12-2006

A Sex-Linked Allele, Autosomal Modifiers and Temperature-Dependence Appear to Regulate Melanism in Male Mosquitofish (Gambusia holbrooki)

Lisa Horth
Old Dominion University, Lhorth@odu.edu

Original Publication Citation

Horth, L. (2006). A sex-linked allele, autosomal modifiers and temperature-dependence appear to regulate melanism in male mosquitofish (*Gambusia holbrooki*). *Journal of Experimental Biology, 209*(24), 4938-4945. doi:10.1242/jeb.02599

The Journal of Experimental Biology 209, 4938-4945
Published by The Company of Biologists 2006
doi:10.1242/jeb.02599

A sex-linked allele, autosomal modifiers and temperature-dependence appear to regulate melanism in male mosquitofish (*Gambusia holbrooki*)

Lisa Horth

Department of Biological Sciences, Old Dominion University, Norfolk, VA 23529, USA

(e-mail: lhorth@odu.edu)

Accepted 16 October 2006

Summary

About 1% of male mosquitofish (*Gambusia holbrooki*) express melanic (mottled-black) body coloration, which differs dramatically from the wild-type, silvery-gray coloration. Here, I report on the genetic inheritance pattern of melanic coloration, which indicates Y-linkage, and at least one autosomal modifier. Phenotypic expression of melanism is also affected by temperature. Expression is constitutive (temperature insensitive) in some populations, inducible (temperature sensitive) in others. Constitutive and inducible expression occur among geographically proximal populations. However, males from any single population demonstrate the same constitutive or inducible expression pattern as one another. The F1 males from inter-population crosses demonstrate temperature-related expression patterns like their sires'. As well, the sex ratio of melanic males' progeny differs among populations. Here, inter-population crosses demonstrate a sex-ratio bias in the same direction as intra-population crosses of the sire population. About 20% of the male progeny of melanic sires express the wild-type phenotype. These silver F1 males sire only silver offspring, suggestive of loss of the melanin gene in F1 males from crossover between sex chromosomes, or control by additional modifiers, or involvement of additional factors. In nature, melanic males persist at very low frequencies. The data collected here on heritability indicate that genetic factors contribute to the rarity of melanic male mosquitofish.

Key words: color, polymorphism, temperature, melanin, melanic, melansitic.

Introduction

Melanin is one of the most widespread pigments in nature (True et al., 1999). It causes the dark coloration found in an enormous array of insects and vertebrates (see Majerus, 1998). Batesian mimicry (Poulton, 1909; Brower, 1958) and industrial melanism (Kettlewell, 1973) are classical examples of adaptive melanism. Additional adaptive properties of melanin include functional camouflage [e.g. Lepidopterans (see Kettlewell, 1973)], background matching [e.g. lower vertebrates (see Hadley, 2000)] and ultraviolet protection [e.g. humans (see Jablonski and Chaplin, 2000)].

The biochemical pathway resulting in the production of melanin is conserved across vertebrate taxa (Hadley, 2000; True et al., 1999). Melanin is formed when tyrosine is converted into the catecholamine 3, 4-dihydroxy-phenylalanine (L-dopa), then into dopamine. Both steps are catalyzed *via* the rate-limiting enzyme, tyrosine hydroxylase [*a.k.a.* tyrosinase or hereafter, TH (Nagatsu et al., 1964; Berne and Levy, 1998)]. The expression of this enzyme differs in melanic and non-melanic tissue of some organisms [e.g. *Xiphophorus* (Kazianis et al., 1999)]. Allelic mutations altering TH result in albinism in cats (Imes et al., 2006) and mice (Halaban et al., 2000). Similarly, in some birds and mammals, mutations in the melanocortin-1 receptor gene (*MC1R*), which produces the receptor for melanocyte stimulating hormone, are correlated with the replacement of wild-type coloration by melanic coloration [e.g. birds (Theron et al., 2001; Mundy et al., 2004), mammals (Robbins et al., 1993; Kijas et al., 2001; Takeuchi et al., 1996; Klunglund et al., 1995) (reviewed by Horth, 2005)].

Temperature often plays a key role in the expression of melanin. In Siamese and Burmese cats, temperature-sensitive (hereafter, TS) TH alleles result in a face-mask and dark pigmentation on extremities (Lyons et al., 2005). TS-TH affects melanic expression in fruit flies (O'Grady and DeSalle, 2000) and mice (Kwon et al., 1989). In nature, a seasonal polymorphism occurs in some *Colias* butterfly species, which is thought to be adaptive: summer broods are composed of individuals with orange/yellow coloration in their wings. This is replaced by melanic coloration in spring and fall broods, allowing individuals to warm-up more quickly in colder temperatures (Watt, 1969). In the laboratory, in mouse melanocyte cell lines, heat shock and cold exposure both reduce TH activity and melanin production (Kim et al., 2001; Kim et al., 2003). As well, UV induction of reactive melanin radicals in skin cells was determined to be a major cause of melanoma in *Xiphophorus* fish (Wood et al., 2006).

Despite exciting advances unveiling the genetic control of melanism in mammals and birds (see Horth, 2005), there exists a depauperate literature on the basic genetic and environmental control of the inheritance of melanism in fish. One recent study has, however, demonstrated an association between relative melanic pigmentation in the lateral stripes of zebrafish and a putative cation exchanger. In the golden zebrafish mutant, lateral stripes are lighter in coloration and have fewer melanophores than the wild-type fish (Lamason et al., 2005). By contrast, little is understood regarding the inheritance of melanism in the Poeciliidae (the live-bearing fish family), which demonstrate strikingly unique melanic expression patterns among species, as well as among conspecific individuals (see Axelrod and Wischnath, 1991). The association between pigmentation and natural selection has been well-studied in this family. Platyfish (*Xiphophorus maculatus*) express few melanic tail-spot patterns (Borowsky, 1978), which serve as valuable disassortative mating traits (Borowsky and Kallman, 1976). Guppy (*Poecilia reticulata*) melanic spot sizes are inversely correlated with orange spot size/brightness – attributes which are important for female choice and male mating success (Endler and Houde, 1995; Houde, 1997; Brooks and Endler, 2001). Both sexes of sailfin mollies (*Poecilia latipinna*) express melanism, but very rarely (Angus, 1983). Melanism is absent in western mosquitofish (*Gambusia affinis*) and is rare in eastern mosquitofish (*G. holbrooki*) (Regan, 1961; Snelson et al., 1986) where it occurs in ~ 0.01% of males in nature (Horth and Travis, 2002). Natural selection acts differentially on silver and melanic eastern male mosquitofish: melanic mosquitofish are subjected to a lower predation rate (and higher recapture rate in nature) than silver males (Horth, 2004) and negative frequency-dependent survival is also associated with melanism in this species (Horth and Travis, 2002).

In melanic eastern mosquitofish, macromelanophores are found in the dermis of the skin (Regan, 1961). Melanin is deposited into these, which produces the black-spotted phenotype (Fig. 1A,C). Melanin is not absent in wild-type fish; they have small micromelanophores with melanin deposits, hence their body coloration remains light in comparison to melanic fish (Fig. 1B,D). For comparison, albino fish have no melanic pigmentation and thus have red eyes and light skin.

The genetic crosses that were conducted in the study were designed to elucidate the inheritance patterns of melanism in eastern mosquitofish and to obtain an understanding of how temperature interacts with genetics to affect melanic expression.

Materials and methods

Fish collection and mating design

Small groups of female ($N\approx10$) and melanic male ($N\approx5$) eastern mosquitofish were periodically collected (additional melanic males were captured as needed), with dipnets, from three natural populations during the period 1996–1999. Collections were made from two north Florida sites: Picnic Pond (Wakulla Co., FL, USA; hereafter PP) and Newport Springs (Wakulla Co., FL, USA; hereafter NS), and from one south Florida site: Miami (Pahayokee, Everglades National Park, Dade Co., FL, USA).

Field-collected males were held in large stock tanks in a hot laboratory and females were placed into individual breeding chambers, one per 22.73 l (5 gallon) aquarium, kept at 31°C. After parturition, approximately four young from each female's brood were randomly selected to grow to maturity (31°C). Each juvenile was housed alone to ensure virginity at maturation. Upon maturation, a virgin female was selected from each maternal line and paired with one melanic male for 1 month (31°C). Afterward, the sire was removed from the aquarium and the gravid female was placed in a breeding chamber to avoid cannibalism of young at birth. Young were typically born within a few days and were periodically checked every few weeks thereafter for maturation. The sex and color of each F1 was tallied: as each juvenile fish matured, it was removed from the tank. When a male matured that did not express melanism, it was transferred to a cold (18°C) laboratory and monitored for ⩾12 weeks [the approximate time required for melanic expression (Angus, 1989a)] or until death.

Intra- and inter-population crosses were conducted. Intra-population crosses (one virgin female × one melanic male) included: (1) NS (N=24 crosses), (2) PP (N=30) and (3) Miami (N=20). Inter-population crosses included: (1) Miami melanic male × PP virgin female (N=35) and (2) PP melanic male × Miami virgin female (N=18).

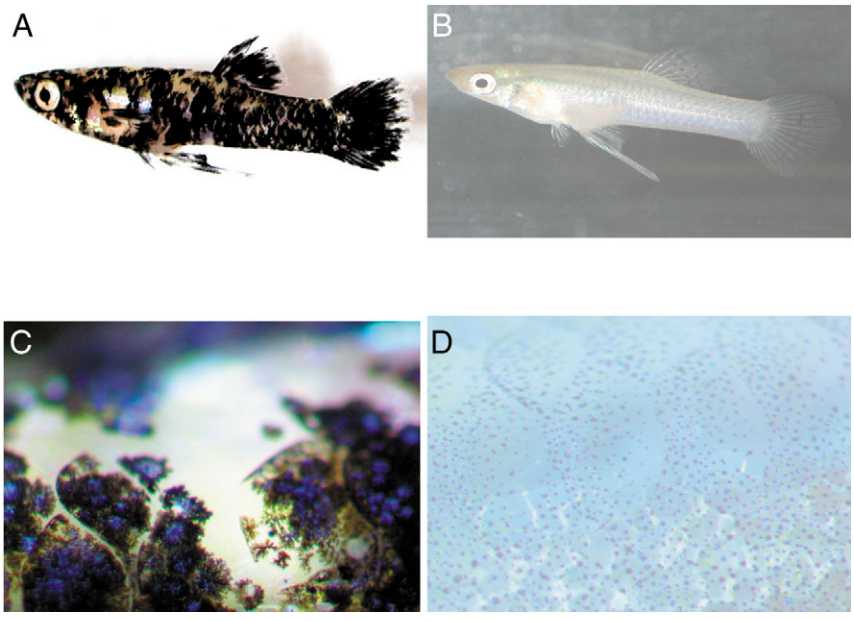

Fig. 1. Male eastern mosquitofish, *Gambusia holbrooki*. (A) Melanic male. (B) Silver male. (C) Magnification (50×) of melanic male dermis, showing melanin deposition in macromelanophores. (D) Magnification (50×) of silver male dermis, showing melanin deposition in micromelanophores.

Silver sons of melanic sires

Not all sons sired by melanic males express melanism, even after cold exposure. To determine the inheritance pattern of melanism in F2 fish sired by silver F1 males (with melanic sires), 17 silver F1 males were mated to virgin females (as described above). Thirteen of the 17 crosses were PP silver F1 males mated to 13 virgin PP females (as single pair crosses). The four remaining single pair crosses were: Miami silver F1 male X Miami female (*N*=2), an F1 son (of a Miami female × PP male) × Miami female (*N*=1), and another F1 son (of a PP female × Miami male) × Miami female (*N*=1). F2 young were reared to maturity at 31°C then housed at 18°C for 3 months (or until death) to identify whether melanic expression was inducible (TS).

Masculinized females

Twenty F1 females sired by different melanic males (a few females from each of the pure-bred populations and from each of the population crosses) were evaluated for the presence of melanin and the formation of a gonopodium (male mating structure) after being fed flake fish-food laced with male-hormone (methyl-testosterone). Females were held for at least 3 months, most much longer (four for >1 yr) at 18°C. Food was prepared by suspending methyl-testosterone in ethanol, adding this mixture to commercial flake-food (TetraMin, That Fish Place, Lancaster, PA, USA), then air-drying to evaporate the alcohol. Fish were fed flakes daily using a final suspension of 0.3 mg testosterone g^{-1} food. Testosterone treatment is used to identify traits with sex-limited expression.

Statistical tests

Two inheritance patterns were evaluated with χ^2 tests: if we assume that all melanic males carry a melanism allele on their Y chromosome (Y_M), but must also have a dominant, autosomal modifier (*A*) to express the melanic phenotype, then when crossing a Y_M male heterozygous for the autosomal, dominant modifier (*Aa*) to a female heterozygous (*Aa*) for this autosomal gene, a 3:1 ratio is the null expectation. Here, males cannot be *aa* genotypes or they would not express melanism. The 3:1 ratio is used when assuming heterozygotes to be more common than homozygotes, presuming the *A* allele is rare due to selection on the melanic phenotype (the same 3:1 ratio is expected if males are heterozygous and all three genotypes are found in females in equal proportions). Alternatively, if there is no fitness cost to the autosomal allele, then the average frequency of melanism is expected to be 0.875 (see Fig. 2). Here, female genotypes (*AA*, *Aa* or *aa*) occur in equal frequency, as do male genotypes (*AA* or *Aa*).

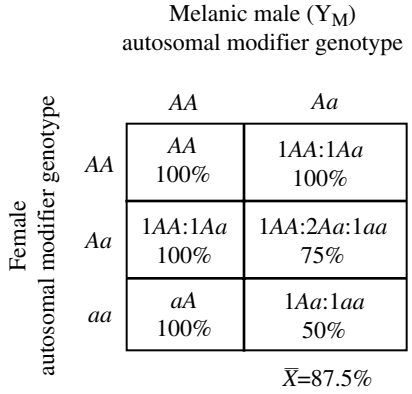

Fig. 2. Potential crosses and outcomes for a dominant, autosomal modifier of a Y-linked melanism gene in *Gambusia holbrooki.* Percentage refers to male fish from a given cross that would have a melanic phenotype. Mean at bottom refers to percentage melanism that would result if sires were heterozygous or homozygous dominant for the autosomal modifier and dams were used from all three diplid genotypes at equal frequency (it is assumed that males are not *aa,* otherwise they would not be melanic).

Results

Intra-population crosses

NS melanic males × NS virgin females

The work presented here shows that melanic expression is constitutive in the NS population. Most sons of melanic sires expressed melanism at 31°C. A minority of sons (see below) remained silver; melanism was not induced in them even after cold exposure (18°C). None of the daughters, in this or any other cross, developed melanism at 31°C or 18°C. Twenty-four NS matings yielded a total of 167 progeny at maturity: 79 melanic males, 19 silver males, and 69 females. Thus 0.81 of the males were melanic (see Table 1). The ratio of melanic:silver males was ~4:1 at 31°C, which does not differ from the classical segregation ratio of 3:1 for Mendelian inheritance of a dominant allele. (χ^2=1.65; 0.10<*P*<0.25). The frequency of melanism of 0.81 also does not deviate from the null expectation of 0.875 for all autosomal crosses (χ^2=0.5313; 0.25<*P*<0.50). The mean number (per brood) of melanic males at maturity was 3.29 (±3.01), of silver males 0.79 (±1.5), and of females 2.87 (±2.75). Brood size at maturity ranged from 0–15 individuals. Nineteen broods comprised all, or primarily, melanic males, and eight broods contained some silver males (Fig. 3). The sex ratio for all broods combined was male biased at ~1.4:1.0 (see Table 1). This differs from the null hypothesis of a 1:1 sex ratio (χ^2=5.0359, 0.01<*P*<0.025).

PP melanic males × PP virgin females

Melanic expression is TS (inducible) in the PP population. Sons of melanic sires did not express melanin at 31°C. Cold exposure induced melanic expression in some, but not all silver F1 males. Thirty matings yielded 224 progeny at maturity (31°C): 0 melanic males, 111 silver males and 113 females. Cold induced melanism in 67 males, 26 remained silver, and 18 died before sufficient time (~12 weeks) elapsed to determine final phenotype. Of the females, 63 lived >12 weeks; none were melanic. Cold induced melanic expression in 0.72 of the F1 males (Table 1). The ratio of melanic:silver males (18°C) was ~2.6:1, which is not significantly different from 3:1 (χ^2=0.4337, 0.50<*P*<0.75) and this frequency of melanism

Table 1. *Sex ratio, and frequency of melanic male progeny from Gambusia holbrooki virgin female × melanic male matings*

Population	Number (male:female)	Sex-ratio (male:female)	Male morph (melanic:silver)	Frequency melanic males @31°C	Frequency melanic males @18°C	N_{total} (number of broods)
Newport Springs	98:69	1.4:1*,a	4:1	0.81	0.00	167 (24)
Picnic Pond (PP)	111:113	0.98:1	2.6:1	0.00	0.72	224 (30)
Miami	134:53	2.5:1*,b	4:1	0.81	0.00	187 (20)
PP female × Miami male	321:167	1.9:1*,c	4:1†	0.81	0.00	488 (35)
Miami female × PP male	76:66	1.1:1	2:1	0.02	0.68	132 (18)

*,a,b,cSignificantly different from 1:1 (a: $\chi^2_{0.05,1}$=5.04, 0.025<*P*<0.01; b: $\chi^2_{0.05,1}$=35.08, *P*<0.001; c: $\chi^2_{0.05,1}$=48.60, *P*<0.001).

Progeny were raised until maturity in a hot (31°C) environment, then if silver, transferred to a cold (18°C) environment to monitor temperature-sensitive melanic expression.

(0.72) is also not significantly different from 0.875 (χ^2=2.539, 0.10<*P*<0.25). The mean number (per brood) of melanic and silver males at maturity at 18°C was 2.79 (±1.91) and 0.90 (±0.90), respectively; that of silver males and females at 31°C was 3.7 (±2.25) and 3.8 (±2.07), respectively. Brood size at maturity ranged from 0 to 15 individuals. Twenty-three broods contained all, or primarily, melanic males; 18 broods contained some silver males. The sex ratio at 18°C was 111 males: 113 females (Table 1), which does not differ from 1:1 (χ^2=0.0179, 0.75<*P*<0.90).

Miami melanic males × Miami virgin females

Melanic expression is constitutive in the Miami population. Most sons of melanic sires expressed melanism at 31°C. Cold exposure did not induce melanism in any silver sons. Twenty matings yielded 187 progeny at maturity: 108 melanic males, 26 silver males and 53 females. 0.81 of the F1 males were melanic (Table 1). The ratio of melanic:silver males was ~4:1. This does not differ statistically from 3:1 (χ^2=2.2388, 0.10<*P*<0.25) and a frequency of melanism of 0.81 does not differ from the null expectation of 0.875 (χ^2=0.7257, 0.25<*P*<0.50). The mean number (per brood) of melanic males at maturity was 5.4 (±4.62), of silver males 1.35 (±1.38), and of females 2.65 (±2.64). Brood sizes at maturity ranged from 1 to 17 individuals. Eighteen broods contained all, or primarily, melanic males and 13 broods contained some silver males (Fig. 4). The sex ratio was male biased at ~2.5:1 (Table 1), which differs from the null expectation of 1:1 (χ^2=35.0855, *P*<0.001).

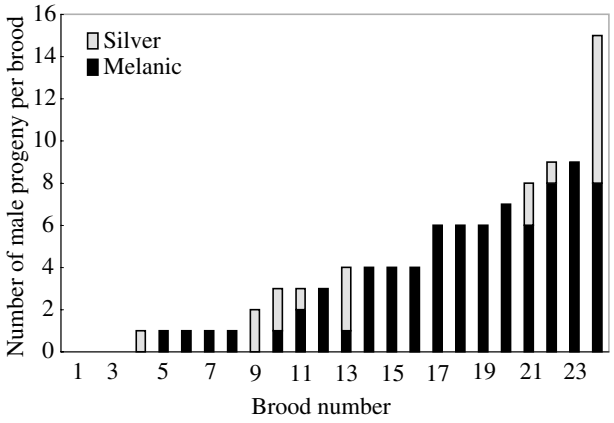

Fig. 3. Number of silver and melanic male progeny per brood for 24 Newport Springs melanic male × virgin female matings.

Inter-population crosses

Miami melanic males × PP virgin females

Melanic expression is constitutive in this south FL by north FL cross, as it was in the sire population. Sons of melanic sires expressed melanism at 31°C and cold exposure did not induce melanism in silver sons. Thirty-five matings yielded a total of 488 progeny at maturity: 259 melanic males, 62 silver males, and 167 females. 0.81 of the F1 males were melanic (see Table 1). The total ratio of melanic:silver male progeny was ~4.2:1, which differs from the dominance expectation of 3:1 (χ^2=5.5337, 0.01<*P*<0.05) but a melanic frequency of 0.81 does not deviate from the expected value of 0.875 (χ^2=1.704, 0.10<*P*<0.25). The mean number (per brood) of melanic males at maturity was 6.76 (±5.57), silver males 1.80 (±2.10), and females 4.72 (±2.76). Brood sizes at maturity ranged from 2 to 29 individuals. Thirty-four broods contained all or mostly melanic males and 20 broods contained some silver males (Fig. 5). The sex ratio was male biased at ~1.9:1.0 (Table 1), which differs from the null expectation of 1:1 (χ^2=48.5984, *P*<0.001).

PP melanic males × Miami virgin females

Melanic expression is inducible in this inter-population cross, just as it is in the sires' population. Sons of melanic sires were silver at 31°C. Cold exposure induces melanic expression in some, but not all, silver F1 males. Unfortunately, >10 crosses resulted in no progeny. In addition, a mechanical failure in the cold-room resulted in many males dying before their expression pattern was known. However, 18 crosses yielded 132 progeny at maturity. These included two males, each with a total of two spots (31°C), 74 silver males, and 66 females. At 18°C, 37 silver males turned melanic, 17 silver males remained silver, and the remainder died before sufficient time elapsed to know whether they would turn melanic. After cold exposure, 0.68 (0.69 if the two males with two spots are included) of the

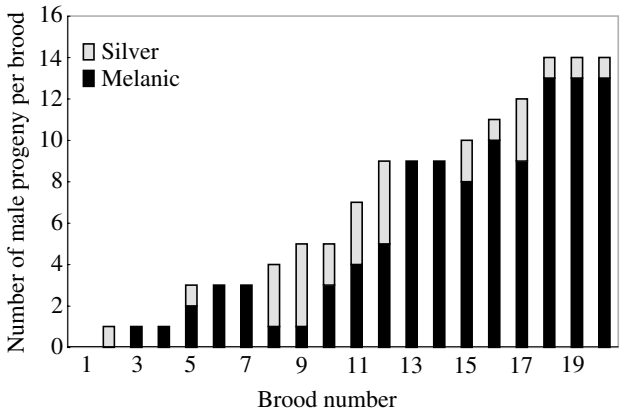

Fig. 4. Number of silver and melanic male progeny per brood for 20 Miami melanic male × virgin female matings.

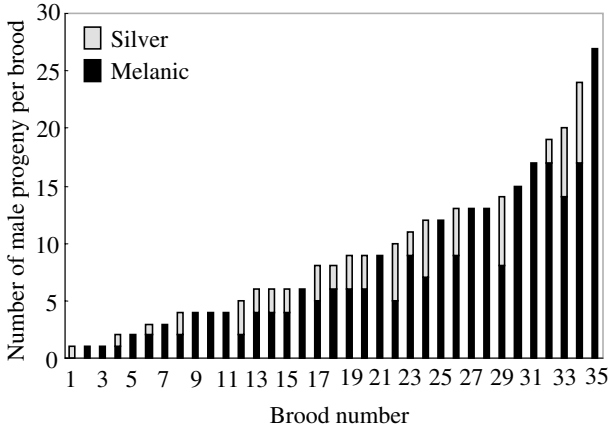

Fig. 5. Number of silver and melanic male progeny per brood for 35 Miami melanic male × Picnic Pond virgin female matings.

F1 males were melanic (Table 1). The total ratio of melanic:silver males in the cold was ~2.2:1.0, which does not differ statistically from 3:1 (χ^2=1.2098, 0.25<*P*<0.50), and the frequency of melanism of 0.68 does not differ from 0.875 (χ^2=2.212, 0.10<*P*<0.25). The mean number (per brood) of melanic males at maturity at 18°C was 2.47 (±2.47), of silver males at 18°C was 1.42 (±1.44), of silver males at 31°C was 4.11 (±3.25), and of females (31°C) was 3.67 (±2.05). Brood sizes at maturity ranged from 1 to 21 individuals. Most broods (*N*=13) contained all, or primarily, melanic males. Some broods (*N*=9) contained no silver males. Overall, the sex ratio (31°C) was nearly equal at 1.15:1.00 (Table 1), which does not deviate from the null expectation of 1:1 (χ^2=0.7042, 0.25<*P*<0.50).

Silver F1 males with melanic sires

About 0.20–0.30 of the male progeny sired by melanic males remain silver, even after cold exposure (>12 weeks). So, 13 PP silver F1 males were mated to 13 PP virgin females. Four additional crosses were conducted: Miami female × Miami silver F1 male (*N*=2), a Miami female × an F1 male (from a Miami female × PP male cross, *N*=1), and a Miami female × an F1 son (from a PP female × Miami male cross, *N*=1). These F1 silver sires produced F2 broods composed of only silver individuals (both sexes). None of the F2 males ever expressed melanic coloration (31° or 18°C). Sixteen F1 silver sons produced no young when crossed to females. Seventeen successful matings yielded 129 progeny at maturity: 55 silver males and 74 females. The mean number of silver males at maturity was 6.11 (±2.61) and females was 8.70 (±4.84). Individual brood sizes ranged from 3 to 25 at maturity. There was a female bias in the overall sex ratio of 0.74:1.00. The sex ratio for the PP population crosses was 0.818:1.00 (36 males:44 females), for the Miami crosses 1:1 (12 males: 12 females) and for the interpopulation crosses, respectively, 0.3:1.0 (3 males: 10 females) and 0.5:1.0 (4 males: 8 females). The PP ratio is not significantly different from 1:1 ($\chi^2_{0.05,1}$=0.455, 0.25<*P*<0.50). The Miami ratio equals 1:1, and the interpopulation crosses are too small for meaningful statistical evaluation. One silver PP F2 was successfully mated to a PP female. They produced one silver son, no melanic sons, and no daughters.

Masculinized F1 female progeny of melanic sires

Testosterone-treated females developed a gonopodium (male sex structure used to transfer sperm), but did not express a dark-spotted phenotype like melanic males.

Discussion

It is important to emphasize that the work conducted in this study was designed to shed light on the basic inheritance patterns of melanism in mosquitofish. Melanic inheritance is more complex than can be entirely resolved from the experimental design of this work. However, four findings are reported here. (1) Some populations have inducible melanism, others constitutive, therefore, more than one genotype produces the melanic phenotype. In Himalayan mice, a point mutation results in TH-misfolding at high temperature (Halaban et al., 2000; Kwon et al., 1989), and a pale phenotype. Mosquitofish with TS-melanism are silver at 31°C so they could carry a similar mutation. This, however, does not account for the ~0.20 silver sons produced by both types of melanic sires. (2) The F1 silver phenotype implies several possibilities (A–E). (A) More than one gene controls melanic expression. Over 120 genes regulate mouse pigmentation (Bennett and Lamoreux, 2003), ⩾40 color-pattern loci exist in guppies (see Endler, 1978; Houde, 1997), and several melanic pattern-forming genes occur in other Poeciliids (Lindholm and Breden, 2002). The relatively constant frequency, across populations, of ~0.20 F1 silver males, is suggestive of an autosomal effect. If mosquitofish melanism is controlled by a Y-linked gene, plus one or more autosomal modifier(s) – and temperature affects expression – this in-part (but not entirely) explains the results obtained in this study, as well as some of the inconsistency revealed in studies on melanism in this species. For example, one study (Regan, 1961) found that all the sons of two south

FL (USA) melanic males were silver, which contrasts with my Miami data (but can be explained if Regan's population is TS). Two additional studies (Martin, 1984; Angus, 1989a) found that about half the sons of a few NC and Central FL (respectively) melanic males were melanic, and in Angus' work, the other half became melanic in the cold.

If melanism is controlled by a dominant, autosomal gene, plus a Y-linked melanic allele, and the autosomal (A) allele is very rare (thus found primarily in the heterozygous state in males and females), the expected melanic:silver phenotypic ratio is 3:1. In my work, one population cross deviates from this ratio. Alternatively, if the autosomal allele is *not* assumed to be rare, and all three diploid states (*AA*, *Aa*, *aa*) persist in equal frequency, the expected frequency of melanism is 0.875 (see Fig. 2). In none of my work do the population crosses deviate from this frequency. However, the F2 males that remained silver (and had melanic grandfathers) cannot be explained by the stated inheritance patterns alone (unless all F1 males were Y_M *aa* genotypes and all were mated only to *aa* females). No account was made of female autosomal genotype in the laboratory crosses, hence one would assume a random sampling of genotypes. Thus, a Y-linked melanic allele, plus one autosomal modifier, in addition to temperature dependence, does not fully explain melanic inheritance patterns.

An alternative is to assume that melanism is dominant, autosomal, and sex-limited. Under this assumption, the dominant melanic allele would have a frequency P=0.005 in nature, resulting in the observed 1% melanic male phenotype. Among melanic males $P/(2–P)$ would be homozygous (*MM*) and a frequency of $(2–2P)/(2–P)$ would be heterozygous (*M*+). If melanic males were mated to females at random, the progeny of *MM* melanic males would all be melanic. However, such males would be present in nature at a frequency of 0.0025, and as such, would be unlikely to be used often in laboratory crosses. *M*+ males' progeny would be melanic at a frequency of $1/2P+1/2=0.5025$. These frequencies are not well reconciled with laboratory cross data where $100\%x+50\%(1–x)=80\%$ melanism, which can only occur if more than half of the melanic sires are *MM*, which is inconsistent with the 0.01 frequency of the melanic phenotype observed in nature. (B) Transposons result in the production of silver F1 males. Some transposons are TS, alter pigmentation (Epperson and Clegg, 1987) and cause multiple changes in biochemical pathways (e.g. Clegg and Durbin, 2000). Helitrons are transposons found in the sex-determining region of the sex chromosomes of platyfish (*Xiphophorus maculatus*) and are posited to play a role in sex chromosome evolution (Zhou et al., 2006). *Jule* is also a transposon found in several live-bearing species that may have a sexually dimorphic pattern in platyfish, where it is found in the subtelomeric regions of the sex chromosomes (Volff et al., 2001). However, classical transposition rates are typically <0.20. (C) Environmental effects on melanic expression are more complex than addressed here. If TS-TH operates on a step-function and some individuals require <18°C for expression, I would not have uncovered this. (D) There is an extremely high rate of spontaneous mutation from black to silver. This is improbable. (E) There is a high rate of sex chromosome recombination (consider the location of *Jule*), and crossover results in loss of the melanic allele (*M*), in silver F1 males. Angus (Angus, 1989a) posited atypical sex determination (XX silver males), or sex chromosome crossover to explain silver males, but his deduction was based upon much lower rates of incomplete penetrance (0.05) than I saw. He also noted that XX silver males would produce all female progeny, which did not occur in my work. For other Poecillidae genera, crossover rates between sex chromosomes are ~0.002–0.003 [*Xiphophorus* (Kallman, 1965)] and 0.01 [*Poecilia* (Angus, 1989b)]. Crossover can explain why F2 males do not express melanism, however, the highest rate reported in Poecillids is ~0.0742 (Lindholm and Breden, 2002), suggesting a rate of 0.20 to be extraordinary. It is noteworthy that in *G. holbrooki's* sister taxa, *G. affinis*, females possess a heteromorphic (sex) chromosome pair that *G. holbrooki* lack (Black and Howell, 1979). Many poeciliids have autosomal sex determination, often associated with a melanism locus (see Meffe and Snelson, 1989). Some species have three sex chromosomes, such that XY and YY are males (WY, WX and XX are females). Here, if all cross types contributed equally to the production of melanic males (Y carries a dominant melanin gene and WY_M females are extremely rare, so not considered), then ~77% of males would be melanic (this is likely an underestimate since few males would probably be homozygous Y_MY_M). If F1 YY males were silver, they would only produce F2 melanic males if they were crossed to WY_M females (presumed very rare). The sex ratio of broods of YY males would be 50:50 for WY and WX females, and 100% male for XX females. Two cross types (XX by YY_M and XX by Y_MY_M) would produce no females, and if all cross types were mated at equal rates, half of the broods would be 50% melanic, the other half, 100% melanic. Whereas the silver F1 male conundrum is resolved by the WXY system, most F1 females would carry a melanic allele, and females sired by melanic males would be expected to turn melanic when exposed to testosterone, which did not occur. (3) When a sex-ratio bias occurs in these crosses, it tends toward the gender bias (male) of the paternal population. These are also the crosses with higher frequencies of melanism (Table 1). In cell culture lines, an androgen receptor activates the TH promoter, and an androgen response element is ~1.4 kb upstream of the promoter (Jeong et al., 2006) suggestive of an association between androgen and TH production. From a similar perspective, mosquitoes (*Aedes aegypti*) demonstrate Y-chromosome meiotic drive due to a distorter gene that is closely linked to the male determining locus on the Y chromosome (Wood and Newton, 1991). Y chromosomes of some strains drive against some X chromosomes, but not others (Owusu-Daaku et al., 1997). A parallel would be Y-chromosome drive when a cross involves a Miami sire but not a PP sire. In the WXY chromosome system, some crosses (XX by Y_MY_M and XX by YY_M) are expected to produce only males. These genotypes might be found in higher frequency in populations with male biased sex ratios. In *X. maculatus*, which has a WXZ sex chromosome system, pigmentation genes are

close to the sex-determining gene, too. *Drosophila simulans* also have autosomal suppressor genes that inhibit the sex-ratio distortion of driving X chromosomes (Atlan et al., 1997). Here the parallel would be driving Y suppressors in populations like PP, but not Miami. Relative overproduction of males by melanic sires could act as compensation for the loss of phenotypic expression in F1 silver males. Female numbers decreased with increasing frequencies of melanic males in empirical mesocosm populations (Horth and Travis, 2002), though whether this occurred solely as a result of female deaths or because of a skewed F1 sex ratio as well, is not known. (4) Melanic expression appears to be sex linked, not sex limited. This is supported by the fact that none of the female progeny with melanic sires express a melanic phenotype, even after ingesting testosterone and being subjected to the cold for at least 12 weeks (see also, Angus, 1989a).

The consequence of loss of expression of melanism is the loss of fitness for the black male phenotype/genotype. If this male color polymorphism were to be maintained *via* mutation-selection balance, a loss of 0.20/generation of the rare allele would imply a huge selective cost to the melanic genotype and an improbably high mutation rate to the melanic phenotype (Lynch and Walsh, 1998). The only potential benefit identified here is possible sex-ratio control. Additional work is underway to explain more about the genetic by environmental control of melanism in mosquitofish.

I thank D. Taylor, D. Houle and J. Travis for intellectual input, R. Angus and W. Loftus for sharing unpublished data, and two anonymous reviewers for constructive comments. This work was funded by National Science Foundation awards DEB99-02312 to J. Travis (Dissertation Improvement Grant for L.H.) and DEB99-03925 to J. Travis.

References

Angus, R. A. (1983). Genetic analysis of melanic spotting in sailfin mollies. *J. Hered.* **74**, 81-84.

Angus, R. A. (1989a). Inheritance patterns of melanic pigmentation in the eastern mosquitofish. *J. Hered.* **80**, 387-392.

Angus, R. A. (1989b). A genetic overview of Poeciliid fishes. In *Ecology and Evolution of Livebearing Fishes* (ed. G. K. Meffe and F. F. Snelson Jr), pp. 51-68. Englewood Cliffs, NJ, USA: Prentice Hall.

Atlan, A., Merçot, H., Landre, C. and Montchamp-Moreau, C. (1997). The sex-ratio trait in *Drosophila simulans:* geographical distribution of distortion and resistance *Evolution* **51**, 1886-1895.

Axelrod, H. and Wischnath, L. (1991). *Swordtails and Platies.* Nepture, NJ, USA: T.F.H. Publications, Inc.

Bennett, D. C. and Lamoreux, M. L. (2003). The color loci of mice – a genetic century. *Pigment Cell Res.* **16**, 333-344.

Berne, R. M. and Levy, M. N. (1998). *Physiology*, 4th edn, pp. 11–31. St Louis, MI, USA: Mosby, Inc.

Black, D. A. and Howell, W. M. (1979). The NorthAmerican mosquitofish, *Gambusia affinis*: a unique case in sex chromosome evolution. *Copeia* **1979**, 509-513.

Borowsky, R. (1978). The tailspot polymorphism of *Xiphophorus* (Pisces: Poeciliidae). *Evolution* **32**, 886-893.

Borowsky, R. and Kallman, K. D. (1976). Patterns of mating in natural populations of *Xiphophorus* (Pisces: Poeciliidae). 1, *X. maculatus* from Belize and Mexico. *Evolution* **30**, 693-706.

Brooks, R. and Endler, J. A. (2001). Direct and indirect sexual selection and quantitative genetics of male traits in guppies (*Poecilia reticulata*). *Evolution* **55**, 1002-1015.

Clegg, M. T. and Durbin, M. L. (2000). Flower color variation: A model for the experimental study of evolution. *Proc. Natl. Acad. Sci. USA* **97**, 7016-7023.

Endler, J. A. (1978). A predator's view of animal color patterns. *Evol. Biol.* **11**, 319-364.

Endler, J. A. and Houde, A. (1995). Geographic variation in female preferences for male traits in *Poecilia reticulata. Evolution* **49**, 456-468.

Epperson, B. K. and Clegg, M. T. (1987). Instability at a flower color locus in the morning glory. *J. Heredity* **78**, 346-352.

Hadley, M. E. (2000). The melanotropic hormones. In *Endocrinology*, 5th Edn. Upper Saddle River, NJ: Prentice-Hall.

Halaban, R., Svedine, S., Cheng, E., Smicun, Y., Aron, R. and Hebert, D. (2000). Endoplasmic reticulum retention is a common defect associated with tyrosinase-negative albinism. *Proc. Natl. Acad. Sci. USA* **97**, 5889-5894.

Horth, L. (2001). The maintenance of a genetic body-color polymorphism in male mosquitofish, *Gambusia holbrooki.* PhD dissertation, Florida State University, Tallahassee, FL, USA.

Horth, L. (2005). Melanism and melanocortin-1 receptor mutations in vertebrates. *Proc. Indian Natl. Sci. Acad. B* **70**, 499-515.

Horth, L. and Travis, J. (2002). Frequency-dependent numerical dynamics in mosquitofish. *Proc. R. Soc. London B* **269**, 2239-2247.

Houde, A. E. (1997). *Sex, Color and Mate Choice in Guppies*, 207pp. Princeton, NJ, USA: Princeton University Press.

Imes, D. L., Geary L. A., Grahn, R. A. and Lyons, L. A. (2006). Albinism in the domestic cat (*Felis catus*) is associated with a tyrosinase (TYR) mutation. *Animal Genet.* **37**, 175-178.

Jablonski, N. G. and Chaplin, G. (2000). The evolution of human skin coloration. *J. Hum. Evol.* **39**, 57-106.

Jeong, H., Kim, M. S., Kwon, J., Kim, K. S. and Seol, W. (2006). Regulation of transcriptional activity of the tyrosine hydroxylase gene by androgen receptor. *Neurosci. Lett*. **396**, 57-61.

Kallman, K. D. (1965). Genetics and geography of sex determination in the poeciliid fish, *Xiphophorus maculatus*. *Zoologica* **50**, 151-190.

Kazianis, S. D., Morizot, C., Della Coletta, L., Johnston, D. A., Woolcock, B., Vielkind, J. R. and Nairn, R. S. (1999). Comparative structure and characterization of a CDKN2 gene in a *Xiphophorus* fish melanoma model. *Oncogene* **18**, 5088-5099.

Kettlewell, H. B. D. (1973). *The Evolution of Melanism: A Study of Recurring Necessity*. Oxford, UK: Oxford University Press.

Kijas, J. M. H., Moller, M., Plastow, G. and Andersson, L. (2001). A frameshift mutation in *MC1R* and a high frequency of somatic reversions cause black spotting in pigs. *Genetics* **158**, 779-785.

Kim, D. S., Park, S. H., Kwon, S. B., Joo, Y. H., Youn, S. W., Sohn, U. D. and Park, K. C. (2003). Temperature regulates melanin synthesis in melanocytes. *Arch. Pharm. Res.* **26**, 840-845.

Kim, H.-S, Hong, S. J., LeDoux, M. S. and Kim, K.-S. (2001). Regulation of the tyrosine hydroxylase and dopamine beta-hydroxylase genes by the transcription factor AP-2. *J. Neurochem.* **76**, 280-294.

Klunglund, H., Vage, D. I., Gomez-Raya, L., Adalsteinsson, S. and Lien, S. (1995). The role of melanocyte-stimulating hormone (MSH) receptor in bovine coat color determination. *Mamm. Genome* **6**, 636-639.

Kwon, B., Halaban, R. and Chintamenei, C. (1989). Molecular basis of a mouse Himalayan mutation. *Bioch. Biophys. Res. Commun.* **161**, 252-260.

Lamason, R. L., Mohideen, P. K. M.-A., Mest, J. R., Wong, A. C., Norton, H. L., Aros, M. C., Jurynec, M. J., Mao, X., Humphreville, V. R., Humbert, J. E., et al. (2005). SLC24A5, a putative cation exchanger, affects pigmentation in zebrafish and humans. *Science* **310**, 499-515.

Lindholm, A. and Breden, F. (2002). Sex chromosomes and sexual selection in Poeciliid fishes. *Am. Nat.* **160**, S214-S224.

Lynch, M. and Walsh, B. (1998). *Genetics and Analysis of Quantitative Traits,* 980pp. Sunderland, MA, USA: Sinauer Assoc., Inc.

Lyons, L. A., Imes, D. L., Rah, H. C. and Grahn, R. A. (2005). Tyrosinase mutations associated with Siamese and Burmese patterns in the domestic cat (*Felis catus*)*. Anim. Genet.* **36**, 119-126.

Majerus, M. E. (1998). *Melanism. Evolution in Action*. Oxford, UK: Oxford University Press.

Martin, R. G. (1984). Proportion of melanic offspring resulting from crosses between melanic male mosquitofish and normal female mosquitofish, *Gambusia affinis holbrooki. J. Elisha Mtichell Scientific Society.* **100**, 121-123.

Meffe, G. K. and Snelson, F. F., Jr (1989). *Ecology and Evolution of Livebearing Fishes* (Poeciliidae). Englewood Cliffs, NJ, USA: Prentice Hall.

Mundy, N. I., Badcock, N. S., Hart, T. Scribner, K., Janssen, K. and

Nadeau, J. J. (2004). Conserved genetic basis of a quantitative plumage trait involved in mate choice. *Science* **303**, 1870-1873.

Nagatsu, T., Levitt, M. and Udenfriend, S. (1964). Tyrosine hydroxylase; the initital step in norepinephrine biosynthesis. *J. Biol. Chem.* **239**, 2910-2917.

O'Grady, P. M. and DeSalle, R. (2000). Insect evolution: How the fruit fly changed (some of) its spots. *Curr. Biol.* **10**, R75-R77.

Owusu-Daaku, K. O., Wood, R. J. and Butler, R. D. (1997). Variation in Y chromosome meiotic drive in *Aedes aegypti* (Diptera: Culicidae): a potential genetic approach to mosquito control. *Bull. Entomol. Res.* **87**, 617-623.

Poulton, E. B. (1909). Mimicry in the butterflies of North America. *Ann. Entomol. Soc. Am.* **2**, 203-242.

Regan, J. D. (1961). Melanodimorphism in the poeciliid fish, *Gambusia affinis* (Baird and Girard). *Am. Mid. Nat.* **65**, 139-143.

Robbins, L. S., Nadeau, J. H., Johnson, K. R., Kelly, M. A., Roselli-Rehfuss, L., et al., (1993). Pigmentation phenotypes of variant extension locus alleles result from point mutations that alter MSH receptor function. *Cell* **72**, 827-834.

Snelson, F. F., Jr, Smith, R. E. and Bolt, M. R. (1986). A melanic female mosquitofish, *Gambusia affinis holbrooki. Am. Mid. Nat.* **115**, 413-415.

Takeuchi, S. H., Suzuki, H., Yabuuchi, M. and Takahashi, S. (1996). A possible involvement of melanocortin 1-receptor in regulating feather color pigmentation in the chicken. *Bichim. Biophys. Acta* **1308**, 164-168.

Theron, E., Hawkins, K., Bermingham, E., Ricklefs, R. and Mundy, N. (2001). The molecular basis of an avian plumage polymorphism in the wild: A *melanocortin-1-receptor* point mutation is perfectly associated with the melanic plumage morph of the bananaquit, *Coereba flaveola. Curr. Biol.* **11**, 550-557.

True, J. R., Edwards, K. A., Yamamoto, D. and Carroll, S. (1999). Drosophila wing melanin patterns form by vein-dependent elaboration of enzymatic prepatterns. *Curr. Biol.* **9**, 1382-1391.

Turner, C. L. (1960). The effects of steroid hormones on the development of some secondary sexual characters in cyprinodont fishes. *Trans. Am. Microsc. Soc.* **79**, 320-333.

van Z. Brower, J. (1958). Experimental studies of mimicry in some North American butterflies. II. *Battus philenor* and *Papilio troilus, P. polyxenes* and *P. glaucus. Evolution* **12**, 123-136.

Volff, J.-N., Körting, C., Altshcmeid, J., Duschl, J., Sweeney, K., Wichert, K., Froschauer, A. and Schartl, M. (2001). *Jule* from the fish *Xiphophorus* is the first complete vertebrate *Ty3/Gypsy* retrotransposon from the *Mag* family. *Mol. Biol. Evol.* **18**, 101-111.

Watt, W. B. (1969). Adaptive significance of pigment polymorphisms in *Colias* butterflies. II. Thermoregulation and photoperiodically controlled melanin variation in *Colias eurytheme*. *Proc. Natl. Acad. Sci. USA* **63**, 767-774.

Wood, R. J. and Newton, M. E. (1991). Sex ratio distortion caused by meiotic drive in mosquitos. *Am. Nat.* **137**, 379-391.

Wood, S. R., Berwick, M., Ley, R. D., Walter, R. B., Setlow, R. B. and Timmins, G. S. (2006). UV causation of melanoma in *Xiphophorus* is dominated by melanin photosensitized oxidant production. *Proc. Natl. Acad. Sci. USA* **103**, 4111-4115.

Zhou, Q., Froschauer, A., Schultheis, C., Schmidt, C., Bienert, G. P., Wenning, M., Dettai, A. and Volff, J.-N. (2006). Helitron transposons on the sex chromosomes of the platyfish *Xiphophorus maculatus* and their evolution in animal genomes. *Zebrafish* **3**, 39-52.