Use of Platyfishes and Swordtails in Biological Research

Steven Kazianis, PhD, and Ronald B. Walter, PhD

The use of platyfishes and swordtails as research models is on the rise. The authors review the basic biology and care of these animals and describe their common uses in research.

Kazianis is Assistant Professor of Research and Associate Director of the *Xiphophorus* Genetic Stock Center (XGSC), and Walter is Professor, Mitte Chair, and Director of the XGSC, Southwest Texas State University, Department of Chemistry and Biochemistry, 419 Centennial Hall, 601 University Drive, San Marcos, TX 78666. Please send reprint requests to Kazianis at the above address, or email: sk13@swt.edu.

The fish genus *Xiphophorus* (family Poeciliidae) currently includes 22 species that are naturally distributed in freshwater drainages of Mexico, Guatemala, Belize, and Honduras. These fishes have been placed into three broad phylogenetic groups: platyfishes (Fig. 1A), northern swordtails (Fig. 1B), and southern swordtails (Fig. 1C)[1,2]. Swordtails are thus named because of the pronounced horizontal extension of the caudal fin, referred to as a sword, seen in males.

In most cases, *Xiphophorus* fishes are hybrids between distinct species. Hybrid fish are generated by natural means (*i.e.*, closed matings) or by artificial insemination. Hybrid offspring often show distinct phenotypic characteristics not typically observed in the parent species. Thus, highly ornamental strains can be derived from such matings, making these fishes very popular among hobbyists. In other cases, hybridization leads to abnormalities; hybrid fishes may spontaneously develop malignant tumors or show an increased susceptibility to induction of neoplasia by chemical or physical means. The discovery of such characteristics conceptually steered experiments more than 60 years ago and remains the basis of experiments that employ these fish today.

In addition to their use in cancer research, *Xiphophorus* fishes are used in numerous other fields of study. In fact, there is increasing interest in using the powerful genetics provided by the *Xiphophorus* model system to study multigenetic phenotypes. The number of *Xiphophorus* research studies appearing in peer-reviewed scientific journals has steadily increased from the 1930s to the 1990s and jumped 60% from the 1980s to the 1990s (Fig. 2). Because *Xiphophorus* models hold significant unexploited potential, they undoubtedly will remain the focus of future experimentation, as well.

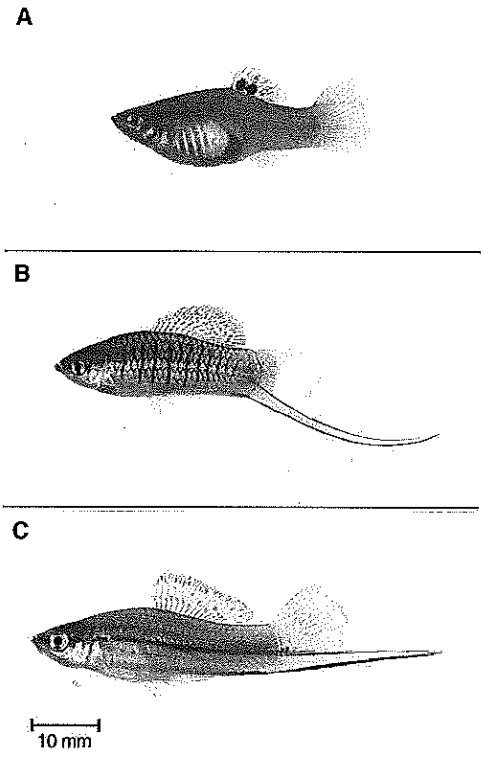

FIGURE 1. Examples of a platyfish, a northern swordtail, and a southern swordtail. (A) *X. maculatus* platyfish (P8 strain); (B) *X. nezahualcoyotl* (Ocampo strain), a northern swordtail; and (C) *X. helleri* (Lance strain), a southern swordtail.

Basic Biology and Care

Xiphophorus fishes are small; adults typically range in size from 20 to 50 mm standard length (measured from the tip of the mouth to the base of the tail). Although no

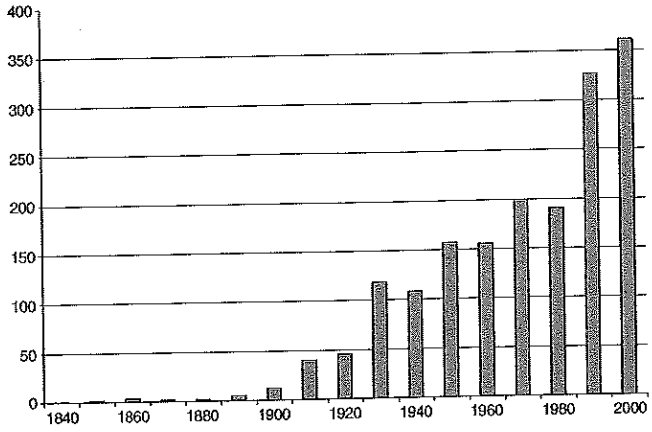

FIGURE 2. Numbers of *Xiphophorus* publications by decade from 1840 to present. Data for the decade beginning with the year 2000 represents an estimate based on manuscripts published in the first two years. These data were derived from a searchable bibliography maintained by the *Xiphophorus* Genetic Stock Center (XGSC) and posted at www.xiphophorus.org. The bibliography currently contains 1,595 *Xiphophorus* citations.

extensive studies have been performed on the lifespan of these fishes, they typically live two to three years.

Depending on the specific strain, these fishes may not show pronounced sexual dimorphism/secondary sexual characteristics; nevertheless, mature males always develop a modified anal fin known as the gonopodium. This highly specialized fin, which differs from one taxon to the next[3,4], is used to transfer sperm packets or "spermatozeugmata" to the females[5]. Embryonic development takes place internally and lasts ~30 days[6]. Inseminated females can store sperm for longer than 10 months, resulting in the possibility of "mixed" broods. Therefore, when breeding *Xiphophorus* fishes for research, one must take great care to avoid unintended inseminations and offspring that are derived from different males.

Gravid *Xiphophorus* females normally bear well-developed, minute (~6 mm) fry, which are quickly capable of procuring food independently. Because *Xiphophorus* fishes occasionally cannibalize their own young, caretakers typically separate newly born broods from their parents. Brood intervals vary between 22 and 45 days. Broods can vary greatly in number from 1 to 160, with such variability derived from species/strain/individual differences and environmental variables.

Xiphophorus fishes develop rapidly within their first few months and typically reach maturation at two to six months. *Xiphophorus* fishes possess a sex-linked locus known as "*P*," which regulates the age at maturation and thus ultimately determines adult size[7]. Allelic combinations of this gene can lead to pronounced differences in size between siblings[7,8]. Perturbation of this locus, such as within certain hybrids (presumably due to inadequate gene regulation), can lead to fishes that do not mature and continue to grow. In extreme cases, these fishes may live out their entire lives without ever reaching maturity[9].

Xiphophorus fishes are typically maintained in aquaria at densities of approximately one fish per 4.4 liters and no greater than one fish per 2 liters. A variety of water sources are used in aquarium systems (*e.g.*, chemically dechlorinated municipal water, carbon-filtered water, ultraviolet (UV)-sterilized water, ozonated water). These fishes are typically maintained in 21°–27°C water at a pH of 6.5–7.5—similar to conditions they encounter over much of their natural geographic range. *Xiphophorus* fishes are omnivorous. They can be fed commercially available foods developed for the pet industry, and have been raised on a diet of flake foods, tubifex worms, freeze-dried or live brine shrimp (*Artemia* sp.), water fleas (*Daphnia* sp.), and "liver paste[10]," or a combina-

TABLE 1. Fish species/stocks maintained at the XGSC.

Platyfish Species	Strain Code(s)	Swordtail Species	Strain Code(s)
X. andersi	andB, andC	*X. alvarezi*	Cand, DL
X. couchianus	Xc	*X. birchmanni*	birchII
X. evelynae	eve	*X. clemenciae*	Tej, Sol, Grande, FincaII
X. maculatus	JpWild, Jp163A, Jp163B, Jp30R, SpSrxo, YSdDrxo, YSdSrxo, YSp, Nigra, Cp, Up, Pp, SR, JpIrBrxo, JpYIr, JpYBr	*X. continens*	conti
X. meyeri	meyerI	*X. cortezi*	cortezi
X. milleri	mll82	*X. helleri*	Lance, Bx, BxII, Hx, Cd, Jalapa, Bel, HeLi, Sara, RA[1], HeAlb[1], Doce
X. variatus	Huich, Zarco, Encino, XPu1Gnbxo	*X. malinche*	Claro
X. xiphidium	Sc, Ps, Aram, RP	*X. montezume*	Rascon, Capuchin, OjoCal
X. gordoni	gordoni	*X. multilineatus*	Multi, CoyII
		X.nezahuacoytl	El Salto, Ocampo
		X. nigrensis	nigrn
		X. pygmaeus	pygTy, pygII
		X. signum	signum

[1] Domesticated strain.

tion of these. Fry do better with small food items, such as live brine shrimp. Overfeeding, which can lead to excessive levels of nitrogenous wastes and undesired bacteria in the aquarium, should be avoided.

Artificial insemination is used to perform certain crosses between individual fish and for the creation of some hybrid fish. In a straightforward procedure originally developed by Eugenie Clark[11], the male is anesthetized and gentle pressure is applied to the ventral regions near and around the gonopodium. Sperm packets are collected by pipetting and can briefly be stored in a 0.7% NaCl solution. The sperm packets are then injected through the urogenital sinus and into the gonoduct of an anesthetized female.

Xiphophorus fishes are typically anesthetized using MS-222 (Sigma-Aldrich, St. Louis, MO) in working concentrations ranging from 0.02-0.04% (pH 7.0). This same compound, when used at higher concentrations (*i.e.*, 0.06% or greater), can be used to euthanize these fishes. The preparation of these working solutions and buffered concentrated stocks is detailed at www.xiphophorus.org/research.htm.

The *Xiphophorus* Genetic Stock Center

Because *Xiphophorus* fish are often used in genetic research, careful husbandry and stock maintenance with exact and traceable records are critical. Research groups in different parts of the world should, when possible, use the same strains to allow direct comparison and potential pooling of experimental results. In the early 1930s, to address the lack of available platyfish, swordtail, and hybrid strains with well-known genetic backgrounds, Myron Gordon, a scientist at Cornell University (Ithaca, NY), traveled to Mexico and neighboring countries and collected fish from their wild habitats. Gordon brought fish, including *X. helleri* and *X. maculatus*, back to New York and bred them in the laboratory[12]. He assigned a unique and sequential number to each experimental cross and maintained meticulous notes regarding phenotype and breeding. Over some 30 years, Gordon's laboratory moved from Cornell University to the New York Aquarium (Brooklyn, NY), and subsequently to the American Museum of Natural History (New York, NY), all the while maintaining a sizeable number of species/strains[12]. Gordon provided fish for many individuals, including researchers and hobbyists, and published hundreds of scientific and lay articles. His laboratory effectively became the *Xiphophorus* Genetic Stock Center (XGSC) and was taken over by his student Klaus D. Kallman, who maintained and expanded the center over the ensuing 32 years[13].

The XGSC, which is presently housed at Southwest Texas State University (San Marcos, TX), carries all 22 described species composing 64 strains of *Xiphophorus* (Table 1). Since these strains are inbred, with some having undergone >90 generations of sib-sib matings, experimental results derived from hybrid crosses can be readily repeated. Information regarding the XGSC can be found at www.xiphophorus.org.

Xiphophorus Genetic Models

The *Xiphophorus* genetic system possesses many of the characteristics of an informative and useful animal model, including: (1) ease of generating large numbers of animals; (2) availability of well-maintained inbred stocks and strains; (3) ability to perform genetic crosses among phenotypically diverse animals, with the capacity for producing fertile offspring; (4) a well-marked gene map; (5) availability of robust genetics allowing assignment of orthology with human genes, and enabling molecular genetic approaches for mechanistic investigation; (6) well-characterized molecular markers and cloned genes that can be used as tools for research investigations among varied scientific disciplines; and (7) availability of established cell lines for *in vitro* studies.

Development of the *Xiphophorus* genetic system and its employment in varied scientific fields can be traced to pioneering studies indicating that after interspecies hybridization, certain hybrid progeny become susceptible to spontaneous tumor development[14–17]. Results from these early *Xiphophorus* tumor crosses established the presence of what we now term "oncogenes" and were the initial indications that loss of gene function could be associated with tumorigenesis, thus indicating the existence of tumor-suppressor genes.

As an example of the ability of the *Xiphophorus* model systems to dissect complex genetic phenomena, we describe the application of *Xiphophorus* interspecies hybrids to the study of "spontaneously developing" (*i.e.*, not chemically or physically induced) melanoma and compare two such crosses. A more detailed treatment of *Xiphophorus* hybrid models can be found in a recent review[18].

Gordon–Kosswig Melanoma Model

The Gordon–Kosswig (G-K) melanoma model[19] serves as a paradigm for studying the role of dominant oncogenes and recessive tumor suppressor genes in tumor development, and has been used in numerous studies of the etiology of melanoma in *Xiphophorus*[18–21]. The *X. maculatus* X chromosome carries a macromelanophore pigment pattern marker gene designated *Sd* (spotted dorsal). Melanocytes derived from *Sd* become hyperplastic within the *X. helleri* genetic background. Thus, backcross hybrids produced by the mating scheme (*X. maculatus* Jp 163 A x *X. helleri*) x *X. helleri* (Fig. 3A) develop spontaneous malignant melanomas. In the first-generation backcross (BC_1), half of the offspring inherit the *Sd* pigment pattern gene, and of those, half (25% of the progeny) exhibit hyperplastic pigment cell expression and half (25% of the progeny) develop malignant melanoma characterized by invasive nodular melanotic lesions[18–21].

Comparative biochemical, histological, and cytological studies of melanin-containing cells from G-K backcross hybrid fishes show a marked decline in the differentiated state of pigment cells in tumors[22]. Within lightly pigmented BC_1 hybrids, cells differentiate only to an intermediate stage and undergo limited cell division. Cells in malignant melanomas derived from heavily pigmented fish are obviously neoplastic and retain rapid, unlimited cell divi-

sion[20,22,23]. Malignant melanomas from these fishes are histopathologically, ultrastructurally, and biochemically very similar to their mammalian counterparts[24–26]. This point is underscored by the observation that fish melanoma cells are able to proliferate in a manner very similar to human melanoma cells when transplanted into athymic mice[24]. These early studies and many others cumulatively document the considerable similarity between melanomas in fishes and those in mammals, including humans.

The current inheritance model for mendelian segregation of phenotypes in the G-K melanoma model predicates two required genetic events: oncogenic potential of the *Sd* macromelanophore pigment pattern gene (or a locus tightly linked to it), and concomitant loss of autosomal tumor suppressor gene copies derived from *X. maculatus*. This tumor suppressor locus is termed *DIFF*, to account for the difference in the degree of differentiation of melanin-producing cells within the BC_1 progeny. Studies conducted over the past several years have demonstrated that the *X. maculatus* X chromosome harboring the Sd macromelanophore pigment pattern contains two tightly linked copies of an epidermal growth factor receptor- (EGFR; HER-*1*-) related sequence[27,28]. These genes are termed *Xmrk-1* and *-2* (ref. 29). *Xmrk-2* is overexpressed (on both RNA and protein levels) in hybrid-derived melanoma tissues relative to the other copy (*Xmrk-1*), which is expressed in most normal tissues at moderate or low levels[27,29–31]. Experiments also indicate that the overexpression is consistent with promoter hypomethylation within melanotic tissues derived from hybrids[32]. In addition, *Xmrk-2* has been shown to harbor two critical mutations in the extracellular domain giving its protein a ligand-independent constitutively active state[33]. The results of these, and other, experiments have cumulatively suggested that *Xmrk-2* is the primary oncogene implicated in the G-K model.

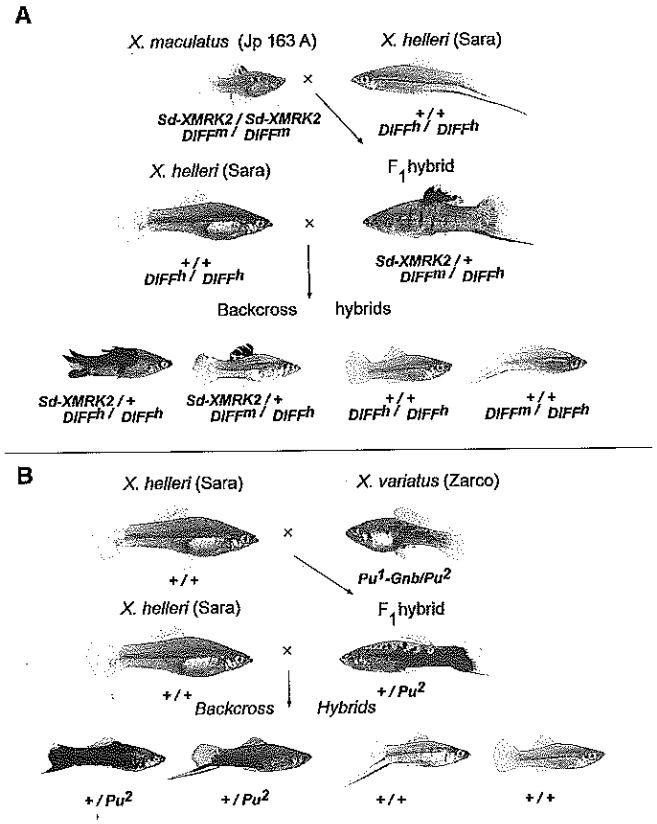

FIGURE 3. Examples of two *Xiphophorus* models of spontaneous melanoma. (A) The Gordon–Kosswig hybrid melanoma cross. The inheritance of two genomic regions: linkage group (LG) XXIV (*Sd*, *Xmrk-2*) and LG V (*DIFF*). Parentheses indicate specific strain codes. The BC_1 hybrid depicted on the bottom left exhibits invasive melanoma causing necrosis of the dorsal fin region, which occurs spontaneously in 25% of BC_1 hybrids simply as a result of the interspecies cross. (B) Cross between *X. variatus* and *X. helleri*, involving the Pu^2 macromelanophore pigment pattern. The pigment pattern is strongly enhanced, and a range of phenotypes is seen in the backcross generation. Fish are one year of age ± two months.

The *DIFF* gene was shown to be autosomal and localized to an area within *Xiphophorus* linkage group (LG) V (refs 23,34–39). A fish homolog in the cyclin-dependent kinase inhibitor gene family (*CDKN2;* alternatively known as *P16, INK4*) was mapped to the *DIFF* position on LG V. This gene is termed *CDKN2X*, and its inheritance has been genetically associated with heavy and light pigmentation phenotypes and the development of melanoma (*i.e.*, the hypothetical expectation for *DIFF*) in a number of different *Xiphophorus* UV-inducible hybrid models, and the G-K spontaneous melanoma model[39–42]. Within BC_1 hybrids, *CDKN2X* allelic copies derived from *X. maculatus* and *X. helleri* are differentially expressed in several tissues including melanomas[41]. Thus, *CDKN2X* is a very strong candidate gene for the hypothetical *DIFF* tumor suppressor[39–42]. Highlighting the relevance of this observation, numerous studies in humans and rodents have also implicated a *CDKN2* gene family member (*CDKN2A, P16*) as the most commonly affected locus in melanomas[43,44]. Thus, association of a related *Xiphophorus* gene with melanoma formation is a provocative discovery.

Pu^2 Model System

Other *Xiphophorus* species harbor macromelanophore pigment pattern loci similar to *Sd*. One such pattern is referred to as Pu^2 and is found in populations of *X. variatus*[45,46]. Pu^2 differs from *Sd* in that it codes for pigment cells on the flanks as opposed to on the dorsal fin. In addition, fish harboring this pigment pattern tend to develop melanomas only at advanced ages (*i.e.*, >1.5 years of age)[46–48]. Backcross hybrids using *X. helleri* as the recurrent parent (Fig. 3B) are either heavily or lightly pigmented, with the former showing a higher incidence of melanoma[37,46]. This result is analogous to the G-K model. Genetic analysis of the Pu^2 model reveals a fundamental difference since evidence indicated an absence of a tumor suppressor effect derived from the LG V genomic region[37]. In contrast to the G-K model, analysis of additional data suggests that another autosomal linkage group (LG

III), is implicated in *Pu*[2]-derived pigment cell proliferation[37,38]. Molecular cloning of an implicated locus or loci within this genomic region remains our future goal.

The potential importance and possible future exploitation of this model should not be underestimated. From 1973 to 1990, the incidence of cutaneous malignant melanoma (CMM) within the US population rose ~94%, more than any other cancer[49,50]. The median age at CMM diagnosis in Caucasian US inhabitants was 57.0 years of age for males and 50.0 years of age for females, although ~35% of diagnoses are in people younger than 45 years of age[49,50]. Because human melanoma studies are inherently retrospective, there is a clear need for experimental animal models suitable for investigation of the interaction of genetic and environmental factors in melanomagenesis, and especially the function of aging as a risk factor for developing such disease. While there are several animal models useful for the study of aging with regard to cancer[51–54], melanoma models are lacking. The *Pu*[2] models should prove highly valuable for studying the development of melanoma late in life.

Other *Xiphophorus* Tumor Models

A total of 231 possible hybrid crosses can be produced between the 22 described species of *Xiphophorus*. However, this number is dwarfed by consideration of the influence of individual and population variability on results observed after hybridization. As an example, Kallman employed two different alleles of *Sd* derived from the same river system in hybrid crosses to *X. couchianus*. The pigment pattern was strongly phenotypically enhanced in one case, but suppressed for the other[12,55]. In addition, experimental manipulations can contribute to the great utility of these models. For example, work conducted within the last decade has shown that hybrid fish can be induced to develop melanoma after exposure to UV radiation[56–58]. It has also been shown that these models are amenable to inclusion of chemical exposure regimens[19,59–62]. In addition to melanoma, the carcinogen N-methyl-N-nitrosourea (MNU) induces a number of other cancers in hybrids (*e.g.*, neuroblastomas, neurofibromas, fibrosarcomas, and rhabdomyosarcomas[19,59–62]). A partial list of *Xiphophorus* crosses resulting in tumor susceptibility is presented in Table 2.

Other Uses of *Xiphophorus*

In addition to their use for cancer research, *Xiphophorus* fishes are actively being used in the study of evolution[1,63,64], sex determination[65–67], endocrinology[68,69], ethology and behavioral ecology[70–73], toxicology[74,75], parasitology[76,77], and immunology[78,79]. In fact, within the US, we estimate that there are 11 laboratories currently employing these fishes for behavioral and evolutionary studies. Over the past 15 years, great strides have been made in understanding the evolutionary relationships of *Xiphophorus* fish as evidenced by several key manuscripts describing new northern swordtail species[2], and delineating the phylogenetic relationships of all 22 species[2,80–82]. These studies also address the evolution of the sword, a male secondary sexual characteristic not present or greatly reduced in platyfish taxa.

Within the behavioral field, use of platyfishes and swordtails has also raised great interest as discoveries have been made regarding female mate preference; these studies raise the possibility that behavioral traits could predate and contribute to the evolution of external morphological characters[72,82–85]. Because of the pronounced interest in this promising field of research, new technologies have recently been developed to facilitate study of these fishes[71–73]. The great diversity of *Xiphophorus* species/strains undoubtedly helps provide the raw material for numerous behavioral studies. It also seems certain that study of hybrids and the effect of hybridization on behavior will be undertaken in the near future. Coupling such work with gene mapping, and the powerful genetics inherent in the *Xiphophorus* system, should result in the identification of key loci implicated in behavioral processes.

TABLE 2. Examples of various *Xiphophorus* tumor models[a].

Species used in cross	Tumor type(s)	Induction method
X. helleri x (*X. maculatus* Jp 163 A x *X. helleri*)	Melanoma	None (spontaneous)
X. helleri x (*X. maculatus* Jp 163 B x *X. helleri*)	Melanoma	MNU[b] and UV[c]
(*X. maculatus* Jp 163 A x *X. andersi*) x *X. andersi*	Melanoma	None (spontaneous)
(*X. maculatus* Jp 163 B x *X. andersi*) x *X. andersi*	Several[d]	MNU
(*X. maculatus* Jp 163 A x *X. couchianus*) x *X. couchianus*	Several[e]	MNU
(*X. maculatus* Jp 163 B x *X. couchianus*) x *X. couchianus*	Melanoma	MNU and UV
X. helleri x (*X. variatus* [Ll] x *X. helleri*)	Several[f]	MNU
X. cortezi [So]	Melanoma	Aging
X. variatus [Pu2]	Melanoma	Aging

[a]The female parent is listed first (i.e., the first cross listed below would be a male F_1 hybrid derived from mating a female *X. maculatus* Jp 163 A with a male *X. helleri* backcrossed to a female *X. helleri*).
[b]N-methyl-N-nitrosourea.
[c]Ultraviolet radiation.
[d]Melanoma and renal adenocarcinoma.
[e]Fibrosarcoma, retinoblastoma, melanotic hyperpigmentation, and schwannoma.
[f]Fibrosarcoma, neuroblastoma.

Summary

Xiphophorus fishes and their hybrids have been used for over 70 years to study a number of biological phenomena. The relative ease of maintenance, small size, and the availability of a stock center resource, greatly contributes to their utility. The ability to create fertile hybrid fish that show useful phenotypic manifestations of traits derived from their parental progenitors uniquely positions them for use as vertebrate animal models. Although researchers have extensively used them for the study of melanoma, increasing interest in studying other cancers, and in altogether other fields of scientific inquiry is driving further usage of these animals in a variety of different model systems. Indeed, it appears that the study of the diminutive fish *Xiphophorus* will continue for some time to come.

Received 1/11/02; accepted 1/25/02.

References

1. Meyer, A. The evolution of sexually selected traits in male swordtail fishes (*Xiphophorus*: Poeciliidae). *Heredity* **79**, 329–337 (1997).
2. Rauchenberger, M., Kallman, K.D. & Morizot, D.C. Monophyly and geography of the Rio Panuco basin swordtails (genus *Xiphophorus*) with descriptions of four new species. *Am. Mus. Novit.* **2975**, 1–41 (1990).
3. Rosen, D.E. & Gordon, M. Functional anatomy and evolution of male genitalia in poeciliid fishes. *Zoologica* **38**, 1–47 (1953).
4. Rodriguez, C.M. Phylogenetic analysis of the tribe Poeciliini (Cyprinodontiformes: Poeciliidae). *Copeia* **1997**, 663–679 (1997).
5. Constantz, G.D. in *Ecology and Evolution of Livebearing Fishes* (eds Meffe, G.K. & Snelson, F.F.) 33–50 (Prentice Hall, Englewood Cliffs, NJ, 1989.)
6. Tavolga, W.N. Embryonic development of the platyfish (*Platypoecilus*), the swordtail (*Xiphophorus*) and their hybrids. *Bull. Am. Mus. Nat. Hist.* **94**, 163–229 (1949).
7. Kallman, K.D. & Schreibman, M.P. A sex-linked gene controlling gonadotrop differentiation and its significance in determining the age of sexual maturation and size of the platyfish, *Xiphophorus maculatus*. *Gen. Comp. Endocrinol.* **21**, 287–304 (1973).
8. Schreibman, M.P. & Kallman, K.D. The genetic control of the pituitary-gonadal axis in the platyfish, *Xiphophorus maculatus*. *J. Exp. Zool.* **200**, 277–293 (1977).
9. Bao, I.Y.& Kallman, K.D. Genetic control of the hypothalamo-pituitary axis and the effect of hybridization on sexual maturation (*Xiphophorus*, Pisces, Poecilidae). *J. Exp. Zool.* **220**, 297–309 (1982).
10. Gordon, M. in *The Care and Breeding of Laboratory Animals* (ed. Farris, E.J.) 345–449 (Wiley & Sons, New York, NY, 1950).
11. Clark, E. A method for artificial insemination in viviparous fishes. *Science* **112**, 722–723 (1950).
12. Kallman, K. How the *Xiphophorus* problem arrived in San Marcos, Texas. *Mar. Biotechnol.* **3**, 6–16 (2001).
13. Atz, J.W. & Kazianis, S. In appreciation of Klaus D. Kallman. *Mar. Biotechnol.* **3**, 3–5 (2001).
14. Kosswig, C. Uber Bastarde der Teleostier Platypoecilus und Xiphophorus. Zeitschrift für induktive Abstammungs- und Vererbungslehre. **44**, 253 (1927).
15. Gordon, M. Hereditary basis of melanosis in hybrid fishes. *Am. J. Cancer* **15**, 1495–1523 (1931).
16. Anders, F. Tumour formation in platyfish-swordtail hybrids as a problem of gene regulation. *Experientia* **23**, 1–10 (1967).
17. Haussler, G. Uber Melanobildung bei Bastarden von *Xiphophorus helleri* und *Platypoecilus maculatus* var. rubra. *Klin. Wochenschr.* **7**, 1561–1562 (1928).
18. Walter, R.B. & Kazianis, S. Xiphophorus interspecies hybrids as genetic models of induced neoplasia. *ILAR J.* **42**, 299–321 (2001).
19. Anders, F. Contributions of the Gordon–Kosswig melanoma system to the present concept of neoplasia. *Pigment Cell Res.* **4**, 7–29 (1991).
20. Vielkind, J.R. & Kallman, K.D. Genetics of melanomas in *Xiphophorus* fishes. *J. Aquat. Anim. Health* **1**, 69–77 (1989).
21. Schartl, M. Platyfish and swordtails: a genetic system for the analysis of molecular mechanisms in tumor formation. *Trends Genet.* **11**, 185–189 (1995).
22. Vielkind, U. Genetic control of cell differentiation in platyfish-swordtail melanomas. *J. Exp. Zool.* **196**, 197–204 (1976).
23. Ahuja, M.R., Schwab, M. & Anders, F. Linkage between a regulatory locus for melanoma cell differentiation and an esterase locus in *Xiphophorus*. *J. Hered.* **71**, 403–407 (1980).
24. Schartl, M. & Peter, R.U. Progressive growth of fish tumors after transplantation into thymus- aplastic (*nu/nu*) mice. *Cancer Res.* **48**, 741–744 (1988).
25. Sobel, H.J., Marquet, E., Kallman, K.D. & Corley, G.J. in *The Pathology of Fishes* (eds Ribelin, W.E. & Migaki, G.) 945–981 (University of Wisconsin Press, Madison, WI, 1975).
26. Felding-Habermann, B., Anders, A., Dippold, W.G., Stallcup, W.B. & Wiegandt, H. Melanoma-associated gangliosides in the fish genus *Xiphophorus*. *Cancer Res.* **48**, 3454–3460 (1988).
27. Wittbrodt, J., *et al.* Novel putative receptor tyrosine kinase encoded by the melanoma-inducing *Tu* locus in *Xiphophorus*. *Nature* **341**, 415–421 (1989).
28. Zechel, C., Schleenbecker, U., Anders, A. & Anders, F. v-erbB related sequences in *Xiphophorus* that map to melanoma determining Mendelian loci and overexpress in a melanoma cell line. *Oncogene* **3**, 605–617 (1988).
29. Woolcock, B.W., Schmidt, B.M., Kallman, K.D. & Vielkind, J.R. Differences in transcription and promoters of *Xmrk-1* and *Xmrk-2* genes suggest a role for *Xmrk-2* in pigment pattern development in the platyfish, *Xiphophorus maculatus*. *Cell Growth Differ.* **5**, 575–583 (1994).
30. Zechel, C., Schleenbecker, U., Anders, A., Pfutz, M. & Anders, F. Search for genes critical for the early and/or late events in carcinogenesis: studies in *Xiphophorus* (Pisces, Teleostei). *Hämatol. Bluttransfus.* **32**, 366–385 (1989).
31. Adam, D., Mauter, W. & Schartl, M. Transcriptional activation of the melanoma inducing Xmrk oncogene in *Xiphophorus*. *Oncogene* **6**, 73–80 (1991).
32. Altschmied, J., Ditzel, L. & Schartl, M. Hypomethylation of the *Xmrk* oncogene promoter in melanoma cells of *Xiphophorus*. *Biol. Chem.* **378**, 1457–1466 (1997).
33. Gomez, A., Wellbrock, C., Gutbrod, H., Dimitrijevic, N. & Schartl, M. Ligand-independent dimerization and activation of the oncogenic Xmrk receptor by two mutations in the extracellular domain. *J. Biol. Chem.* **276**, 3333–3340 (2001).
34. Siciliano, M.J., Morizot, D.C. & Wright, D.A. in *Pigment Cell*, Vol 2 (ed. Riley, V) 47–58 (S. Karger, Basel, 1976).
35. Morizot, D.C. & Siciliano, M.J. Linkage group V of platyfishes and swordtails of the genus *Xiphophorus* (Poeciliidae): linkage of loci for malate dehydrogenase-2 and esterase- 1 and esterase-4 with a gene controlling the severity of hybrid melanomas. *J. Natl. Cancer Inst.* **71**, 809–813 (1983).
36. Fornzler, D., Wittbrodt, J. & Schartl, M. Analysis of an esterase linked to a locus involved in the regulation of the melanoma oncogene and isolation of polymorphic marker sequences in *Xiphophorus*. *Biochem. Genet.* **29**, 509–524 (1991).
37. Kazianis, S., Morizot, D.C., McEntire, B.B., Nairn, R.S. & Borowsky, R.L. Genetic mapping in *Xiphophorus* hybrid fish: assignment of 43 AP-PCR/RAPD and isozyme markers to multipoint linkage groups. *Genome Res.* **6**, 280–289 (1996).
38. Morizot, D.C., Nairn, R.S., Walter, R.B. & Kazianis, S. The linkage map of *Xiphophorus* fishes. *ILAR J.* **39**, 237–248 (1998).
39. Kazianis, S., *et al.* Localization of a *CDKN2* gene in linkage group V of *Xiphophorus* fishes defines it as a candidate for the *DIFF* tumor suppressor. *Genes Chromosom. Cancer* **22**, 210–220 (1998).
40. Nairn, R.S.,*et al.* A *CDKN2*-like polymorphism in *Xiphophorus* LG V is associated with UV-B-induced melanoma formation in platyfish-swordtail hybrids. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 13042–13047 (1996).
41. Kazianis, S., *et al.* Comparative structure and characterization of a *CDKN2* gene in a *Xiphophorus* fish melanoma model. *Oncogene* **18**, 5088–5099 (1999).
42. Kazianis, S., *et al.* Overexpression of a fish *CDKN2* gene in a hereditary melanoma model. *Carcinogenesis* **21**, 599–605 (2000).
43. Ruas, M. & Peters, G. The p16INK4a/CDKN2A tumor suppressor and its relatives. *Biochim. Biophys. Acta* **1378**, F115–F177 (1998).
44. Saida, T. Recent advances in melanoma research. *J. Dermatol. Sci.* **26**, 1–13 (2001).
45. Borowsky, R. in *Evolutionary Genetics of Fishes* (ed. Turner, B.J.) 235–310 (Plenum Press, New York, NY, 1984).
46. Kazianis, S. & Borowsky, R. Stable association of a pigmentation allele with an oncogene: nonhybrid melanomas in *Xiphophorus variatus*. *J. Hered.* **86**, 199–203 (1995).

47. Schartl, A., Malitschek, B., Kazianis, S., Borowsky, R. & Schartl, M. Spontaneous melanoma formation in nonhybrid *Xiphophorus*. *Cancer Res.* **55**, 159–165 (1995).
48. Borowsky, R. Melanomas in *Xiphophorus variatus* (Pisces, Poeciliidae) in the absence of hybridization. *Experientia* **29**, 1431–1433 (1973).
49. Miller, B., *et al.*, eds. *SEER Cancer Statistics Review: 1973–1989* (National Cancer Institute, Bethesda, MD, 1992).
50. Miller, B. *et al.*, eds. *SEER Cancer Statistics Review: 1973–1990* (National Cancer Institute, Bethesda, MD, 1993).
51. Cui, J., Chen, D., Misfeldt, M.L., Swinfard, R.W. & Bystryn, J.C. Antimelanoma antibodies in swine with spontaneously regressing melanoma. *Pigment Cell Res.* **8**, 60–63 (1995).
52. Garrett, I.R., Dallas, S., Radl, J. & Mundy, G.R. A murine model of human myeloma bone disease. *Bone* **20**, 515–520 (1997).
53. Dalefield, R.R., Palmer, D.N. & Jolly, R.D. The frequent occurrence of thyroid tumours in aged horses. *J. Comp. Pathol.* **110**, 57–64 (1994).
54. Sugimura, Y., Sakurai, M., Hayashi, N., Yamashita, A. & Kawamura, J. Age-related changes of the prostate gland in the senescence-accelerated mouse. *Prostate* **24**, 24–32 (1994).
55. Kallman, K.D. Different genetic basis of identical pigment patterns in two populations of platyfish, *Xiphophorus maculatus*. *Copeia* **1970**, 472–487 (1970).
56. Setlow, R.B. & Woodhead, A.D. Temporal changes in the incidence of malignant melanoma: explanation from action spectra. *Mutat. Res.* **307**, 365–374 (1994).
57. Setlow, R.B., Grist, E., Thompson, K. & Woodhead, A.D. Wavelengths effective in induction of malignant melanoma. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 6666–6670 (1993).
58. Nairn, R.S., Morizot, D.C., Kazianis, S., Woodhead, A.D. & Setlow, R.B. Nonmammalian models for sunlight carcinogenesis: genetic analysis of melanoma formation in *Xiphophorus* hybrid fish. *Photochem. Photobiol.* **64**, 440–448 (1996).
59. Schwab, M., *et al.* Genetic basis of susceptibility for neuroblastoma following treatment with N-methyl-N-nitrosourea and X-rays in *Xiphophorus*. *Cancer Res.* **39**, 519–526 (1979).
60. Schwab, M., *et al.* Genetic basis of susceptibility for development of neoplasms following treatment with N-methyl-N-nitrosourea (MNU) or x-rays in the platyfish/swordtail system. *Experientia* **34**, 780–782 (1978).
61. Kazianis, S., *et al.* MNU induction of neoplasia in a platyfish model. *Lab. Invest.* **81**, 1191–1198, 2001.
62. Kazianis, S. *et al.* Genetic analysis of neoplasia induced by N-nitroso-N-methylurea in *Xiphophorus* hybrid fish. *Mar. Biotechnol.* **3**, 37–43 (2001).
63. Meyer, A., Morrissey, J.M. & Schartl, A. Recurrent origin of a sexually selected trait in *Xiphophorus* fishes inferred from a molecular phylogeny [see comments]. *Nature* **368**, 539–542 (1994).
64. Morizot, D.C. in *Genomes* (ed. Gustafson, P.) 43–60 (Kluwer Academic/Plenum Publishers, New York, NY, 2000).
65. Kallman, K.D. The sex determining mechanism of the poeciliid fish, *Xiphophorus montezumae*, and the genetic control of the sexual maturation process and adult size. *Copeia* **1983**, 755–769 (1983).
66. Kallman, K.D. in *Evolutionary Genetics of Fishes* (ed. Turner, B.J.) 95–171 (Plenum Publishing, New York, NY, 1984).
67. Tiersch, T.R., Chandler, R.W., Kallman, K.D. & Wachtel, S.S. Estimation of nuclear DNA content by flow cytometry in fishes of the genus *Xiphophorus*. *Comp. Biochem. Physiol.* **94B**, 465–468 (1989).
68. Flynn, K.M., Schreibman, M.P., Yablonsky-Alter, E. & Banerjee, S.J. Sexually dimorphic development and binding characteristics of NMDA receptors in the brain of the platyfish. *Gen. Comp. Endocrinol.* **115**, 282–291 (1999).
69. Breuckmann, A., Paris, F., Schreibman, M.P. & Blüm, V. Immunoreactive gonadotropin-releasing hormone (GnRH) in the brain and pituitary of adult and juvenile swordtails (*Xiphophorus helleri*, Teleostei, Poeciliidae). *J. Morphol.* **230**, 55–67 (1996).
70. Beaugrand, J.P. & Goulet, C. Distinguishing kinds of prior dominance and subordination experiences in males of green swordtail fish (*Xiphophorus helleri*). *Behav. Processes* **50**, 131–142 (2000).
71. Hoefler, C.D. & Morris, M.R. A technique for the temporary application and augmentation of pigment patterns in fish. *Ethology* **105**, 431–438 (1999).
72. Rosenthal, G.G. & Evans, C.S. Female preference for swords in *Xiphophorus helleri* reflects a bias for large apparent size. *Proc. Natl. Acad. Sci. USA* **95**, 4431–4436 (1998).
73. Trainor, B.C. & Basolo, A.L. An evaluation of video playback using *Xiphophorus helleri*. *Anim. Behav.* **59**, 83–89 (2000).
74. de Wolf, W., Seinen, W. & Hermens, J.L. *N*-acetyltransferase activity in rainbow trout liver and *in vitro* biotransformation of chlorinated anilines and benzenes in fish. *Xenobiotica* **23**, 1045–1056 (1993).
75. Gandzyura, V.P., *et al.* The effect of lead and ammonium ions on bioproductional parameters of fishes offspring. [Russian]. *Gidrobiologicheskii Zhurnal* **34**, 85–90, 1998.
76. Dove, A.D. Richness patterns in the parasite communities of exotic poeciliid fishes. *Parasitol.* **120**, 609–623 (2000).
77. Schmahl, G., Schmidt, H. & Ritter, G. The control of ichthyophthiriasis by a medicated food containing quinine: efficacy tests and ultrastructure investigations. *Parasitol. Res.* **82**, 697–705 (1996).
78. Hogarth, P.J. Protection of the developing foetus from immunological attack in *Xiphophorus helleri*. *J. Reprod. Fertil.* **23**, 532 (1970).
79. McConnell, T.J., *et al.* Identification and mapping of two divergent, unlinked major histocompatibility complex class II B genes in *Xiphophorus* fishes. *Genetics* **149**, 1921–1934 (1998).
80. Meyer, A., Morrissey, J.M. & Schartl, M. Recurrent origin of sexually selected trait in *Xiphophorus* fishes inferred from a molecular phylogeny. *Nature* **368**, 539–542 (1994).
81. Borowsky, R.L., McClelland, M. Cheng, R. & Welsh, J. Arbitrarily primed DNA fingerprinting for phylogenetic reconstruction in vertebrates: the *Xiphophorus* model. *Mol. Biol. Evol.* **12**, 1022–1032 (1995).
82. Morris, M.R., de Queiroz, K. & Morizot, D. Phylogenetic relationships among populations of Northern swordtails (*Xiphophorus*) as inferred from allozyme data. *Copeia* **2001**, 65–81 (2001).
83. Basolo, A.L. Female preference predates the evolution of the sword in swordtail fish. *Science* **250**, 808–810 (1990).
84. Basolo, A.L. The phylogenetic distribution of a female preference. *System. Biol.* **45**, 290–307 (1996).
85. Morris, M.R. & Casey, K. Female swordtail fish prefer symmetrical sexual signal. *Anim. Behav.* **55**, 33–39 (1998).