Review Article

Pigmentation in *Xiphophorus*: An Emerging System in Ecological and Evolutionary Genetics

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Abstract

The genus *Xiphophorus* has great potential to contribute to the study of vertebrate pigmentation and elucidating the relative influence of ecology, physiology, and behavior on evolution at the molecular level. More importantly, the association between pigmentation and a functional oncogene offers the potential to understand the evolution and maintenance of cancer-causing genetic elements. Using criteria laid out recently in the literature, I demonstrate the power of the *Xiphophorus* system for studying pigment evolution through integrative organismal biology. Using the most recent phylogeny, the phylogenetic distribution of several important pigmentation loci are reevaluated. I then review support for existing hypotheses of the functional importance of pigmentation. Finally, new observations and hypotheses regarding some of the characteristics of pigment patterns in natural populations and open questions and future directions in the study of the evolution of these traits are discussed.

Introduction

VARIATION IN PIGMENTATION is one of the most striking examples of polymorphism in the natural world. This variation is of considerable evolutionary importance due to the wide range of mechanisms of both natural and sexual selection that act on these traits as summarized by a number of recent reviews.[1–5] The causes and consequences of color and pattern variation span a gamut of behavioral, ecological, and physiological contexts, as well as the developmental pathways that lead to pigment phenotypes. Perhaps most importantly, the study of pigment variation facilitates investigation not just of the functional consequences of variation in color and pattern, but of evolution at the genetic level that gives rise to this variation. In fact, the study of pigmentation traits and their underlying genes is among the most tractable fields to better understand molecular evolution.[1]

Several recent reviews have discussed pigmentation traits in a variety of contexts, including their signaling properties, effects on behavior, and power for ecological and evolutionary studies. The purpose of this review is not to rehash their same points, although they are summarized in brief detail below and can be accessed for further information. Rather, the purpose of this review was to describe an emerging, but often overlooked, system with the potential to contribute substantially to the study of ecological and evolutionary genetics of pigmentation.

Interest in pigmentation has arguably increased over the last decade and a number of reviews have focused on the potential for study of pigmentation to address important areas in evolutionary biology, including visual ecology and signaling,[2] behavior,[3] and ecology.[4] Two recent reviews by Hubbard *et al.*[4] and Kronforst *et al.*[1] provide excellent summaries of the power and potential for pigmentation traits to advance our understanding of evolutionary ecology, particularly at the genetic level. Studying these traits provides an opportunity to understand the genetic basis of morphological variation with functional consequences for fitness stemming from behavioral, ecological, and physiological sources. The common thread that emerges from these reviews is that the systems poised to make the greatest contributions are those in which both genetic and ecological investigations of pigmentation are possible. In fact, Kronforst *et al.*[1] point out that the two most important considerations for emerging systems in pigmentation research are the degree of polymorphism in color and patterns and the feasibility of genetic studies. The authors went on to list six important characteristics that make pigmentation research more feasible in emerging systems, which I will paraphrase here: (1) polymorphism within or among populations of a species or between species, (2) amenable to captive breeding designs, (3) small size, short generation times, and high fecundity, (4) availability of genomic resources for the focal species or a close relative, (5) *in situ*

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hybridization capability, and (6) ability to conduct gene knockdown or overexpression.

The Potential of *Xiphophorus* for the Study of Pigmentation

Xiphophorus is a genus of small livebearing fishes distributed from NE Mexico to Guatemala and Honduras.[6] They have been collected and studied for over 160 years beginning with *Xiphophorus hellerii*, perhaps the most well known member of the genus. *Xiphophorus* first became popular with aquarists who valued the striking variation in morphology, including melanin-based pigmentation traits. Those melanistic traits quickly drew interest from researchers due to the ease of inducing melanomas in both hybrid and nonhybrid situations due to the conservation of a functional oncogene, *Xmrk*, in many species of the genus.[7] This sparked a century of ongoing research on melanoma formation and genetics. More recently, dating back to the late 1980s, the genus has become a model system for studies of sexual selection and particularly female mate choice and sexual signaling. This is due in large part to the considerable variation within and among species in morphological traits, specifically the sword ornament. One of the most variable components of *Xiphophorus* morphology are the numerous pigmentation traits, of which 40 can be found in the southern platyfish, *Xiphophorus maculatus*, alone (reviewed by Basolo[8]). This review will focus on melanin-based pigment traits because these are the traits most commonly shared by two or more members of the genus, which enables comparative studies of the functional importance and genetic basis of these traits. Nonetheless, even within only the melanin-based traits, there is considerable variation in *Xiphophorus* with at least 21 such traits.[7–9]

An important characteristic of the genus *Xiphophorus* is that it meets all of the six criteria laid out by Kronforst *et al.*[1] for emerging systems in the study of pigmentation. First, melanin-based traits are polymorphic within and among populations of species and polymorphic between species. Second, *Xiphophorus* adapt well to captivity and controlled breeding designs for genetic mapping and related experiments have been successful for over 50 years.[10–12] Third, these species reproduce readily in aquaria at short intervals (30–60 days or less), are small enough (∼2–8 cm) to maintain many breeding individuals in a relatively small space, and produce multiple offspring per brood several of which approach 20 fry per brood with occasional broods reaching 50–60 fry in some species. Fourth, the annotated platyfish genome (*X. maculatus*) was recently completed,[13] simplifying the assembly of transcriptomes[14] and genomes[15] of other *Xiphophorus* species and providing an important new resource for pigmentation genetics and comparative studies in the genus. Fifth, *in situ* hybridization capabilities exist and have been used in a variety of studies.[16–18] Sixth, while not yet commonly performed, gene knockdown methodologies have been applied to *Xiphophorus* cell lines.[19] While gene knockout is not currently possible, functional assays can typically be conducted in the closely related *Medaka*. *Xiphophorus* also has two significant advantages not mentioned in the six criteria of Kronforst *et al.*[1] Those are (1) rich historical records from populations in the wild and potential for field and ecological studies and (2) rarely paralleled capability of behavioral studies. Ecology and behavior are critical aspects in the study

of color polymorphism.[3,4] The study of coat color polymorphism in mice had been primarily laboratory-based studies of genetics until the more recent emphasis on ecological context. Several studies on different species of mice in the past 10 years have shed light on the power of combining laboratory models with genomic resources and the study of pigmentation in species' natural habitats (reviewed in Hubbard *et al.*[4]).

Although ecology has been an understudied discipline in *Xiphophorus*, as will be discussed in more detail later, the potential for ecological study in the genus is undeniable. Within and among species, there is heterogeneity in abiotic environments, including climate, visual ecology (temporal variation in turbidity), substrate type, color, and size; heterogeneity in fish communities, including sympatry and allopatry with a variety of congeners and other poeciliids; and variation in predator regimens. As mentioned elsewhere throughout this review, inter- and intra-sexual selections are widely studied in *Xiphophorus*—although currently less so in the context of pigmentation. The resources and knowledge that have developed over the past two decades will greatly facilitate the study of the behavioral aspects of pigment patterns in the genus.

Melanin-Based Traits in *Xiphophorus*

The melanin-based traits of *Xiphophorus* traits are shared among many members of the genus and exhibit striking polymorphism. Additionally, the genetics of these traits are generally better understood than other pigment traits owing largely to the long history of studies on melanoma. In *Xiphophorus*, melanistic traits are divided into two categories: macromelanophore and micromelanophore. Many species of *Xiphophorus* exhibit dots or blotches of black pigment that are formed by macromelanophores (up to 500 μm). These patterns take a wide variety of forms from speckles to large blotches producing a mottled appearance and occur primarily on the body (Fig. 1) but sometimes on the fins (e.g., spotted dorsal of *X. maculatus*). There are at least 17 macromelanophore patterns among members of the genus.[7,8] These patterns are coded by the macromelanphore determining locus (*Mdl*). All of the populations where these patterns are known to occur are polymorphic with only a subset of individuals in a population exhibiting macromelanophores at any given time. However, with the exception of spotted caudal (Sc), the forces maintaining polymorphism in these traits are not well understood at the present. The Sc pattern consists of macromelanophore spots that occur at the junction of the caudal peduncle and fin (Fig. 1). Although occurring in only a few members of the genus, this pattern has received more attention in the context of sexual selection,[20,21] which will be discussed in subsequent sections.

Since the time that a two-locus model for melanoma formation and suppression was proposed, it was believed that the "tumor locus"—later described as *Xmrk*—was itself the gene that also gave rise to macromelanophores (for review, see Weis and Schartl[7] and Anders[22]). Schartl[23] provides further information regarding *Xmrk* and the pathways involved in the formation of melanoma in species with *Mdl*. Considerable progress in cancer and pigmentation genetics was made when *Mdl* was discovered to be a distinct locus from the *Xmrk* oncogene.[7] Furthermore, work done on the recently completed genome of *X. maculatus* has highlighted a
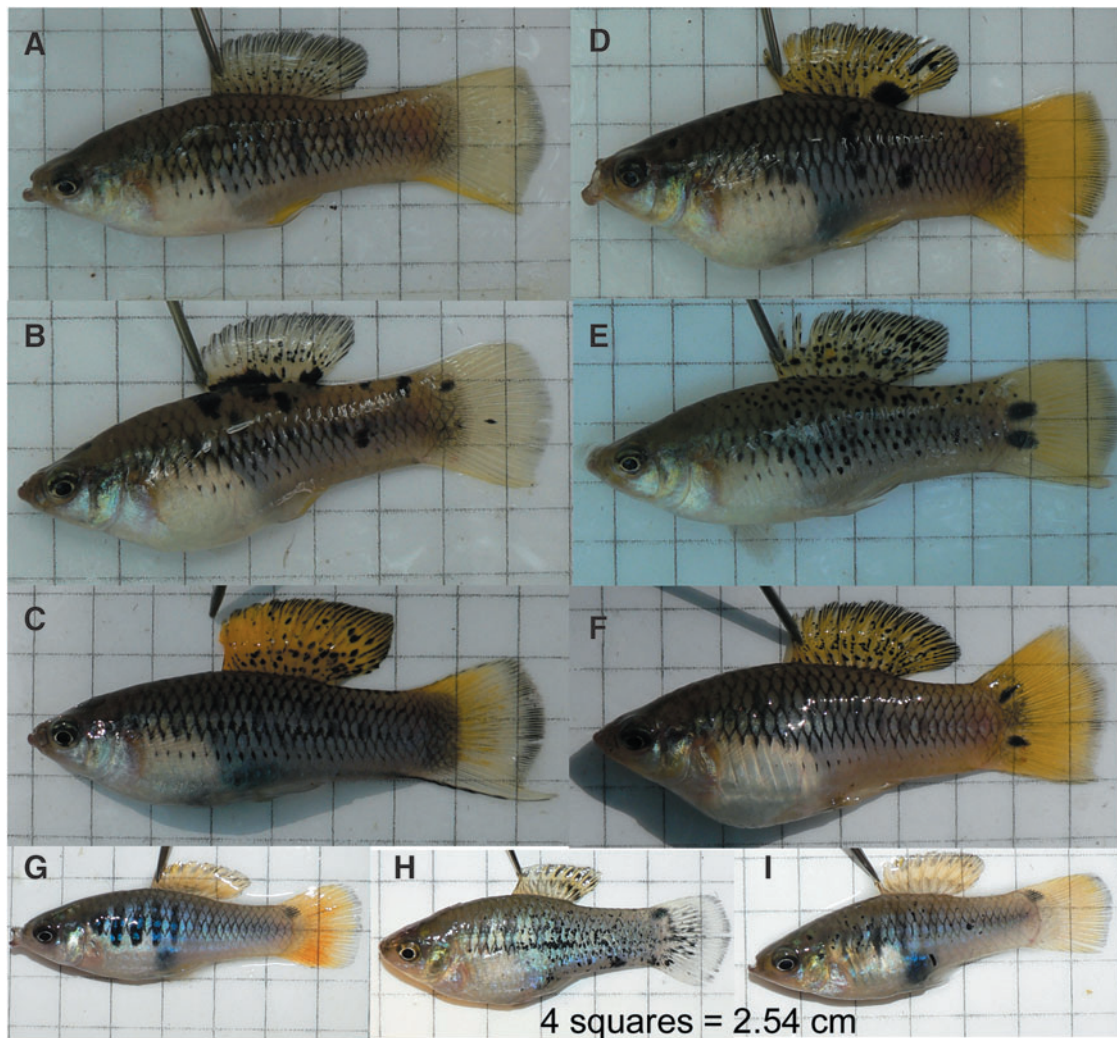
FIG. 1. Male *Xiphophorus* from a population of natural hybrids between *Xiphophorus birchmanni* and *Xiphophorus malinche* exhibits a variety of different melanin-based pigment traits **(A–F)** and *Xiphophorus variatus* exhibiting different tailspot patterns **(G–I)**: macromelanophore (M) patterns including spotted caudal (Sc), and micromelanphore patterns caudal blotch (Cb), tailspot locus (Ts), and vertical bars (Vb). These males demonstrate the variation that exists in the size and shape of the different traits among individuals, even within populations in the case of fish **(A–F)** all from the same locality and collection date. **(A)** Vb, no other pattern. **(B)** M. **(C)** Cb, faint Vb. **(D)** M, possible Cb. **(E)** M, Sc, Vb. **(F)** Sc, faint Vb. **(G)** Ts, Vb. **(H)** M, Ts, Vb. **(I)** M, Ts, Vb. Color images available online at www.liebertpub.com/zeb

candidate gene for *Mdl* that has known involvement in pigmentation and that maps closely to the location of *Xmrk* on the X chromosome of the platyfish.[13] In addition to determining that *Mdl* is a separate locus from *Xmrk*, Weis and Schartl[7] plotted the distribution of those loci and inferred that both were likely present in the most recent common ancestor to the genus. They estimated the age of *Mdl* and *Xmrk* to be at least 5–6 million years old, which is broadly consistent with an estimated age of the genus at 4–6 million years ago (MYA) (R. Cui, pers. comm.). Their analysis revealed that pigmentation traits (i.e., *Mdl*) appeared to be gained and lost multiple times in the genus and in an uneven manner. Specifically, the presence/absence of *Mdl* did not coincide with monophyletic groups, suggesting a more complex evolutionary history (gain/loss) of the locus than might be expected if monophyletic groups share similar life history or ecological traits. This pattern could be due partly to demographic effects or extensive reticulate evolution that has occurred in the genus and will be discussed in more detail below.

A second class of pigment patterns found in *Xiphophorus* is composed of smaller cells called micromelanophores. Although overlapping in size, the pigment cells of micromelanophores are characterized by being smaller on average than macromelanophores. The micromelanophore patterns include caudal blotch (Cb), vertical bars, and a diversity of "tailspot" patterns (Fig. 1). With the exception of tailspots, micromelanophore patterns differ from macromelanophore patterns in that their expression is affected by the nervous system. Expression is often increased and patterns are darker during social encounters but lighter in social isolation.[24,25] The Cb trait is perhaps the least well known and least studied of the melanin-based traits. It stretches across the caudal peduncle from top to bottom stopping at the uppermost fin ray of the sword. The homology of Cb among species and with

other patterns is not well documented nor is its functional significance understood. Tailspot patterns are found only among members of the platyfish clade and are one of the most polymorphic patterns (within species) in the genus with as many as eight patterns in *X. maculatus* and six in *Xiphophorus variatus*.[25,26] These patterns are controlled by single alleles at the tailspot locus (herein termed *Ts*) with three known modifier loci.[25,26]

Vertical bars are deep lying micromelanophores arranged in a series of vertical stripes or bars that typically straddle the lateral line of the fish but vary greatly in size and shape among species.[9] I should note that while "melanism" in the literature typically refers to dark, uniformly expressed coloration. Vertical bars therefore differ slightly from this traditional definition of "melanistic traits." However, they will be discussed here due to the fact that they are melanin-based and are constitutively expressed in some species. These vertical bars were originally called "parr" markings and are found in most species of *Xiphophorus*. Vertical bars are present in both sexes, but they are often less frequent and their expression less pronounced in females. Males of a variety of species have the ability to vary the expression of pigment in vertical bars, which is often associated with social encounters and particularly aggressive behavior between males. Not all species exhibit the ability to vary expression and bar darkening is therefore not an honest signal of escalating aggression in all species.[24] All *Xiphophorus* with vertical bars are polymorphic and the frequency of individuals with bars varies among species and populations. Interestingly, Zimmerer and Kallman[27] presented evidence that, at least in *Xiphophorus nigrensis*, the absence of bars is due to the presence of a suppressor gene that prevents bar expression. The genetic factors giving rise to bars are likely than actually monomorphic, but the suppressor gene is polymorphic, producing polymorphism in the expression of the trait. More work is needed to fully understand this phenomenon and the extent to which it may play a role in polymorphism of other traits and the extent to which mutation in any of the genes involved in the pathways producing vertical bars might lead to a suppression effect.

In their landmark work, Weis and Schartl[7] demonstrated that *Mdl* is separate from *Xmrk*, representing perhaps the most prominent step forward in the study of pigmentation genetics in *Xiphophorus* in decades. Together, Weis and Schartl[7] and Schartl[23] provided the first phylogenetic examinations of the macromelanophore encoding locus *Mdl* and the oncogene *Xmrk*. Since that time, and even in the 5 years since Schartl,[23] multiple phylogenies with higher resolution have been constructed with the greatest confidence in evolutionary relationships among species of the genus to date. These phylogenetic advances provide the opportunity to resolve the distributions of *Mdl* and other pigment traits and their underlying loci and to determine their evolutionary histories and test hypotheses regarding their origin and maintenance over some 4–5 million years of evolution. A variety of hypotheses have been either directly proposed or could be implicated for the evolutionary maintenance of these melanic pigment traits. However, these hypotheses have gone largely untested, and the work that has been conducted has yet to be synthesized. With the laboratory, genetic, and ecological resources available, *Xiphophorus* can be a potent emerging system in the study of pigmentation combining the power of a laboratory cancer model like the zebrafish (*Danio rerio*[28,29]) with the ecology of the

mouse complex (*Chaetodipus*, *Mus*, *Peromyscus*[5]). Together with genomic and transcriptomic resources, a rich history of established methodology for behavioral assays, and success in measuring reproductive success in the wild, *Xiphophorus* has the potential to become a leading model system in ecological and evolutionary genetics.

Revisiting the Phylogentic Distribution of Traits

A number of phylogenies based on diverse morphological and genetic characters exist for *Xiphophorus*.[9,14,30–36] Herein, I use the recent phylogeny of Jones *et al.*[36] constructed from >60,000 single-nucleotide polymorphisms derived from genome-wide restriction site-associated DNA sequencing with 15× coverage per individual and locus. The recent genome-wide phylogenies[14,36] differ only slightly in overall topology. These differences do not change the inferences of this review. The phylogeny of Jones *et al.*[36] is the first to contain all 26 species of *Xiphophorus*.

Using published data on the presence or absence of *Mdl* and macromelanophore patterns among species (Weis and Schartl,[7] Schartl,[23] and original species descriptions), and mapping it onto the phylogeny of Jones *et al.*,[36] produces an evolutionary history with subtle but important differences from previous studies. With only two primary exceptions, the presence or absence of *Mdl* is broadly consistent with major evolutionary lineages in the genus (Fig. 2). Assuming that the origin of *Mdl* preceded the divergence of *Xiphophorus*[7] and that *Mdl* was present in the most recent common ancestor, it appears to have been lost in three primary groups: the Rio Grande platyfish, the southern swordtails, and the lineage of northern swordtails leading to *Xiphophorus multilineatus*, *X. nigrensis*, *Xiphophorus continens*, and *Xiphophorus pygmaeus*. The distribution of *Mdl* therefore corresponds well with monophyletic groups in the genus rather than having a mosaic distribution with more haphazard gain/loss inferred from older phylogenies. The exceptions are as follows: *Mdl* was lost in the southern platyfish *Xiphophorus andersi* and gained in the southern swordtail *X. hellerii*.

Mdl was lost in *X. andersi*, which also lacks the micromelanophore tailspot patterns that are typical of most platyfish. Neither *Mdl* nor tailspots are present in the three species of Rio Grande platyfish, *Xiphophorus couchianus*, *Xiphophorus gordoni*, and *Xiphophorus meyeri*. Whether these loses could be due to founder effects during range expansion of an ancestral form, result from a bottleneck or from genetic drift in historically small populations would require more investigation. Given that *X. maculatus* is likely the most basal of the platyfish, and that *Xiphophorus clemenciae* is correctly placed among the southern swordtails rather than platyfish,[14,36] it suggests that *Mdl* was ancestral to all platyfish and lost in *X. andersi* and the Rio Grande platyfish. The placement of *X. clemenciae* with southern swordtails is well supported and makes sense from the standpoint of *Mdl*, which is absent in *X. clemenciae* and almost all members of the monophyletic southern swordtails. I should draw attention to the fact that *X. hellerii* is the only species of the southern swordtails that has been thoroughly studied. Although Kallman *et al.*[37] conducted extensive sampling in the ranges of *X. clemenciae*, *Xiphophorus monticolus*, and *Xiphophorus mixei* and did not report any evidence of any macromelanophores that would suggest the presence of *Mdl* in those species.
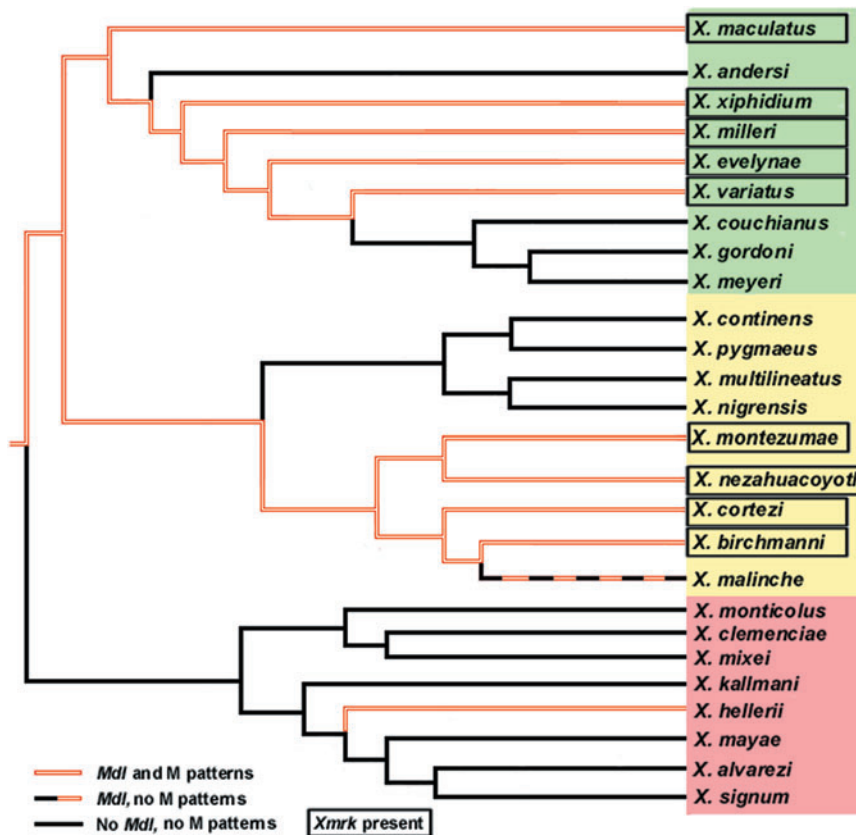
FIG. 2. The distribution of macromelanphore determining locus (*Mdl*), macromelanophore (M) patterns, and *Xmrk* plotted on a total evidence phylogeny. *Open branches* indicate lineages where *Mdl* is present, *filled branches* indicate where *Mdl* is absent, and *open* and *filled* indicates that *Mdl* is present but macromelanophore patterns do not occur. Boxes around species names denote those that have the oncogene *Xmrk*. Note: The phylogenetic tree comes from Jones *et al.*[36] (see Revisiting the Phylogenetic Distribution of Traits) and has been altered with the author's permission. The tree does not necessarily indicate the evolutionary history of the plotted traits themselves. Color images available online at www.liebertpub.com/zeb

The case of *X. hellerii* is more complex. *X. hellerii* is the only species that has *Mdl* in an otherwise *Mdl*-absent clade. It seems unlikely that this locus would be under sufficient selection to be retained in *X. hellerii* since the split with platyfish and northern swordtails, yet be sufficiently neutral as to be lost in six other lineages of southern swordtails nor to be under such negative selection among most species but escape selection in *X. hellerii* and many northern swordtails and platyfish. The presence of *Mdl* within a single lineage of southern swordtails alternatively could be a result of historical gene flow with platyfish. Cui *et al.*[14] documented considerable reticulate evolution within the genus and evidence for historical gene flow within and among many lineages, including gene exchange between platyfish and southern swordtails. More specifically, Schumer *et al.*[15] found evidence for significant gene flow—as much as 4%—between *X. maculatus* and *X. hellerii*. *Mdl* is not the only locus to show a similar pattern (see Phylogenetic Distribution of Ts and its Modifiers). If *Mdl* and associated pigmentation is under selection,[38] one can postulate that selection for macromelanophores could maintain the locus in *X. hellerii* following historical genetic exchange.

Further evidence of potential genetic exchange comes from the absence of *Xmrk* in *X. hellerii*, despite the presence of *Mdl* and macromelanophore patterns. Weis and Schartl[7] and later Schartl[23] demonstrated that every species known to have *Xmrk* also exhibits macromelanophore patterns. That is, all species known to have *Mdl* and macromelanophore patterns also have *Xmrk*. The only two exceptions were *X. hellerii* and *Xiphophorus nezahualcoyotl*.[23] *Xiphophorus malinche* is not included here due to the fact that macromelanophore patterns

are not observed in natural populations (Zachary W. Culumber, pers. obs.), even though the genetic entity *Mdl* is present in the species.[7] Regarding *X. nezahualcoyotl*, Fernandez and Bowser[39] presented evidence that this species may be polymorphic for *Xmrk*, meaning that specimens typed previously by Weis and Schartl[7] may have simply not carried *Xmrk*. Among their evidence was the formation of nonhybrid melanoma associated with *X. nezahualcoyotl* with the spotted side (Ss) pattern. Nonhybrid melanomas have been reported in two other species, *Xiphophorus cortezi* and *X. variatus*, both of which have *Xmrk*. Second, the histological characterisitics of the lesions closely matched those of other melanomas associated with *Xmrk* in *Xiphophorus*. Finally, *X. nezahualcoyotl* has *Mdl*, which is a necessary condition for the presence of *Xmrk*. All species known to have *Xmrk* are polymorphic for its presence.[23] This may not be surprising given that *X. nezahualcoyotl* may be the result of ancient admixture between *X. cortezi* and and *Xiphophorus montezumae*,[14] both of which have *Mdl* and *Xmrk*. If *X. nezahualcoyotl* is assumed to have *Xmrk*, *X. hellerii* represents the only species known to have macromelanophore patterns but not *Xmrk*. Given that *X. hellerii* has been used in hybridizations for the study of melanoma for decades and is one of the most intensely sampled and studied species of the genus, it seems unlikely that *Xmrk* would have not been discovered in *X. hellerii* if polymorphic. The presence of macromelanophores and absence of *Xmrk* is consistent with historical gene flow as mating between *X. maculatus* (or a platy ancestor) and *X. hellerii* would have broken apart the otherwise tight linkage observed between the two loci in other species, such as *X. maculatus*. Thus, *Mdl* would be free of the potentially hitch-hiking *Xmrk* and could be maintained in the

lineage if macromelanophores confer a selective advantage in *X. hellerii* (*sensu* Fernandez and Bowser[39]).

Phylogenetic Distribution of Cb

Cb is restricted to the nine member of the northern swordtail clade and is not known to occur in platyfish nor in southern swordtails. Six of the nine northern swordtails exhibit Cb and occur in sympatry with at least one other species of swordtail or the platyfish *X. variatus* (Fig. 3). Given its distribution, Cb appears to have arisen in the most recent common ancestor of the northern swordtails. It was subsequently lost in the lineage leading to *X. continens* and *X. pygmaeus* and that became *Xiphophorus birchmanni*. Interestingly, the distribution of Cb shows a strikingly close relationship with the distribution of size, and preference for, the sword trait (Fig. 3). Cui *et al.*[14] compiled the sword index (SI) for all *Xiphophorus* where the SI is equal to the sword extension length divided by the standard length. They assigned a value of 0.275 for species with no sword. Here, I have adjusted the values by subtracting 0.275 from all SIs, such that species with no sword have an SI of 0.0, and scaled all remaining values to 1.0. *X. birchmanni* has been a assigned a value of 0.0 due to the fact that well below 1% of the adult male population exhibits a caudal extension <1 mm (Rauchenberger *et al.*,[9] Zachary W. Culumber, pers. obs.). The most important point is that *X. birchmanni*, *X. continens*, and *X. pygmaeus* represent the three shortest swords in the clade. Species that have a highly reduced or no sword—and which typically have reduced or no preference for swords—also lack Cb. Figure 3 shows the "relative sword index" for each species. Whether Cb could be linked to sword or preference genes cannot be said, but the co-absence of Cb and swords/preference appears nonrandom. Laboratory crosses between species and future genetic studies are necessary to determine whether there is an association between these traits. However, of the 128 genes identified as potentially involved in sword production, none appear to have a role in pigmentation.[40]

The Sc trait was not mapped to the phylogeny owing to the fact that only three species exhibit it. Two northern swordtails, *X. birchmanni* and *X. cortezi*, and the platyfish *X. variatus* exhibit a Sc pattern. The Sc of *X. variatus* is found only in the Río Tuxpan and Río Cazones drainage[6] and is likely not homologous to the Sc of the northern swordtails.[25] Weis and Schartl[7]

used different nomenclature for this pattern calling it "spotted caudal" (Sc) in *X. cortezi* but "caudal spotted" (Cs) in *X. birchmanni*. Given their close phylogenetic relationship, it is plausible that the Sc and Cs patterns are homologous in *X. cortezi* and *X. birchmanni*, but no known critical cross has been conducted to confirm whether these patterns are homologous and allelic or not. However, the patterns in these two species are very similar in appearance. If it is assumed that Sc in *X. birchmanni* and *X. cortezi* are homologous, this pattern most likely arose in the most recent common ancestor, which would include *X. malinche*. None of the known populations of *X. malinche* exhibit Sc, meaning that the pattern was likely secondarily lost. Interestingly, despite the fact that *X. birchmanni* has *Xmrk* and Sc, nonhybrid melanomas associated with Sc either do not occur or are exceedingly rare as none have ever been reported from the wild despite considerable sampling and study of the species. Why Sc and *Xmrk* would result in melanoma in *X. cortezi* but not in *X. birchmanni* is not clear. The patterns may be separate genetic entities in the two species, and melanoma formation is specific to *X. cortezi*. Or the patterns may be homologous, but the development of melanoma may result from differences in some other form of physiological or genetic regulation that differs between the two species.

Phylogenetic Distribution of *Ts* and Its Modifiers

Tailspots are a micromelanophore trait that is unique to the platyfish, although they have been lost multiple times (Fig. 4). It should be noted that the homology of the locus encoding tailspots (*Ts*) is not completely known. However, the homology of *Ts* among three of the four platyfish with tailspots, *X. maculatus*, *Xiphophorus milleri*, and *X. variatus*, is very probable.[11] Given its strictly platy distribution, it seems most likely that *Ts* was present in the most recent common ancestor. If *Ts* were present in northern or southern swordtails, but fixed for a wild-type state, crosses with patterned platyfish would produce some patterned offspring due to the fact that pattern alleles are dominant over the wild type. This is not yet known to have been reported. If this is true and *Ts* was in the most recent common ancestor of the platyfish, this would put its age at ∼2.6–5 MYA, taking 5 MYA as the age of the most recent common ancestor of *Xiphophorus*[7] and 2.6 MYA as an approximate age of platyfish (R. Cui, pers. comm.; but see
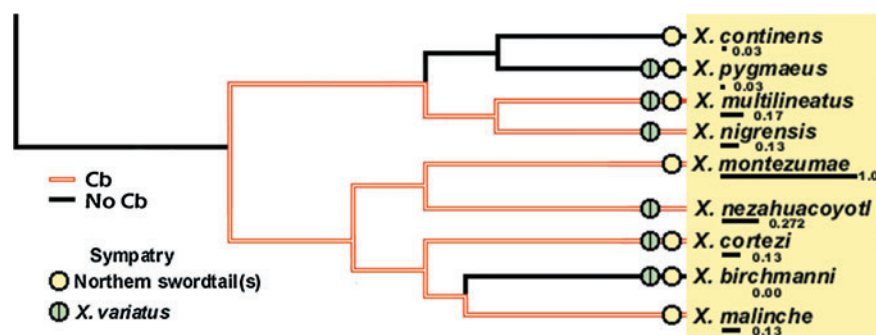

FIG. 3. The phylogenetic distribution of Cb and patterns of sympatry in the northern swordtails. *Red branches* indicate the presence and *black* the absence of the trait. *Yellow circles* on a branch denote that the species is sympatric with at least one other northern swordtail in part or all of its range. *Green circles* denote the same but for the platyfish, *X. variatus*, which is the only platyfish in sympatry with northern swordtails. Bars and values underneath each species name indicate the "relative sword index" (see Phylogenetic Distribution of Cb). See note on Figure 2 regarding tree topology. Color images available online at www.liebertpub.com/zeb
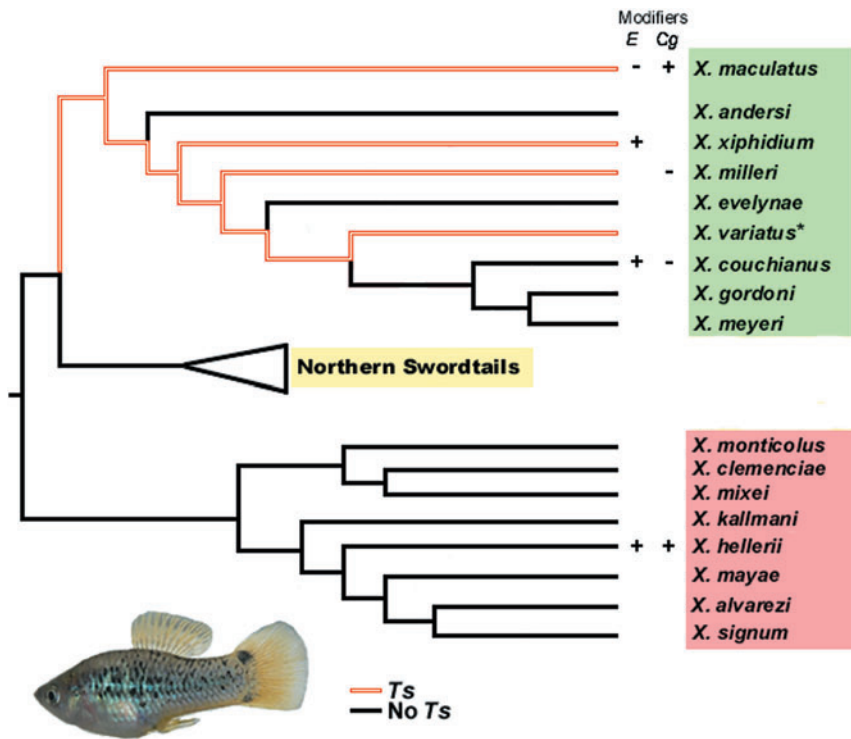
FIG. 4. The phylogenetic distribution of the *Ts* and its modifiers. Plus and minus signs indicate the presence and absence, respectively, of the indicated modifier loci. No signs mean that information is not available. Pictured is an *X. variatus* male with the cut crescent tailspot pattern. **X. variatus* has a third modifier, *Mod-1*, which is not shown here because its presence has not been tested in any other species. See note on Figure 2 regarding tree topology. Color images available online at www.liebertpub.com/zeb

Jones *et al.*[36]). With the exception of *X. milleri*, which has a small distribution but three tailspot patterns, the loss of tailspots coincides with species of restricted range (*X. andersi* and *Xiphophorus evelynae*) or that colonized distant localities (Rio Grande platyfish). It is not yet clear whether the loss of tailspots and tailspot variation is due to demographic effects, limited environmental variability or other factors. It should also be noted that there is some controversy over the designation of *X. evelynae* as a distinct species. Chen and Borowsky[41] noted that fish collected in the some of the same localities as the original description of *X. evelynae* bore a strong resemblance to *X. variatus*, including tailspot patterns. Tailspot patterns were originally considered to be absent in *X. evelynae*.

There are also three loci that modify specific tailspot patterns. Two such loci have been described for tailspots of *X. maculatus*. Of these, the modifier loci extensor (*E*) and Guatemala crescent (*Cg*) have an interesting distribution. Specifically, although *X. hellerii* does not have tailspots, natural populations of *X. hellerii* contain both *E* and *Cg*. In *X. maculatus*, the *Cg* modifier is restricted to only the Lake Peten population in Guatemala and nowhere else throughout its range.[42,43] The phylogenetic distribution of *Cg* is incompletely known, but neither *X. couchianus*, *X. nigrensis*, nor *X. milleri* exhibit *Cg*-like modification when hybridized with *X. maculatus*.[25] The *E* modifier is found in *X. hellerii* but is not known to occur in natural populations of *X. maculatus*. An *E*-like gene exists in both *Xiphophorus xiphidium* and *X. couchianus*, but their homology with the *E* of *X. hellerii* is not known. A third locus, *Mod-1*, has been described in *X. variatus*, but its geographic and phylogenetic distributions are incompletely known.[26]

How *X. hellerii* has attained two loci that modify *Ts*, a locus restricted to the platyfish, is not known. One possibility is that the modifier loci predated the evolution of *Ts*. This cannot be

entirely ruled out without further crosses with swordtails. The distribution of modifier loci may be explained by reticulate evolution. As discussed above, there is strong evidence of historical gene flow between platyfish and southern swordtails, including gene exchange between *X. maculatus* or its ancestor and *X. hellerii*. In addition, as with *Mdl*, there is evidence that modifier loci may be under selection. A gene transferred to *X. hellerii* that is under selection could be maintained through evolutionary time. As discussed in more detail below, effects of *Ts* on physiology play a role in the maintenance of tailspot polymorphism. Borowsky[26] presented evidence that *Mod-1* of *X. variatus* may also have effects on physiology. This provides a potential mechanism for selection to maintain modifier loci without *Ts*. More laboratory crosses among members of the genus are ultimately necessary to better understand the distribution of these modifier loci and rule out competing hypotheses regarding their evolutionary histories, as well as better understand any link that may exist between modifiers and physiology or other potential sources of selection that may favor their maintenance.

Hypotheses for the Maintenance of Polymorphism

Here, I focus on the primary hypotheses for why pigmentation patterns exist and are maintained in natural populations. I have placed these hypotheses into three broad categories: behavior, ecology, and physiology. Within each category, there are undoubtedly unexplored themes. The intention here is to touch upon hypotheses that have been explicitly proposed, common mechanisms that could impose selection, and provide a general overview of the work that has been conducted in each of these areas as they pertain to pigment patterns in the genus. For reference, in addition to the hypotheses described in works cited in this review, Gray and McKinnon[44] discussed many of the potential mechanisms for

the maintenance of color polymorphisms. It is worth noting that the selection favoring the maintenance of *Mdl* and its patterns has been proposed in large part due to the intimate association of *Xmrk*, a potentially injurious functional oncogene, with *Mdl*. Specifically, Weis and Schartl[7] suggested that the maintenance of *Xmrk* in so many members of the genus might be an example of genetic hitchhiking, in which the selection favoring the maintenance of *Mdl* would simultaneously maintain the tightly linked *Xmrk* locus.

Behavior

Sexual selection and female mate choice have been widely studied in *Xiphophorus* since the early 1990s (for review, see Rosenthal and Garcia de Leon[45]), with particular emphasis on conspicuous traits like body size,[46,47] dorsal fin size,[48,49] and the sword ornament.[50–52] Despite the precedent of visual traits playing an important role in female mate choice, comparatively less has been investigated into preferences that might promote polymorphism in melanic pigmentation traits. However, a small handful of examples that have been conducted suggest that preferences may be species- and context-dependent.

In one of the most interesting examples, female *X. cortezi* prefers males with the Sc pattern. The Sc pattern is often associated with the *Xmrk* oncogene leading to the formation of melanoma.[20] Males with Sc in natural populations also have larger body size than males without the pattern, which reinforces selection for Sc and *Xmrk* since female *Xiphophorus* prefer larger body size.[21] Female preferences therefore provide a mechanism to not only maintain the Sc pattern but a functional oncogene. Several studies have been conducted on female preferences for vertical bars. Preferences appear to be polymorphic with some females exhibiting strong preferences for bars, others against bars, and others with no preference.[53,54] This suggests that the maintenance of polymorphic bars may be tied largely to females preferences. However, in *X. pygmaeus*, a preference for males without bars may have led to the secondary loss of vertical bars.[55] Sister species *X. nigrensis* also has no bars, but females exhibit significant preference for males when bars are present,[56] which makes it difficult to determine whether lack of the trait or preference against the trait was present in their common ancestor. Franck *et al.*[38] evaluated female preferences for males with macromelanophore spots and found that preference for spotted males existed only in turbid water. Finally, in a recent study on the micromelanophore tailspots of *X. variatus*, Culumber and Rosenthal[57] demonstrated that patterned and unpatterned females had no preferences between patterned and unpatterned males, suggesting that nonrandom mate preferences do not maintain polymorphism in tailspots. Their data demonstrating a lack of preference for tailspots are consistent with mating patterns from natural populations that suggest random mating with respect to tailspots.[58] Although *X. maculatus* may exhibit some degree of nonrandom mating for tailspots,[59] the study of behavioral preferences has not been conducted. Preferences have not been tested for the vast majority of macromelanophore patterns nor for the micromelanophore pattern Cb. Furthermore, female preferences for vertical bars have been tested primarily in northern swordtails.

It has been suggested that a relationship between melanism and aggression in a variety of vertebrates including fish may

be the result of pleiotropy.[60] With the exception of vertical bars, the relationship between melanism and aggression in *Xiphophorus* is not well described. In fact, no studies of aggression or intrasexual signaling have been published on melanistic patterns other than vertical bars. Vertical bar expression is affected by social encounters with bars becoming darker during interactions between males, for example, but lighter when fish are in isolation and not interacting with other individuals. Moretz and Morris[24] showed that increased bar expression is a signal of aggressive intent in several species of swordtails. However, contrary to the typical link between melanism and increased aggression, Moretz[61] suggested that naturally barless males were more aggressive than males with bars as they bit more frequently and were more likely to win contests against barred opponents. Evidence of increased aggression exists in another live-bearer, *Gambusia holbrooki*. Melanic males are more aggressive toward females and spend more time chasing females and attempting matings than their siblings with no melanic coloration.[62] This aggressive behavior appears to translate to an effect on intersexual selection as females avoid mating attempts by melanic males to a greater extent than silver males. Melanic males may be maintained due to increased survivorship from predation.[63]

Pigmentation traits could facilitate location or recognition of conspecific individuals or potential mates. A visual signal that increases your probability of detection by conspecifics should increase your fitness and decrease the likelihood of hybridization with heterospecifics. This hypothesis could be relevant for *Xiphophorus* because multiple live-bearers often occur in sympatry in part and sometimes all of their natural range and co-occurence of two or three species of *Xiphophorus* in the same locality is also common.[6,64] However, there is an inherent problem with this hypothesis and that is that all of the melanin-based traits discussed here are polymorphic such that only a subset of the population has them at any one time. This does not entirely invalidate this hypothesis, but at least suggests that some form of selection must balance any benefit of increased recognition by conspecifics and that other traits may be more important in recognizing conspecifics than melanin-based traits.

Very few studies have been conducted in this regard, but both have suggested a role of two different traits in social/species recognition context. In *X. hellerii*, Franck *et al.*[38] demonstrated that shoaling preference for fish with macromelanophore spots and female preference for males with spots occurred only in turbid water. In clear water, females did not exhibit a preference for nor against spotted fish. Their findings suggest that macromelanophores increase detection in turbid water but provide no advantage in clear water. This would lead to a very clear prediction about the frequency of patterned fish during turbid conditions and about their reproductive success. Following the findings of Franck *et al.*,[38] males with patterns should have greater reproductive success when streams are turbid because they are more easily detected by females than males with no pattern. This produces a testable hypothesis that could be evaluated with molecular markers. One might expect females with patterns also to have greater reproductive success, if their pattern enables males to find them in turbid water.

A second study was conducted with *X. pygmaeus*, a smaller species that occurs in sympatry with *X. cortezi* (comparatively

larger body size) in part of its range. *X. pygmaeus* do not have vertical bars, whereas *X. cortezi* are polymorphic for bars.[9] As with females of most members of the genus, *X. pygmaeus* females have a preference for large body size.[65] By conducting mate choice trials with multiple signals, Hankison and Morris[66] were able to demonstrate that when presented with body size alone (no vertical bars) *X. pygmaeus* females preferred larger heterospecific males. When presented with males differing in body size but for which the larger, heterospecific *X. cortezi* male had vertical bars, *X. pygmaeus* females exhibited a preference for the smaller conspecific males. This suggests that in natural populations where these species co-occur vertical bars could be an informative trait helping to prevent hybridization between these species. Since the presence of vertical bars is polymorphic, vertical bars may not be a reliable signal. Any time a heterospecific male does not have vertical bars or other species-specific pigmentation trait, females would have to rely on chemical cues, for which preferences tend to be strongly in favor of conspecifics.[67–69] Given that chemical cues are always available for species recognition (but see Fisher *et al.*[69]), this would appear to render pigment patterns an unnecessary and potentially costly trait to produce and bear if there is a chance for increased predation or melanoma formation. This begs the question whether species or mate recognition alone can explain the maintenance of these patterns.

We can gain a better idea of the potential for these traits to function in species recognition by examining the phylogenetic distribution of these traits and comparing it to patterns of sympatry among species of the genus (Fig. 3). Within the northern swordtails alone, Cb and Sc would be informative in species-recognition in only three of five cases. *X. cortezi* has Cb and is sometimes sympatric with *X. multilineatus*, which also has Cb. The same is true for the Sc pattern. The only two northern swordtails to exhibit Sc, *X. cortezi* and *X. birchmanni*, have a small area of sympatry and are suspected to hybridize.[6] Four of the six northern swordtails that occur in sympatry with the platyfish *X. variatus* exhibit swords, which can presumably be used to detect conspecifics regardless of whether Cb is present since *X. variatus* has no sword. Two species that have no or a highly reduced sword, *X. pygmaeus* and *X. birchmanni*, also occur in sympatry with *X. variatus*. Neither of these species have Cb, which would be informative for species recognition had it been maintained in those lineages. If those two cases are included, the presence of Cb is only informative in species recognition in three of seven cases of sympatry. Similarly, sympatric populations of two morphologically similar species, *X. birchmanni* and *X. variatus*, are both polymorphic for macromelanophore body spots, which would be an unreliable indicator of species recognition. Although Kallman and Kazianis[6] pointed out that body size differs in many of the sympatric species pairs throughout the genus, which could be a prezygotic barrier (*sensu* Marcus and McCune[31]), and that body size is more similar in the known cases of natural hybridization, all available evidence suggests that body size would not be sufficient to prevent hybridization since females typically prefer larger body size even if when the larger male is a heterospecific.[66] The picture that emerges is that pigmentation trait may be single component of a suite of traits used in a hierarchy for species or mate recognition. This is particularly true given that preferences for visual cues of heterospecifics are common in northern swordtails[51,54,65] and some species with very different morphology, including multiple pigment patterns naturally hybridize in the wild.[6,70,71] Olfactory cues are likely the most reliable signal in species and mate recognition in natural populations.[67–69] There must therefore be some additional form of selection favoring the maintenance of polymorphism in these pigmentation traits in natural populations.

Ecology

Ecology is a understudied discipline in poeciliid biology, although considerable work has been conducted in the guppy, *Poecilia reticulata*.[72,73] Although investigation of predation effects on pigment patterns in *Xiphophorus* has been mentioned on multiple occasions,[8,72] the relationship between predation and pigment patterns remains the least studied of the potential mechanisms favoring the maintenance of polymorphism in melanistic traits. Whether fish with melanistic traits would be at an advantage or disadvantage is not clear. Predation could be negative frequency-dependent in which case the most abundant type, for example, unpatterned fish, would be consumed at a higher rate until patterned fish are at a higher frequency. This may be due to predators forming a search image based on the most common morph.[74,75] Having or not having a pattern may also help individuals blend into the environmental background more easily (i.e., crypticity). This could also confer an advantage as noncryptic individuals would be more conspicuous to visual predators. These scenarios may be complicated by the fact that *Xiphophorus* often live in variable habitats with heterogeneous backgrounds, variation in amount of vegetation, and by relationships between certain pattern genes and body size (discussed by Endler[72]).

These hypotheses have not been investigated in *Xiphophorus* in the context of pigmentation traits, although a few studies of predation based on sexual ornaments have been conducted.[51,76,77] Throughout the range of *Xiphophorus*, there are a variety of known fish predators, including numerous fish species,[76,77] invertebrate predators, such as crayfish (Zachary W. Culumber, pers. obs.), dragonfly larvae,[63] giant water bugs,[78] and avian predators, such as kingfishers. Predation habits and rates of these different species on *Xiphophorus* are not well documented let alone whether predation pressure differs among individuals with and without melanistic traits. Horth[63] demonstrated that melanic males of *G. holbrooki*, which occur at very low frequency in nature, are consumed less by predators than wild-type silver males in both experimental conditions and natural populations. The reason for this is not clear but could be due to higher reflectance and visibility of silver fish to predators. Differences in behavior, odor, or other traits could also explain their results and could not be ruled out. The extremely low frequency of the melanistic males in natural populations nonetheless suggests a significant cost to having pigmentation. Similar studies have yet to be conducted in *Xiphophorus*, although melanin-based patterns in *Xiphophorus* are typically at considerably higher frequencies and are typically less conspicuous than melanic male *G. holbrooki*.

It is worthwhile to mention the effect of parasites on fish pigmentation. Like many other species, when ectoparasites such as metacercariae of *Uvulifer* sp. attach to the body, *Xiphophorus* produce a cyst and melanic pigment surrounding

the infected site. These spots of black pigment surrounding individual parasites give rise to the name "black spot disease" (BSD). Individuals in a given population vary greatly in the number of these parasite-induced black spots (Fig. 5). BSD and visually similar pigmentation patterns have not yet been studied in *Xiphophorus.* Some studies have been conducted in other poeciliids. Females of the asexual *Poecilia formosa* prefer males without black spots, suggesting a role of sexual selection against this parasite-induced melanism.[79] In *Gambusia affinis*, BSD not only affects individual shoaling decisions, but both healthy and infected fish prefer to shoal with healthy individuals.[80] From those studies, it is not clear whether females were responding to the parasitized state of the fish or spots of black pigment in general, particularly since fish were shown animations of males with black spots and females were naive with respect to BSD. It would be interesting to decouple the visual and olfactory preferences of parasitized and non-parasitized fish, as well as test female preferences for males with BSD compared to males with melanophores equal in size to those of the BSD-infected fish. Tobler *et al.*[79] demonstrated visual preferences against BSD using animated stimuli. Thus, whether females simply have preferences against pigmented individuals or avoid pigmented individuals due to an association with parasitism (e.g., olfactory) is not known. As with genetically controlled pigmentation traits, black spots could affect detection rates by visual predators. This could also affect mate and shoaling preferences related to BSD-infected fish. How this variation in black spots affects fitness in terms of social encounters or detection by predators is not yet clear nor is the extent to which generalities can be made across poeciliid genera.

Physiology

The bulk of the evidence for a relationship between melanism and physiology comes from associations between tailspot alleles and environmental variables that give rise to differences in physiological performance. The *Ts* of the platyfish clade exhibits pleiotropic effects in that it produces different melanic tailspot patterns and also gives rise to differences in physiology among allelic variants. Pattern alleles are dominant over the wild type but co-dominant to one another. Different alleles at *Ts* have different physiological response to dissolved oxygen[26,81] and temperature[82] in the variable platyfish, *X. variatus*. Wild-type and cut crescent in-

dividuals differ in their metabolic rate,[26] giving rise to nonrandom spatial patterns and fitness differences in natural populations where concentrations of dissolved oxygen can differ among pools.[81] In the same species, wild-type (unpatterned) fish exhibit significantly reduced heat tolerance compared to three pattern alleles—crescent, cut crescent, and upper cut crescent—collected from the same population.[82] These findings highlight the potential for both spatial and temporal variation in the abiotic environment to effect tailspot frequencies. Environmental variation also correlates with variation in adult male size of different tailspot patterns in *X. variatus*,[26] consistent with the hypothesis that tailspot patterns have different physiological niches and respond differently to environmental factors.

Outside the platyfish, the link between pigment patterns and physiology is less clear. In the one known study examining a relationship between a macromelanophore pattern and metabolism in *X. hellerii*, there was no difference in metabolism between individuals with macromelanophores and those with no pattern.[83] A relationship between melanism and physiology has been reported in at least one other live-bearer, *G. holbrooki*, in which temperature determines whether melanism is expressed in some populations.[84] The genes and pathways involved and whether these differences also result in differences in physiological performance for individuals carrying alleles for melanism have not been demonstrated. Fitness benefits have been reported for melanistic males of other species including increased body and gonopodium size, increased survival with predators, and increased competitive ability have been reported.[63,85] The genetic and physiological basis of those associations nor of the association between Sc and body size in *X. cortezi* are not known.

In a review of pigment patterns in fishes, Price *et al.*[3] discussed the potential for melanism to confer a thermoregulatory advantage for fish in shallow water or even provide protection from harmful UV rays. As pointed out therein, these aspects of melanisn have not been widely studied and there are few known examples. As Endler[72] pointed out, there may be little room for a thermoregulatory benefit of melanism in many poeciliids owing to their largely neotropical distribution. Nonetheless, the precedent exists for a link between pigment traits and physiology in *Xiphophorus*. More work is needed to understand if and how other traits and their underlying loci affect physiological performance.

FIG. 5. Black spot disease: melanin production around the site of parasitism by metacaraciae of *Uvulifer* sp. on an *X. malinche* female gives rise to spots on the body and fins that look similar to a macromelanophore pattern. Notice the similarity with the spots in Figure 1E, H, and I, which are not caused by parasites. Color images available online at www.liebertpub.com/zeb

The Future of Pigmentation Research in *Xiphophorus*

The common theme that arises upon examining the existing evidence is that few studies have demonstrated mechanisms that have definite contributions to the evolutionary maintenance of pigmentation traits in *Xiphophorus*. The notable exceptions being the physiological differences among tailspot patterns in platyfish, at least one study demonstrating polymorphic female preference for males with vertical bars in *X. cortezi*, and preferences for macromelanophores in turbid water in *X. hellerii*. However, these existing studies are species- or context-specific. What maintains polymorphism in the majority of currently described melanin-based traits is still unknown. Even for those on which work has been done, such as macromelanophores in turbid water, the forces that keep the frequency of patterned fish balanced are not clear. This highlights the great potential for a wealth of discovery yet to

be made in *Xiphophorus* and considerable opportunity to contribute to our understanding of pigment trait evolution, animal communication, and evolutionary biology more generally. *Xiphophorus* and the larger family of live-bearers are an interesting group from an evolutionary perspective. These important pigmentation traits that play important roles in behavior, ecology, and physiology largely remain a black box. Despite the advances made in other taxa (for review, see Kronforst *et al.*[1] and Hoekstra *et al.*[5] and references therein) much remains to be learned about the evolutionary ecology of pigmentation. Due to ongoing research in other taxa including other live-bearers with some shared pigmentation traits, great potential exists to examine the basis of convergent evolution in divergent taxa.

Area for significant improvement exists in terms of understanding the role of pigmentation traits in behavior and ecology. I highlight these two disciplines for specific reasons. First, it is well established that *Xiphophorus* are amenable to behavioral studies owing to the significant body of literature on sexual selection that has demonstrated an ability to elicit and measure preferences in the laboratory. The methods needed to be able to test for and detect preferences for or against melanin-based traits have been standard practice for many years but are almost entirely applied to structural morphology (e.g., swords, dorsal fins, and body size). In addition, a new era of video playback of animated stimuli enables high-throughput testing. Development of new tools such as the anyFish software platform[86] will continue to enable greater than ever ability to manipulate specific traits, while controlling background morphology of realistic animated stimuli that match the morphology, coloration, and locomotion of wild-caught fish. With the methodological and infrastructural framework that exists, a variety of work regarding the role of pigmentation traits in species recognition, mate choice, and intrasexual signaling will be increasingly feasible.

Second, the role of ecology in the evolution of *Xiphophorus* and other live-bearers has long been a neglected area of research with notable exceptions mentioned above regarding predation and environmental variation in *P. reticulata*, male–male competition in *Gambusia* and *Xiphophorus*, and environmental effects on the *Ts* in platyfish. However, the role of ecological communities and associated factors, such as predation, competition, and particularly adaptation to environmental variation in the evolution of live-bearers and specifically the genus *Xiphophorus*, has not been widely studied. The feasibility of studying these fish in their natural habitat has been demonstrated through extensive field sampling since the 1800s including well-documented natural ranges and occurrence data. Field studies could take advantage of this and of relatively high abundance of these fish in their natural habitats to track mortality with mark-recapture and correlate changes in the frequency of pigmentation patterns with environmental fluctuations, predation regimens, and other ecological factors. These studies would not only aid in understanding evolution of pigmentation but in terms of expanding the knowledge of evolutionary ecology of *Xiphophorus*.

The study of pigmentation in *Xiphophorus* will be greatly aided by the recent completion of the genome of the platyfish, *X. maculatus*.[13] That work has already led to advancement in pigmentation research and highlighted a potential candidate gene for the macromelanophore determining locus. The work of Schartl *et al.*[13] indicates that *Egfrb* is both on the proper chromosome and close enough to the oncogene *Xmrk* to potentially be *Mdl*. Although further investigation is needed to confirm whether *Egfrb* is *Mdl*, the existence of this genomic resource will facilitate the study of this and other pigmentation genes in *X. maculatus*, as well as other *Xiphophorus* and poeciliids. These genomic resources place *Xiphophorus* among a handful of systems in terms of the power and potential to investigate ecological and evolutionary genetics at the molecular level. Previous work has already demonstrated the ability to use molecular markers to track reproductive success in natural populations of *Xiphophorus*.[87] The recently completed genome will provide unprecedented potential to develop molecular markers and open the door to population genomics of pigmentation.[88] The ultimate goal should be the integration of behavior, ecology, physiology, and population genomics to understand the interplay of these forces on the evolution of morphology and adaptation at the molecular level.

Summary

Notable advances have been made in our understanding of pigment trait evolution in *Xiphophorus* over the past 20 years, but much remains to be unraveled. Further studies are needed to determine the major forces balancing these polymorphic traits and the extent to which these selective forces are the same or different among populations and species. Fish are important models of environmental genomics due to intimate physiological contact with their environment[89] and pigmentation offers a rarely paralleled opportunity to understand molecular evolution stemming from diverse selective forces.[1] The recently sequenced genome of *X. maculatus* combined with a high degree of polymorphism in melanin-based traits make *Xiphophorus* a particularly strong emerging system in pigmentation evolutionary genetics. This emerging system is poised to yield insight into ecology and evolution and to advance our understanding of adaptation, convergence, and divergence in vertebrate pigmentation.

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