Proposal for Constructing BAC Libraries for *Xiphophorus* Species

Paul B. Samollow, Department of Genetics, Southwest Foundation for Biomedical Research
Ronald B. Walter, *Xiphophorus* Genetic Stock Center, Texas State University | San Marcos
Steven Kazianis, Wistar Institute, University of Pennsylvania
Chris T. Amemiya, Genome Resource Center, Benaroya Research Institute at Virginia Mason

A. Background and rationale

The genus *Xiphophorus* comprises 23 recognized (and two unnamed) species of live-bearing fishes that populate freshwater habitats on the Atlantic slopes of Mexico, Guatemala, Belize, and Honduras. Members of this genus possess a relatively low degree of post-fertilization (post-zygotic) reproductive isolation with one another. In native habitats combinations of spatial, ecological, behavioral, and anatomic factors discourage cross-species mating, such that naturally occurring interspecific *Xiphophorus* hybrids are virtually unknown (ROSEN 1979; ROSENTHAL *et al.* 2003). In the laboratory however, most members of the genus are inter-fertile with one another and produce fertile offspring, either through interspecific pairings in aquaria, or by means of artificial insemination procedures. This capability has led to the experimental crossing/backcrossing of many different species combinations and has revealed a diversity of cross-specific developmental perturbations influencing the regulation of pigmentation patterns and intensity, growth and maturation rates, behavioral patterns, DNA repair rates, and susceptibilities to spontaneous, ultraviolet radiation (UVR)-induced, and chemical mutagen-induced neoplasias. Consequently, the *Xiphophorus* species complex represents an easily manipulated and astoundingly diverse model system for examining the evolution of interacting gene complexes, and for dissecting the genetic bases of developmental programs, behaviors, and the molecular processes underlying neoplastic disease initiation and progression.

We propose the construction of BAC libraries for six species from distinct evolutionary branches (clades) of *Xiphophorus*. The rationale is to enable comparisons of gene content and structure in genomic regions that are implicated in the regulation of complex characteristics as manifested by interspecific genetic incompatibilities in hybrid crosses. Specifically, the ability to segregate quantitatively and qualitatively variable interspecies-hybrid backcross offspring, and the existence of extensive linkage mapping resources for this genus (section F) enables the genetic mapping of Mendelian and quantitative trait loci (QTLs) that are involved in the regulation of cellular differentiation and cell cycle regulation, DNA damage and repair, tumorigenesis, sex determination and the development of secondary sexual characteristics, and complex visual and olfactory communication signaling pathways. As the genomic regions harboring such genes are identified, BAC analyses will make it possible to characterize the organizational differences in gene content and structure within these regions with the goal of correlating recently evolved changes in gene sequence and structure with alterations in gene function and phenotypic outcome. In addition, the availability of *Xiphophorus* BAC libraries will allow investigators to take advantage of the extensive genomic database resources of the pufferfish (*Fugu rubripes)*, zebrafish (*Danio rerio*), and medaka (*Oryzias latipes*) to predict the genomic structures and gene contents of regions found to harbor developmental and cancer-related QTLs, thereby facilitating the discovery of candidate genes in these regions. Finally, examination of the structures and sequences of targeted *Xiphophorus* genomic regions will enhance the utility of other vertebrate (fish and non-fish) genome data by enabling the extrapolation of novel findings in *Xiphophorus* to other species.

B. Significance of the *Xiphophorus* Model System

The successful development and growth of an organism result from the coordinated interactions of genes that have co-evolved during the evolutionary history of the species (i.e., co-adapted gene complexes). When new species arise from a common progenitor, their genomes begin to diverge through random and adaptive processes. As the genomes of the separate lineages continue to evolve, genes must (and, in successful lineages, do) evolve in coordinate ways to maintain the ability to bring about a viable and fertile adult individual capable of reproduction. Nevertheless, when the genomes of closely related

species are mixed, as in the case of interspecific hybridization or other means (e.g., transgenesis), it is often that case that suites of genes that performed harmoniously in the ancestral species, and which co-evolved to maintain normal physiologic and developmental function in each of the daughter species, now interact in ways that result in physiologic and developmental abnormalities. The evolutionary mechanisms that enable suites of genes to co-evolve in ways that preserve the ability to produce normal, adaptive phenotypes are poorly understood, as are the specific structural and regulatory changes that manifest as hybrid incompatibilities among genes that derive from the divergent genomes of related species. The ability to combine divergent genomes *in vivo* can provide a powerful experimental system for examining both of these aspects of genomic divergence, as well as a system for probing the specific genetic bases of developmental and other regulatory systems.

The *Xiphophorus* tumorigenesis model: Many species of *Xiphophorus* exhibit striking polymorphism of black skin pigmentation patterns resulting from the differentiation and genetically determined localization of large melanin-bearing cells called macromelanophores. The presence (or absence) and distribution of these cells, and the pigment patterns they define, are controlled by components of a complex genetic locus, *Tu*, which lies near the sex-determining locus on the homomorphic "X" and "Y" sex chromosomes (linkage group 24). While these macromelanophore patterns are clearly and strictly defined within a species, they can become wildly altered in interspecific hybrids and their backcross progeny. The so-called Gordon-Kosswig (G-K) model of interspecific hybrid tumorigenesis (KOSSWIG 1927; HAUSSLER 1928; GORDON 1931; ANDERS 1967), provides an elegant example of this kind of interspecific hybrid incompatibility. In this cross platyfish, *X. maculatus,* bearing one of the several pigmentation alleles of *Tu* (e.g.: *Sd*, spotted dorsal) are crossed to *X. helleri*, a swordtail species that lacks *Tu*-based macromelanophore phenotypes. The resulting F_1 hybrids exhibit a vastly enhanced macromelanophore phenotype resulting from increased proliferation and dispersion of macromelanophore cells. When the F_1 is backcrossed to *X. helleri*, half of the backcross offspring exhibit no macromelanophore development (as in pure *X. helleri*), whereas the other half show enhanced pigmentation similar to that seen in the F_1 (due to *Sd*-derived macromelanophore proliferation). Among the pigmented progeny half (25% of the total progeny) exhibit even greater *Sd* enhancement than the F_1 and go on to develop malignant melanoma. The other half of the pigmented progeny seldom develops melanoma, and the unpigmented fish never do. The segregation of unpigmented, pigmented, and pigmented/tumorous backcross phenotypes is explained by a two locus genetic model (ANDERS 1991) comprising the dominant sex-linked *Tu* (*Sd*) gene that determines the position and extent of macromelanophore differentiation, and a recessive autosomal locus termed *DIFF* (VIELKIND 1976) that modulates the expression of the *Tu* gene. Recent studies have identified *Xmrk-2*, an aberrantly regulated receptor tyrosine kinase gene, as the *Tu* component responsible for the macromelanophore phenotype (KAZIANIS and WALTER 2002; WELLBROCK *et al.* 2002). The *DIFF* locus has been mapped to *Xiphophorus* linkage group 5, and recent evidence suggests it might be *CDNK2X*, a *Xiphophorus* homolog of human *CDKN2A* (KAZIANIS *et al.* 1999; KAZIANIS *et al.* 2000; NAIRN *et al.* 2001).

The simple G-K model of genetically determined spontaneous melanoma is but one of several interspecific hybridization schemes that alter normal macromelanophore patterns and increase susceptibility to melanoma (WALTER and KAZIANIS 2001). These alternative crosses also lead to enhanced macromelanophore proliferation and dispersion, but do not result in development of melanoma unless the animals are also exposed to UVR or chemical mutagens (SCHWAB *et al.* 1978; KAZIANIS *et al.* 2001a; KAZIANIS *et al.* 2001c). Backcross progeny from such crosses also exhibit increased susceptibility to non-*Tu* related neoplasias when exposed to UVR and chemical mutagens. These various interspecific hybrid crosses provide model systems for the genetic and molecular dissection of environmentally induced carcinogenesis. Finally, some crosses actually result in suppression of *Tu* expression rather than enhancement (WALTER and KAZIANIS 2001), thereby demonstrating that the genetic bases of multilocus interactions controlling macromelanophore compartmentalization and development are quite varied among species.

Xiphophorus as research models: The foregoing examples illustrate the capacity of the *Xiphophorus* interspecific hybrid system for delving the genetic regulation of cellular differentiation, dispersion, and

proliferation and provide a solid research paradigm for understanding developmental anomalies contributing to neoplastic disease. The application of similar strategies can (and has) also enable(d) the genetic dissection of other complex phenotypes such as the regulation of other pigmentation patterns (WELLBROCK *et al.* 2002), resistance to DNA damage (MEADOR *et al.* 2000; MITCHELL *et al.* 2001), variation in DNA repair capabilities (WALTER *et al.* 2001), susceptibility to non-melanoma neoplasia (KAZIANIS *et al.* 2001b), the multigenic basis of sex determination (VOLFF and SCHARTL 2001), genetic control of growth rates and age at maturation (KALLMAN 1985), and more. More fundamentally, comparisons of the levels of genetic incompatibility between different pairs of *Xiphophorus* species, representing different stages of evolutionary divergence, can enable the testing of hypotheses regarding the importance of adaptive versus random (neutral) processes in the evolutionary trajectories of interacting genes and gene complexes that influence a broad range of physiologic, developmental, and behavioral characteristics.

In addition to these hybridization-based research areas, recent research utilizing *Xiphophorus* species as experimental models includes: numerous genetic topics (e.g., molecular evolution of oncogene-tumor suppressor systems; gene structure, regulation, and expression; genome evolution, photobiology [DNA damage and repair]; molecular mechanisms and genetic control of DNA repair efficiency and rate; genetics of susceptibility to environmental [UVR and chemical] carcinogenesis; genome instability; antigen receptor structure and evolution); cell migration and differentiation; evolution of visual and olfactory communication; sexual selection and mate choice; genetic, endocrine, and social influences on growth and maturation; physiology of weightlessness; endocrinology; parasitology; and more.

C. Potential uses of *Xiphophorus* BACs

While it is impossible to predict the varied uses to which a set of phylogenetically diverse *Xiphophorus* BAC libraries might be put, the areas of research described below illustrate some specific applications of BAC technology, and hint at their potential in the broader *Xiphophorus* system.

Genetic and molecular analysis of tumorigenesis: Several projects within an NIH funded Program Project (CA75137: Genetic Determinants of Tumorigenesis; Ronald B. Walter, P.L.) are presently investigating new models (alternative interspecific crosses) for studying the molecular mechanisms of DNA damage and repair, the spectrum of dysplastic and neoplastic outcomes of different environmental carcinogens, and the genetic underpinnings of susceptibility to neoplastic development. These divers systems show distinctive differences from one another and from the “classic” G-K model in patterns of genetic control of tumorigenesis-related phenotypes, most notably in the involvement of genes other than the classic *DIFF* locus in modulating tumor susceptibility. BAC analysis will be used to characterize candidate gene regions that are found, through linkage analysis in the specific *Xiphophorus* crosses, to harbor QTLs that influence DNA damage and repair characteristics and susceptibility to UVR- or chemical-induced melanoma and hypermelanotic phenotypes. Determining the gene content and structure of these regions is a first step toward identifying candidate genes for these QTLs. BAC libraries will also provide a means to analyze genomic regions known from studies in other species to contain genes involved in DNA repair, cell cycle regulation, cell growth and differentiation, apoptosis, immunosuppression, and other processes and mechanisms that contribute to neoplastic transformation and proliferation.

Genetic control and molecular mechanisms of sex-determination: Sex in *Xiphophorus* fishes is determined by poorly understood interactions between the major sex-determining locus (*SDL*) on linkage group 24, and at least one autosomal locus (*ASD1*) that modifies the effects of *SDL* in some, but not all species and interspecific hybrids (reviewed by KALLMAN 1984; VOLFF and SCHARTL 2001; KAZIANIS *et al.* in press). The autosome-like (fully recombining) “X” and “Y” sex chromosomes are defined in a given cross by allelic alternatives for macromelanophore pigmentation patterns and skin color variants at the *Tu* and *RY* loci, respectively, both of which are closely linked to *SDL*. In addition, a W sex chromosome devoid of pigmentation and color genes is present in some species, and all possible combinations of X, Y, and W pairs are possible though interspecific hybridization approaches. Recent molecular analyses of

the *SDL* region (NANDA *et al.* 2000; FROSCHAUER *et al.* 2002) have begun to characterize the organizational relationships and structures of the RY - *SDL* - *Tu* (*Xmrk-2*) complex and have suggested hypotheses about the origins and evolution of the genes in this region. Current linkage mapping efforts (KAZIANIS *et al.* submitted) and planned QTL analyses are likely to pinpoint the autosomal regions that harbor sex-determining modifiers in the near future. Detailed comparative molecular analyses of the *SDL* region in species with different variants of the X, Y, W system, as well as characterization of the autosomal *ASD1* region which influences sex in some but not all species, will be enormously facilitated by the availability of BACs from a variety of *Xiphophorus* species.

Characterization of QTL-bearing regions identified through linkage analysis of interspecific backcross hybrids: In addition to the uses of BACs discussed in connection with the major research areas above, numerous smaller studies that use interspecific hybrid models to dissect the genetic control of a broad array of morphologic, physiologic, and behavioral characteristics can also benefit from the use of BACs. The generation of novel phenotypic variation through the interspecific hybridization process provides quantitative phenotypes that can potentially enable the mapping of QTLs for the characteristics of interest to specific genomic regions. BAC clones corresponding to these genomic regions can be examined to characterize the organization and arrangement of genes within them, and thereby help to identify potential candidate genes that contribute to normal regulation of the process(es) in question. Examples of such research topics include: immune function; endocrine influences on genetically controlled growth rates and age at maturation; genetic bases of numerous physical characteristics (spot patterns, coloration, body shape, etc.); olfactory and visual communication; the evolutionary and developmental origins of swordtail “swords” (size, shape, presence/absence), and complex behaviors related to mate choice and mating strategies.

Comparative genomics / Genome evolution: The availability of *Xiphophorus* BAC libraries would facilitate targeted comparative genomic analyses with the major fish genome models (zebrafish, pufferfish, and medaka) by providing access to large genomic regions that could either be scanned for specific gene content and synteny, or sequenced for complete sequence comparisons. Because extensive genome sequence is already available for pufferfish (fully sequenced), zebrafish (partially sequenced) and medaka (partially sequenced), prediction of the genic content and approximate sequence characteristics of specific *Xiphophorus* BAC clones (identified by QTL mapping and probing with known *Xiphophorus* STS marker sequences) can be inferred from consensus sequence data compiled from fish and mammalian databases. One example of the potential use of BAC sequences would be to better understand the evolution of repetitive element families that are common in *Xiphophorus* and other fishes, and which may be related to the acquisition of novel gene functions (Nanda et al. 2000; Froschauer et al. 2001).

D. Size and interest of the research community that will use the BAC resources

The research community that has expressed interest in the production of *Xiphophorus* BAC resources is best defined as the body of investigators working on a number of different fish species, including guppy (*Poecilia reticulata*), pufferfish, medaka, zebrafish, and other (usually small) aquatic organisms that serve as models for basic research on genetic, cellular, and molecular processes related to human health and disease. The recent (Sept. 29 – Oct. 2, 2003) “Aquatic Animal Models of Human Disease” meeting, sponsored by the National Center for Research Resources (NCRR), hosted more than 100 investigators from around the world who discussed the use of aquarium fish models for the study of comparative and functional genomics, gene regulation/expression, environmental mutagenesis, molecular mechanisms of carcinogenesis, transgenic models of disease, and molecular toxicology. Several of these investigators have furnished letters that express their enthusiasm for the production and distribution of *Xiphophorus* BAC resources, and describe ways in which such resources could benefit their research programs and those of the broader research community. A total of 22 such letters, from these and other investigators are included in the accompanying Appendix. Another metric of the size of the *Xiphophorus* research community is publication volume. A scan of the PubMed literature database indicates that 126 biomedically oriented articles specifically concerning *Xiphophorus* were published in the five-year period

ending Oct. 4, 2003. Expanding the search to include all poeciliid fishes (same family as *Xiphophorus*) revealed 335 such articles in this same time period. Moreover, a search of the comprehensive *Xiphophorus* Genetic Stock Center publications archive revealed that all research publications concerning *Xiphophorus* in the decade ending in 2000 rose 70% over the previous decade. Projections for publication numbers in the current decade are even higher.

E. Previous proposals for BAC-based genomic sequencing

To our knowledge, no *Xiphophorus* species has been proposed for BAC-based genomic sequencing.

F. Other genomic resources for these species

Xiphophorus linkage maps: One goal of an ongoing NIH funded Program Project (CA075137: Genetic Determinants of Tumorigenesis; R B. Walter, P.L.) is to complete a high density, genus consensus linkage map for QTL mapping of physiologic and developmental variation (especially tumor-related QTLs) in interspecific hybrid crosses. There are currently two separate maps with a total of 640 unique loci including 371 microsatellite and 192 AP-PCR markers (http://www.xiphophorus.org/mapping.htm; KAZIANIS *et al.* submitted; WALTER *et al.* submitted). Efforts are underway to combine these maps into a single comprehensive linkage map resource. Because these maps also include many highly conserved Type I functional gene homologs, they also provide comparative information regarding the evolution of gene synteny and linkage relationships among distantly related fish and other vertebrate species.

Xiphophorus Genetic Stock Center: The NIH supported XGSC (RR017072; The *Xiphophorus* Genetic Stock Center; R. B. Walter, P.I.) (http://www.xiphophorus.org/xgsc.htm) provides fish from more than 70 genetic lineages/strains to investigators in more than 30 laboratories in the United States, Canada, Mexico, Japan, and Germany. In addition to lineages of 21 of the recognized species, the XGSC maintains several inbred strains, some of which have attained more than 95 generations of full-sib mating.

Xiphophorus.org: The Xiphophorus.org website (http://www.xiphophorus.org/) provides a wealth of information and links on *Xiphophorus* biology, genetics, research tools, natural history, biogeography, and other information that is useful to investigators and interested hobbyists. It also maintains a regularly updated *Xiphophorus* bibliography.

Additional resources include 418 *Xiphophorus* and 704 other poeciliid nucleotide sequence entries in GenBank (Oct. 10, 2003), and normal and tumor-derived cell lines available from investigators and commercial sources. A recent NIH grant to Dr. Kazianis (RR017336; Enhanced Mapping and Cell Culture for *Xiphophorus* Fishes; S. Kazianis, P.I.) supports the development of a murine–*Xiphophorus* radiation hybrid panel for use in the construction of a *Xiphophorus* radiation hybrid map.

G. Choice of *Xiphophorus* species for BAC library construction

The choice of species requested for BAC library construction is based on three fundamental criteria. 1. Phylogenetic representation: the genus comprises four putatively monophyletic clades: northern platyfishes (3 species), northern swordtails (9 species), southern platyfishes (6 species), southern swordtails (5 species) (RAUCHENBERGER *et al.* 1990; MEYER *et al.* 1994; MORRIS *et al.* 2001). The northern swordtails and southern platyfish are further divided into major subclades within their respective branches. We propose the construction of a BAC library from a representative of each major clade, and from two subclades within the northern swordtails and southern platyfishes. 2. Research utility: several *Xiphophorus* species are currently the foci of major research programs in carcinogenesis, evolution of tumor suppressor/oncogene systems, sex determination, and other biomedically related topics. It makes sense to target these species to the extent that it does not compromise phylogenetic breadth. 3. Availability: species that are unavailable or are not in abundance at the XGSC are avoided.

Our priorities for BAC construction are:

X. helleri (http://www.xiphophorus.org/assets/hyb1.jpg): southern swordtail; historically and currently the most commonly used macromelanophore-lacking parent in interspecific hybrid crosses of all kinds; XGSC – abundant; multiple strains. (HIGHEST PRIORITY)

X maculatus (http://www.xiphophorus.org/assets/hyb1.jpg): maculatus subclade of southern platyfish; most commonly used macromelanophore-bearing parent in environmentally induced carcinogenesis studies; XGSC- abundant; multiple strains. (HIGHEST PRIORITY)

X. couchianus (http://www.xiphophorus.org/assets/hyb7.jpg): northern platyfish; macromelanophore-lacking parent in carcinogenesis studies – yields different pattern of susceptibility to tumorigenesis among backcross offspring than crosses using *X. helleri* as parent; XGSC- abundant. (VERY HIGH PRIORITY)

X. montezumae (http://www.xiphophorus.org/assets/rascon.jpg): montezumae subclade of northern swordtails; used in crosses to study genetics of color variation and potentially useful as macromelanophore-lacking parent in carcinogenesis studies; XGSC – abundant; multiple strains. (VERY HIGH PRIORITY).

X. variatus (http://www.xiphophorus.org/assets/hyb15.jpg): variatus subclade of southern platyfish: alternative macromelanophore-bearing parent for carcinogenesis studies; pattern of susceptibility to tumorigenesis among backcross offspring not fully documented; XGSC – abundant; multiple strains. (HIGH PRIORITY)

X. cortezi (http://www.xiphophorus.org/assets/cortezi.jpg): cortezi subclade of northern swordtails; potential use for phylogenetic studies and examination of fin pigment regulation in relation to carcinogenesis; XGSC – abundant. (HIGH PRIORITY)

H. Size of the *Xiphophorus* genome

Xiphophorus genomes are estimated at ~830 mb (TIERSCH *et al.* 1989; GREGORY 2001), or about 25% the size of the human genome.

I. Availability and quality of source DNAs

The *Xiphophorus* species/strains for which BAC libraries are proposed are all available at the *Xiphophorus* Genetic Stock Center located at Texas State University-San Marcos (Texas) (http://www.xiphophorus.org/xgsc.htm). To prepare high molecular weight DNA, animals will be anesthetized and bled via cardiac puncture. Blood has been shown to be the best source of genomic DNA for preparing BAC libraries from lower vertebrates (AMEMIYA *et al.* 1996). Blood (~50-100 µl) from one large individual will suffice for preparing a BAC library. Preparation and processing of agarose-embedded cells are routinely done in Dr. Amemiya's laboratory. Tissues and carcasses from each individual processed will be stored (-80° C) for possible future reference. No problems in genomic cloning are anticipated because many teleost BAC libraries have already been generated.

J. Specifications of the libraries and supporting rationale

A 10X coverage BAC library will be made using *Eco*RI partial digests with average insert size of 150 kb. Due to the relatively small sizes of *Xiphophorus* genomes, it is anticipated that such libraries will comprise roughly 150 384-well microtiter dishes (assuming insert sizes of 150 kb). Numerous genomic markers are available to assess coverage of the libraries.

K. Time frame for library availability

Research programs are already awaiting the availability of BACs for investigating the structures of genomic regions in connection with the genetic control and molecular mechanism of carcinogenesis, structure and evolution of the *Xiphophorus* sex-determining region, and comparative analysis of the *Xiphophorus* sex-linked oncogene/sex-determining region.

L. Other support for BAC library construction

None.

M. Availability of other *Xiphophorus* BAC libraries

A *Xiphophorus* BAC library has been established by the *Xiphophorus* research group in Würzburg, Germany (FROSCHAUER *et al.* 2002). This high-quality, fully arrayed library was derived from the *X. maculatus* Jp 163A strain, but is not publicly available at this time (J.-N. Volff, private communication). Because of the uncertainty regarding future access to this BAC library from this critically important species (sections B and G), we have proposed making a new, publicly accessible *X. maculatus* BAC library from a closely related but genetically distinct strain: *X. maculatus* Jp 163B.

REFERENCES

AMEMIYA, C. T., T. OTA and G. W. LITMAN, 1996 Construction of P1 artificial chromosome (PAC) libraries from lower vertebrates, pp. 223-256 in *Nonmammalian Genomic Analysis: A Practical Guide*, edited by B. BIRREN and E. LAI. Academic Press, New York.

ANDERS, F., 1967 Tumour formation in platyfish-swordtail hybrids as a problem of gene regulation. Experientia **23:** 1-10.

ANDERS, F., 1991 Contributions of the Gordon-Kosswig melanoma system to the present concept of neoplasia. Pigment Cell Research **3:** 7-29.

FROSCHAUER, A., C. KORTING, T. KATAGIRI, T. AOKI, S. ASAKAWA, N. SHIMIZU, M. SCHARTL and J. VOLFF, 2002 Construction and initial analysis of bacterial artificial chromosome (BAC) contigs from the sex-determining region of the platyfish Xiphophorus maculatus. Gene **295:** 247-254.

GORDON, M., 1931 Hereditary basis of melanosis in hybrid fishes. The American Journal of Cancer **15:** 1495-1523.

GREGORY, T. R., 2001 Animal Genome Size Database. http://www.genomesize.com/.

HAUSSLER, G., 1928 Uber melanombildungen bei Bastarden Xiphophorus helleri und Platypoecilus maculatus var. rubra,. Klin Wochenschr **7:** 1561-1562.

KALLMAN, K., 1985 Genetic control of sexual maturation in fishes of the *Xiphophorus*. IXth Int. Congress Comp. Endocrinol. **2:** 1175-1177.

KALLMAN, K. D., 1984 A new look at sex determination in poeciliid fishes, pp. 95-171 in *Evolutionary Genetics of Fishes*, edited by B. J. TURNER. Plenum Publishing Corporation, New York.

KAZIANIS, S., L. D. COLETTA, D. C. MORIZOT, D. A. JOHNSTON, E. A. OSTERNDORFF and R. S. NAIRN, 2000 Overexpression of a fish *CDKN2* gene in a hereditary melanoma model. Carcinogenesis **21:** 599-605.

KAZIANIS, S., I. GIMENEZ-CONTI, R. B. SETLOW, A. D. WOODHEAD, J. C. HARSHBARGER, D. TRONO, M. LEDESMA, R. S. NAIRN and R. B. WALTER, 2001a MNU induction of neoplasia in a platyfish model. Lab Invest **81:** 1191-1198.

KAZIANIS, S., I. B. GIMENEZ-CONTI, R. B. SETLOW, A. D. WOODHEAD, J. C. HARSHBARGER, D. TRONO, M. LEDESMA, R. S. NAIRN and R. B. WALTER, 2001b MNU induction of neoplasia in a platyfish model. Lab Invest**: 81**:1191-1198.

KAZIANIS, S., I. B. GIMENEZ-CONTI, D. TRONO, A. PEDROZA, L. B. CHOVANEC, D. C. MORIZOT, R. S. NAIRN and R. B. WALTER, 2001c Genetic Analysis of Neoplasia Induced by N-Nitroso-N-methylurea in Xiphophorus Hybrid Fish. Marine Biotechnology **3:** S37-S43.

KAZIANIS, S., D. C. MORIZOT, L. D. COLETTA, D. A. JOHNSTON, B. WOOLCOCK, J. R. VIELKIND and R. S. NAIRN, 1999 Comparative structure and characterization of a *CDKN2* gene in a *Xiphophorus* fish melanoma model. Oncogene **18:** 5088-5099.

KAZIANIS, S., D. C. MORIZOT, J. VIELKIND, B. WOOLCOCK, M. WIGLER, R. LUCITO, R. S. NAIRN, J. RICHARDS, A. PEDROZA, L. HOLLEK, L. HAZELWOOD, R. B. WALTER and K. D. KALLMAN, Sex-Determination in Platyfishes and Swordtails. 2nd International Symposium on Live-bearing Fishes. in press.

KAZIANIS, S., R. S. NAIRN, R. B. WALTER, D. A. JOHNSTON, J. KUMAR, D. TRONO, L. DELLA-COLLETA, I. GIMENEZ-CONTI, J. D. RAINS, E. L. WILLIAMS, M. M. MAMEROW, K. J. KOCHAN, M. SCHARTL, J. R. VIELKIND, J.-N. VOLFF, B. WOOLCOCK and D. C. MORIZOT, The genetic map of Xiphophorus fishes represented by 24 multipoint linkage groups. Submitted.

KAZIANIS, S., and R. B. WALTER, 2002 Use of platyfishes and swordtails in biological research. Lab Animal **31:** 46-52.

KOSSWIG, C., 1927 Uber bastarde der teleostier *Platypoecilus* und *Xiphophorus*. Zeitschrift fur induktive Abstammungs- und Vererbungslehre **44:** 253.

MEADOR, J. A., R. B. WALTER and D. L. MITCHELL, 2000 Induction, distribution and repair of UV photodamage in the platyfish, *Xiphophorus signum*. Photoechmistry and Photobiology **72:** 260-266.

MEYER, A., J. M. MORRISSEY and M. SCHARTL, 1994 Molecular phylogeny of fishes of the genus Xiphophorus suggests repeated evolution of a sexually selected trait. Nature **368:** 539-541.

MITCHELL, D. L., J. A. MEADOR, M. BYROM and R. B. WALTER, 2001 Resolution of UV-Induced DNA Damage in Xiphophorus Fishes. Marine Biotechnology **3:** S61-S71.

MORRIS, M. R., K. DE QUEIROZ and D. C. MORIZOT, 2001 Phylogenetic Relationships among Populations of Northern Swordtails (*Xiphophorus*) as Inferred from Allozyme Data. Copeia **1:** 65-81.

NAIRN, R. S., S. KAZIANIS, L. D. COLETTA, D. TRONO, A. P. BUTLER, R. B. WALTER and D. C. MORIZOT, 2001 Genetic analysis of susceptibility to spontaneous and UV-Induced carcinogenesis in *Xiphophorus* hybrid fish. Marine Biotechnology **3:** S24-S36.

NANDA, I., J. N. VOLFF, S. WEIS, C. KORTING, A. FROSCHAUER, M. SCHMID and M. SCHARTL, 2000 Amplification of a long terminal repeat-like element on the Y chromosome of the platyfish, *Xiphophorus maculatus*. Chromosoma **109:** 173-180.

RAUCHENBERGER, M., K. D. KALLMAN and D. C. MORIZOT, 1990 Monophyly and geography of the Rio Panuco basin swordtails (Genus *Xiphophorus*) with descriptions of four new species. American Museum Novitates **2975:** 1-41.

ROSEN, D. E., 1979 Fishes from the uplands and intermontane basins of Guatemala: revisionary studies and comparative geography. Bull. Am. Mus. Nat. His. **162:** 271-375.

ROSENTHAL, G. G., X. F. DE LA ROSA RENYA, S. KAZIANIS, M. J. STEPHENS, D. C. MORIZOT, M. J. RYAN and F. J. GRACÍA DE LEON, 2003 Dissolution of sexual signal complexes in a hybrid zone between the swordtails *Xiphophorus birchmanni* and *Xiphophorus malinche* (Poeciliidae). Copeia **in press**.

SCHWAB, M., S. HAAS, M. R. AHUJA, G. KOLLINGER, A. ANDERS and F. ANDERS, 1978 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.

TIERSCH, T. R., R. W. CHANDLER, K. D. KALLMAN and S. S. WACHTEL, 1989 Estimation of nuclear DNA content by flow cytometry in fishes of the genus *Xiphophorus*. Comp Biochem Physiol [B] **94:** 465-468.

VIELKIND, U., 1976 Genetic control of cell differentiation in platyfish-swordtail melanomas. Journal of Experimental Zoology **196:** 197-204.

VOLFF, J., and M. SCHARTL, 2001 Variability of genetic sex determination in poeciliid fishes. Genetica **111:** 101-110.

WALTER, R. B., and S. KAZIANIS, 2001 Xiphophorus Interspecies Hybrids as Genetic Models of Induced Neoplasia. Institute for Laboratory Animal Research **42:** 299-321.

WALTER, R. B., J. D. RAINS, J. E. RUSSELL, T. M. GUERRA, C. DANIELS, D. A. JOHNSTON, J. KUMAR, A. WHEELER, K. KELNAR, V. A. KHANOLKAR, E. L. WILLIAMS, J. L. HORNECKER, L. HOLLEK, M. M. MAMEROW, A. PEDROZA and S. KAZIANIS, A microsatellite map for *Xiphophorus*. Submitted.

WALTER, R. B., H.-M. SUNG, R. D. OBERMOELLER, D. L. MITCHELL, G. W. INTANO and C. A. WALTER, 2001 Relative Base Excision Repair in *Xiphophorus* Fish Tissue Extracts. Marine Biotechnology **3:** S50-S60.

WELLBROCK, C., A. GOMEZ and M. SCHARTL, 2002 Melanoma development and pigment cell transformation in *Xiphophorus*. Microscopy Research and Technique **58:** 456-463.