2.32	-2.38	0.049
thrusts (mating attempts)	8.00	3.25	-2.49	0.041
number of chases	45.00	45.87	-0.11	0.917

^a Indicates log +1 transformation.

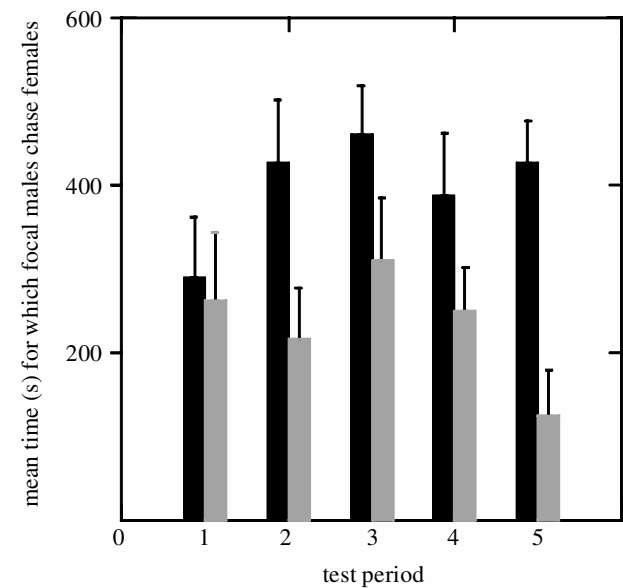


Figure 3. Time (s) for which focal male morphs chased females. Black bars, melanic male chase times; grey bars, silver male chase times.

(c) *Social background males*

Background males interacted differently with the two colour morphs. Background males chased focal melanic males far fewer times than focal silver males (table 4; melanic = 0.95, silver = 3.69; $t_{0.05(2)7} = -4.16$, $p = 0.004$). The number of times focal morphs chased background fishes did not differ by colour (melanic = 21.37, silver = 16.75; $t_{0.05(2)7} = -0.62$, $p = 0.555$). Male–male aggressive chases can have fitness consequences: one male chased a focal male, grabbed his gonopodium and pulled him a distance of several body lengths. Such behaviour may result in death, as it did here.

4. DISCUSSION

(a) *Correlated traits*

This work is the first demonstration, to my knowledge, that colour and behaviour are correlated traits in temperature-sensitive mosquitofishes expressing two phenotypes. This example is particularly interesting as both colour phenotypes are expressions of the same genotype. If indirect selection occurs on aggressive behaviour as a result of a correlation with melanic coloration, the response to selection may be hastened or slowed, depending on whether direct selection on aggression operates in the same direction (Lande & Arnold 1983). Natu-

ral selection was not investigated in this study; however, differential survival for the two colour genotypes has been documented (constitutive black males have higher survival than silver males in the presence of three major predators; Horth 2001). Also, relatively aggressive (melanic) males may have a mating advantage resulting from increased thrusting behaviour (this work; Bisazza & Pilastro 1997). So, indirect and direct selection on colour and behaviour occur in the same direction (in this example) and may contribute to the persistence of rare melanic males. When temperature-sensitive males are melanic, traits that are not favoured (e.g. silver colour and dampened aggression) side-step selection by not being expressed. However, they will be expressed in the F₁ generation under suitable (warm) conditions. If one or both of these traits (silver, dampened aggression) is then favoured under a different selective scenario (e.g. increased female cooperation during mating), an opportunity is provided for the inducible temperature-dependent phenotype to be maintained.

Constitutive melanic males endure fitness trade-offs associated with negative frequency-dependent selection, which is known to maintain polymorphisms (Ayala & Campbell 1974). These males had relatively high survival, compared with silver males, at low frequency, but relatively few (melanic) progeny at high frequency (Horth 2001). Behavioural differences between these colour morphs were observed (Horth 2001). If a frequency-dependent effect also occurs for the inducible temperature-sensitive phenotype, fitness advantages for each colour morph under different selective regimes could permit the persistence of this temperature-sensitive genotype.

It may be that the tight biochemical connection between melanic expression and dopamine production (Hadley 1996) is the primary link causing the correlation between colour and behaviour. Indeed, α -MSH stimulates melanin production and affects aggression and mating behaviour in other organisms (Gonzalez *et al.* 1996), and tyrosinase is the rate-limiting enzyme in the production of dopamine and melanin (True *et al.* 1999). In mosquitofishes, the correlation between melanin and aggression may or may not be adaptive, but a pleiotropic relationship involving important steps in the biochemical pathway producing melanin and dopamine probably affects both traits.

(b) *Focal male behaviour*

The two colour morphs differ dramatically in the number and duration of their mating-related interactions with females, which could provide the temperature-sensitive genotype with greater opportunity for successful mating (when melanic via more thrusting; Bisazza & Pilastro

Table 3. Female behaviour towards focal male colour morphs.

female behaviour	focal male		$t_{0.05(2)7}$	p
	melanic mean	silver mean		
approach or follow focal ^a	1.23	2.21	-4.63	0.002
aggressive chase of focal ^a	3.15	2.52	-1.43	0.196
female tilts and hops	4.87	2.00	-3.29	0.013

^a Indicates log transformation.

Table 4. Focal male colour morph's interactions with background males.

behavioural interaction	focal male		$t_{0.05(2)7}$	$p_{\text{days } 1-5}$
	melanic mean	silver mean		
background male chases focal male ^a	0.95	3.69	-4.16	0.004
focal male chases background male	21.37	16.75	-0.62	0.555

^a Indicates log +1 transformation.

1997). However, melanic males must avoid more female antagonistic behaviour than silver males, so important trade-offs probably exist (cf. Bisazza *et al.* 2001). Male aggression is expressed by melanic phenotypes in several ways, including interactions with other males in the presence of females. Generally, male-male aggression is thought to be especially important when it plays a part in sexual selection (e.g. Cox & Le Boeuf 1977; Andersson 1994).

Males expressing melanism were subjected to a different temperature from silver males while maturing, so it should be mentioned that the difference and change in temperature for melanic fishes could possibly affect behaviour. However, most fishes acclimatize faster to warmer temperatures than to colder ones, and small fishes adapt particularly rapidly (e.g. within a few days; Sylvester 1972; Chung 1981, 2000; Sidell *et al.* 1983; Lutterschmidt 1997; Lutterschmidt & Hutchison 1997). When the acclimatization temperature is increased from 10 °C to 30 °C (approximately the range used here) the *Gambusia* 'lethal-temperature tolerance' remains unchanged (Al-Habbib & Yacoob 1993). Further, heat resistance, which is important for adapting to temperature change, is known to be especially high in *Gambusia* (Al-Habbib & Yacoob 1993). Marked diurnal changes in small freshwater lake water temperature in the autumn and spring may allow *Gambusia* to adapt to relatively rapid temperature changes.

Other live-bearing species (and many other taxa) harbour melanic morphs (e.g. swordtails (Borowsky 1973), mollies (Angus 1983) and platyfishes (Axelrod & Wischnath 1991)). Indeed, a negative correlation has recently been documented between the area of black coloration and orange-spot brightness on male guppies, for whom orange coloration signals sexual attractiveness (Brooks & Endler 2001). Also, a negative correlation was found between melanic throat-patch size and reproductive success (Griffith *et al.* 1999) despite the fact that females in some populations prefer males with large throat patches (Moller 1988, but see Griffith *et al.* 1999). Species

expressing melanism vary widely in mating systems (from female choice to no cooperation). Thus, it would be interesting to determine whether aggressive mating traits are associated with melanic expression and how this affects reproductive success in other species.

(c) Females

Although the meaning of female approaches is not entirely clear, it is evident that they are not hostile acts. Whether female *Gambusia* choose their mates is a debated topic: forcible inseminations were historically thought to be the primary mode of reproduction (e.g. Peden 1973). In choice tests, Martin (1986) and Nelson & Planes (1993) observed no preference by females for either colour morph. More recent work, however, suggests that females prefer silver males (Taylor *et al.* 1996; Bisazza *et al.* 2001), particularly after interacting with melanic males (Taylor *et al.* 1996).

The active avoidance by females (tilts and hops away from the male) of males, especially melanic ones, could be a type of mating preference, or a response to gonopodial hooks (figure 4). Hooks allow males to latch onto females when mating and can injure females (Rosen & Tucker 1961; Constantz 1989). Melanic males attempt just over twice as many thrusts as silver males, and females avoid melanic males just over twice as often as they avoid silver males. After repeated mating attempts, the female gonopore may tear (personal observation). Such injurious effects are noted in the females of other live-bearing species where males have hooks: guppies show blood clots after mating, and swordtails bleed after gonopodial withdrawal (Constantz 1989).

Although nips are very rare, melanic males are nipped most, which may be relevant as wounded males typically withdraw from social activity altogether, occasionally for hours. Thus, it is worth reiterating that nipping has been observed in other experiments, and in nature (personal observation).

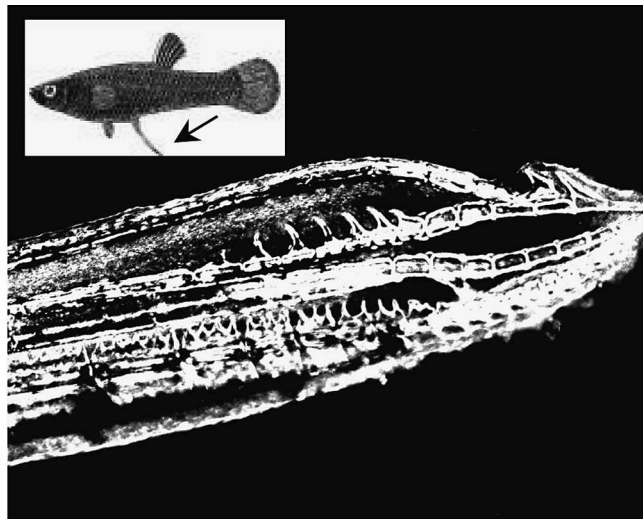


Figure 4. Tip of male mosquitofish gonopodium (5 \times magnification, brightfield microscopy). The arrow on the insert shows the location of the magnified photograph.

(d) *Social background males*

Aggressive chases can result in injury, social withdrawal and time away from mating and foraging. Silver (background) males may chase silver (focal) males more than melanic males because when melanic males fight, they are extremely aggressive and much more likely to win contests. Thus, silver males may actively avoid melanic males in an effort to avoid conflict and the associated risks.

(e) *Summary*

Sibling males expressing different colour phenotypes clearly differ in the behaviours that can affect fitness. From these experiments it cannot be definitively determined whether one phenotype has a relative fitness advantage overall. Precisely how the melanic phenotype's benefit (e.g. time with females and number of mating attempts) to cost (e.g. female avoidance in hops and nips) ratio plays out compared with the silver phenotype's (e.g. female non-aggressive approaches versus chases by other males) is unknown.

The adaptive benefits of an inducible melanic genotype may result: (i) from a combination of behavioural differences and selective pressures that benefit one morph during one period (e.g. melanism during winter may allow higher survival in the presence of predators) and the other morph during a different period (e.g. silver coloration during the summer may mean more female approaches, allowing higher reproductive success); or (ii) from selective pressures not addressed here (e.g. light absorbed by melanin may actually damage DNA in live-bearing fishes (Setlow *et al.* 1993; Setlow & Woodhead 2001), making it beneficial to be silver in high-light (e.g. warm) conditions). Finally, it is also possible that inducible expression is merely an exaptation (Gould & Lewontin 1979). The link between colour and behaviour may be decoupled in nature simply by the effect of seasonal temperatures. Sibling males will experience differing selective pressures if they are born at different times of the year. A male born in early summer will mature silver, mate that season and experience the selective regime unique to the silver morph. By contrast, that same male, if born in

August, would mature in the autumn, turn melanic over winter and experience the selective regime of the aggressive melanic male until death. Important social interactions and the resultant long-term fitness consequences may vary for a great proportion of a male's lifetime contingent upon his body colour and behavioural repertoire. Now that the association between colour and behaviour is established, future work may demonstrate whether, and how, the temperature-sensitive phenotype contributes to the persistence of the melanic morph in nature.

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APPENDIX A: BEHAVIOURAL DEFINITIONS

(a) *Focal male*

- (i) Chase of female: average number of times that the focal male morph directly pursues female(s), remaining within three body lengths of her. This includes changing swimming direction to follow a female, and/or hovering near her, usually just before or after conducting a rapid chase of her. This behaviour was also quantified as the cumulative number of seconds (of the 600 s d⁻¹ maximum, for each of 5 days) for which a male conducted this behaviour.
- (ii) Rapid chase of female: average number of times that a focal male morph swims at a highly accelerated speed chasing a female; often ends with a gonopodial thrust, or a series of thrusts.
- (iii) Thrust at female: average number of times that a focal male morph thrusts his gonopodium (anal fin modified to transfer sperm) towards a female's gonopore in a mating attempt. Thrusts do not necessarily indicate successful sperm transfer.

(b) *Female*

- (i) Approach or follow focal: average number of times that a female non-aggressively swims after a focal male morph, changing swimming directions to follow him, or slowly swimming up to within two body lengths of him, looking directly at him, but never attacking him.
- (ii) Aggressive chase of focal: average number of times that a female rapidly chases a focal male morph, swimming within two body lengths or less of him, often nipping at him, or darting at him.
- (iii) Tilts/hops: average number of times that a female tilts her abdomen sideways or hops out of the water when a focal male morph is attempting to mate with her.
- (iv) Nip: a female pulls a body part of a focal male morph (e.g. fin, gonopodium, head) into her mouth. Nips can tear fins, damage other body parts and

cause the victim to withdraw socially. Males can also nip other males.

(c) *Background males*

- (i) Silver background male chases focal: average number of times that a silver male from the background group rapidly and aggressively chases a focal male morph, sometimes nipping him, sometimes pursuing him because he has come near the female that the group male was attempting to mate.
- (ii) Focal chases background male: same definition as (i) above, except that the focal morph is the aggressor and the background male is being chased.

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