The *Xiphophorus* Genetic Stock Center Manual

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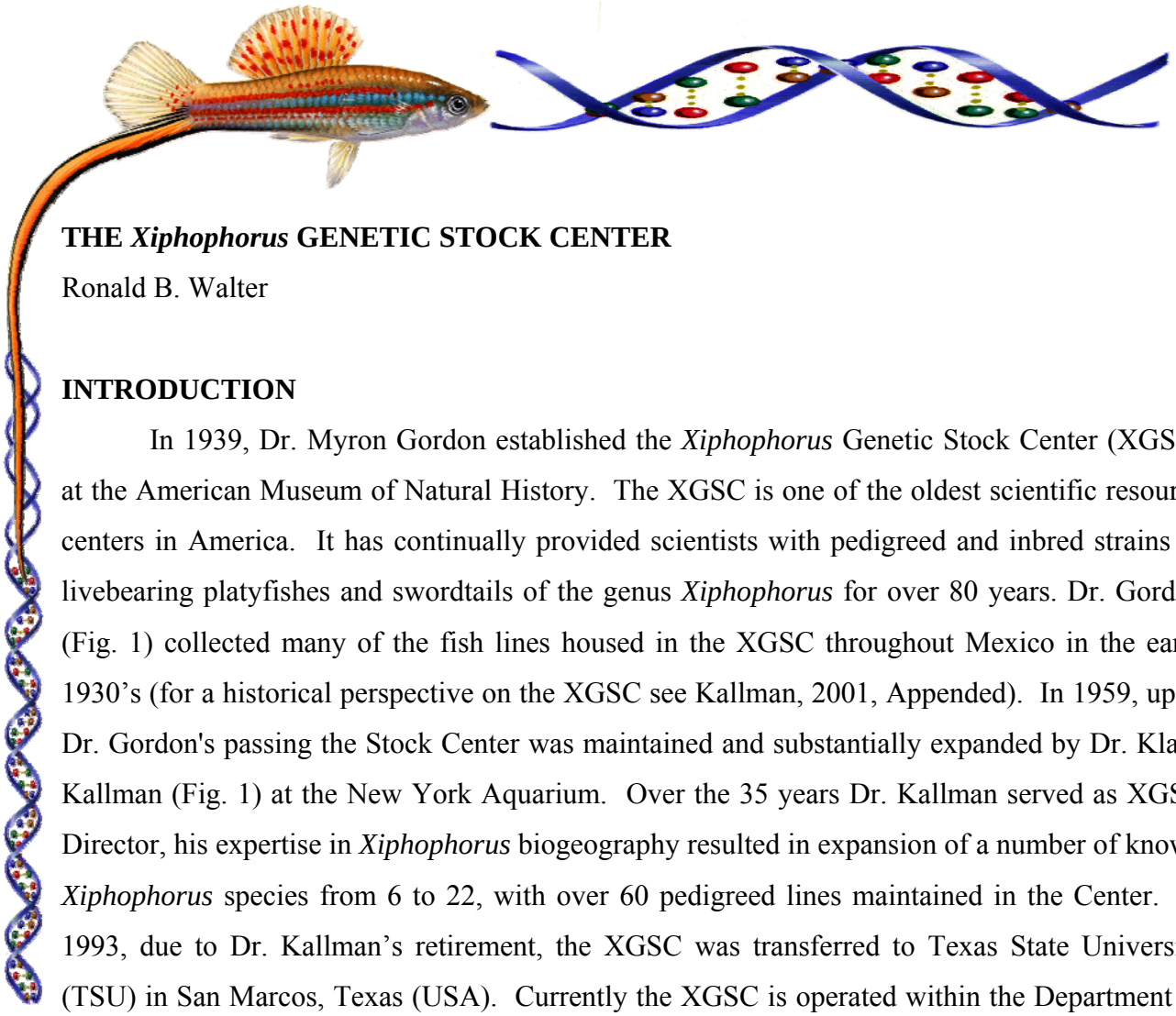

THE *Xiphophorus* GENETIC STOCK CENTER

Ronald B. Walter

INTRODUCTION

In 1939, Dr. Myron Gordon established the *Xiphophorus* Genetic Stock Center (XGSC) at the American Museum of Natural History. The XGSC is one of the oldest scientific resource centers in America. It has continually provided scientists with pedigreed and inbred strains of livebearing platyfishes and swordtails of the genus *Xiphophorus* for over 80 years. Dr. Gordon (Fig. 1) collected many of the fish lines housed in the XGSC throughout Mexico in the early 1930's (for a historical perspective on the XGSC see Kallman, 2001, Appended). In 1959, upon Dr. Gordon's passing the Stock Center was maintained and substantially expanded by Dr. Klaus Kallman (Fig. 1) at the New York Aquarium. Over the 35 years Dr. Kallman served as XGSC Director, his expertise in *Xiphophorus* biogeography resulted in expansion of a number of known *Xiphophorus* species from 6 to 22, with over 60 pedigreed lines maintained in the Center. In 1993, due to Dr. Kallman's retirement, the XGSC was transferred to Texas State University (TSU) in San Marcos, Texas (USA). Currently the XGSC is operated within the Department of Chemistry & Biochemistry and as part of the TSU Molecular Biosciences Research Group (MBRG).

The *Xiphophorus* System: Research Model

To better understand heritable factors involved in multigenic or complex traits (e.g., behavior, disease resistance, tumor development, etc.), experimental animal models are of considerable importance. Characteristics of useful and informative experimental animal model systems include factors such as: (1) commonality with human molecular genetics and molecular physiology; (2) availability of large numbers of animals at reasonable cost; (3) background knowledge concerning the genetics of the animal model, (4) availability of well-maintained inbred and non-inbred lines; (5) the ability to perform genetic crosses among phenotypically diverse lines producing fertile offspring; (6) a well marked gene map, and (7) characterized

Figure 1, top: Dr. Myron Gordon established the Stock Center and maintained it until his death in 1959. He initiated the study of *Xiphophorus* Genetics from collections he made in Mexico from 1929-1959

Figure 2, bottom: Dr. Klaus Kallman, greatly expanded and enhanced the Center serving as XGSC Director for over 35 years.

molecular markers and cloned genes that can be utilized as tools for research investigations among varied scientific disciplines. Due to the efforts of many scientists over the past 80+ years, *Xiphophorus* fishes possess all of these desirable attributes.

Xiphophorus have emerged as a contemporary research tool and the *Xiphophorus* genetic system, as a research model, is now receiving unprecedented attention.

Xiphophorus fishes and hybrids are used in many areas of contemporary research including behavioral genetics, mechanisms of sexual selection, bio-geographical systematics, study of molecular events leading to speciation, and other topics. *Xiphophorus* interspecies hybrids are almost always fertile, and are extremely valuable for their genetic variability. Extensive use of these hybrids for gene mapping (Morizot et al., 1998, Walter et al., 2004;

Kazianis et al., 2004) has resulted in a robust *Xiphophorus* gene map further expanding the utility of this model to address complex genetic questions. Current MBRG/XGSC research efforts are aimed at gene map expansion and tagging of chromosomal regions with PCR markers.

- **Table 1: Examples of institutes that have received fish from the XGSC**
- Benaroya Research Institute at Virginia Mason
- Brookhaven National Laboratories
- California State University, Northridge
- Cornell University
- East Carolina University
- George Washington University, Medical School
- Louisiana State University
- Marine Biological Laboratory at Woods Hole, MA
- Mount Desert Island Marine Laboratory, ME
- North Carolina State University
- Ohio State University
- Princeton University
- Queens College
- Simon Fraser University, British Columbia
- Texas State University
- UT-MD Anderson Cancer Center (Smithville, TX)
- University of Connecticut
- University of Florida
- University of Kaposvar (Hungary)
- University of Nebraska
- University of New Mexico
- University of Oklahoma
- University of Puerto Rico
- University of Southern Maine
- University of Texas, Austin
- University of Tulsa
- University of Wuerzburg (Germany)
- University of Warmia and Mazury (Poland)
- W.T. Henning High School (Sulphur, LA)
- Wistar Institute (Univ. Penn.)

In addition to other areas of research inquiry, *Xiphophorus* fish possess a historical contribution to understanding the genetics underlying tumorigenesis. Genetic control of tumor

susceptibility in *Xiphophorus* has been discovered both in pure strains and in particular interspecific hybrids for a variety of spontaneous and induced neoplasias, including at least eight types of malignant melanomas and a long list of less well-studied tumors such as neuroblastomas, renal tumors, thyroid tumors, fibrosarcomas, and rhabdomyosarcomas (Walter and Kazianis, 2001, Kazianis et al., 2001a,b, Anders et al., 1991, Anders, 1991; Schwab et al., 1978, 1979; Setlow et al., 1989). A list of *Xiphophorus* crosses leading to tumor susceptibility is presented in Table 1.

SERVICES OF THE STOCK CENTER

The *Xiphophorus* Genetic Stock Center provides fish to scientists and aquarists around the world (Table 1). Scientists in more than 30 laboratories in the United States and eight other countries have received animals from the Center. In addition to supplying animals and consultation on husbandry and genetic questions, the XGSC produces custom interspecies hybrids for a variety of research projects. Researchers anticipating use of *Xiphophorus* lines of hybrids are encouraged to contact the XGSC manager at the earliest stages of planning to ensure scale up of breeding regimen for needed fish production. The Stock Center will gladly assist in preparing contracts for fish supply and will provide needed support letters for grant/contract proposals.

Facilities and Operation

The XGSC occupies about 3,500 sq. ft. on the fourth floor of Centennial Hall on the Texas State University campus. This facility was newly rennovated and occupied in 2000. The Center maintains ≈ 1050 5-gallon and 150 ten to 50-gallon aquaria for stock perpetuation. An additional 200 aquaria are dedicated for research project utilization. While this number may seem large, tank space is premium and each new genetic strain or research project necessitates some redesign of the overall Stock Center management plan.

Quality control at the Stock Center must be impeccable to maintain integrity of the large numbers of genetic strains. Each strain possesses a unique phenotype or biochemical genetic "signature" which has been checked for assurance of stock purity. Individual broods are never mixed, and males and females are separated prior to sexual maturity. Parents of broods used in stock perpetuation are preserved in ethanol for reference in the event of later questions.

Figure 2: Standard photo (left) and fish eye view (right) of aquaria racks in the current XGSC.

The XGSC staff routinely collect data on brood size, sex ratios, and phenotypic scoring for each fish line. Records of these data can be traced back to the collection date for some stocks. Current XGSC efforts involve computer entry, search engine sorting and retrieval for the large database of *Xiphophorus* stock information some of which is continuous and dates back over 50 years.

Stock Management:

Management of each *Xiphophorus* stock begins with assignment of a pedigree number to a brood born to a pedigreed male and a pedigreed female. All *Xiphophorus* are livebearing fish and brood sizes typically range from 5-35 individuals. Broods are generally maintained for a week or more in the mating tank where they were born after both parents have been removed to a separate aquarium. If the brood size is deemed insufficient to maintain Center or research project needs, the parental male and female are simply moved into another mating tank to continue reproduction. Generally, producing females will drop new broods about every 30 days. When production levels are met, parental animals are sacrificed and flash frozen for storage at -80^{o}C, or stored in ethanol in the event of future questions regarding the pedigree.

Broods are assigned a sexing date no more than two months from the date of birth. Generally, if the fish are kept at a density of one individual per gallon, two months is the optimal time (for platyfish) to separate males from females based on the developing male gonopodium. At this time, males and females are placed in separate aquaria. The stock perpetuation cycle is completed when a sexually mature pedigreed male and pedigreed female are placed into a mating

tank to produce the next generation. In platyfishes, the cycle from birth to sexual maturity may take from 3-6 months, whereas in swordtails, this cycle may take a year or more.

With the exception of crosses between *X. helleri* and *X. maculatus* most interspecies hybrid crosses do not require artificial insemination. However, when artificial insemination is required, it is relatively simple requiring only sedation of the female followed by injection of male sperm into the female urogenital sinus by means of a sterile plastic micropipet (Clarke, 1950).

Some *Xiphophorus* stocks (e.g., *X. couchianus*) represent species now suspected to be extinct or on the verge of extinction in the wild. Habitat destruction throughout the range of *Xiphophorus* (Mexico and Central America) may eventually lead to the demise of many of the 26 presently recognized species. Thus, the *Xiphophorus* Genetic Stock Center also serves as a worldwide repository for treasured genetic material.

Stocks Available

The Stock Center currently houses 24 of the 26 identified species of platyfish and swordtails in the genus *Xiphophorus*, and 61 genetic lines representing these species. At the time of this printing, the two *X. maculatus* strains, Jp 163 A and Jp 163 B, are in their 102nd and 96th generations of inbreeding, respectively. *X. couchianus* is in its 72nd generation of inbreeding. The 'oldest' strain in this collection is the *X. helleri* 'Cd' line which was collected in the 1930's; however due to a much longer generation times for swordtails, this line is approximately in its 56th generation of inbreeding.

Web Page Information

A very informative *Xiphophorus* web page can be found by searching for www.Xiphophorus.org.

This web page includes links to other fish resource centers and *Xiphophorus* research investigators world wide. In addition, a searchable literature bibliography containing >2,000 *Xiphophorus* publications is provided and freely accessible. The web page also contains digital images of many *Xiphophorus* species, images of several hybrid cross diagrams, on-line status of the *Xiphophorus* gene map, and an educational location providing information about *Xiphophorus* research projects.

Contact Information

Ms. Leona Hazlewood serves as XGSC, Operational Manager and is the point of contact for most XGSC questions and for setting up fish needs and shipments [lh24@txstate.edu]. In additon, Ms. Markita Savage serves as XGSC technician and is familar with species and lines [ms39@txstate.edu]. The main XGSC contact phone number is (512) 245-8469 and facsimile number is (512) 245-1922. Dr. Ronald B. Walter provides XGSC oversight and may be contacted for information regarding the XGSC operation [RWalter@txstate.edu] (512) 245-0357.

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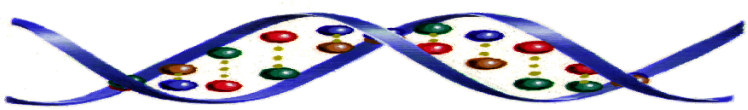


Xiphophorus Species and Stocks

Xiphophorus maculatus

General Introduction:

This poeciliid fish is native to southern Mexico and adjacent parts of Central America. Representatives of this species were first found in museum records in 1866, in collections made by Gunther in Central America. Additional specimens appeared in collections in the Smithsonian Museum in 1867 and the Chicago Natural History Museum in 1902. During the first half of the 20th century, several collectors sampled several thousand specimens throughout the species' distribution. From these early collections, it is apparent that members of this species display a wide array of color variations and pattern markings (Kallman, 1968).

Based on the complement of sex-chromosomes, three types of females and two types of males are seen in this species. Females may be XX, WX, WY, while males can be XY or YY (Kallman, 1968). The Stock Center currently houses 14 stocks of *Xiphophorus maculatus*. Differences among stocks include collection site and history, pigment patterns, and P-alleles (genes that regulate hormones affecting sexual differentiation, i.e., the age of maturation).

Xiphophorus maculatus, Jp 163 A

Female

Male

Strain code: Jp 163 A

Phenotypes scored: Dorsal red (Dr), spotted dorsal (Sd), anal red (Ar)
Striped side (Sr)
Shoulder spot (ss), and dot (D).

Introduction:

Ancestors of this stock were collected from the Rio Jamapa, Veracruz, Mexico in 1939 by Dr. James W. Atz. This line was established in the early Stock Center in New York with offspring from a single female from that collection. After nine generations, this stock was split into two lines which have been maintained separately by inbreeding since that time (Kallman, 1968). These two stocks are designated Jp 163 A and Jp 163 B. The Jp 163 A stock at the Stock Center is currently in the 101st generation of inbreeding. This stock is also used in the production of four (or more) hybrids.

Six phenotypic traits are maintained in this stock, four sex-linked traits and two autosomal traits (Gordon, 1953; Kallman, 1970). The X-chromosome in this stock carries two linked pigment pattern genes, one that produces a red dorsal fin (Dr) and a second that encodes the expression of macromelanophores in the dorsal fin (Sd). Two additional pattern genes are linked on the Y-chromosome, stripe-sided (Sr) and a gene that produces a red anal fin (Ar). The shoulder spot (ss) develops on the anterior, dorsal part of the flank. The shoulder spot trait is determined by two gene loci (let's call them arbitrarily m and n), one sex linked and the other one autosomal. The trait is expressed when the fish is homozygous recessive at both loci. In Jp 163 A and B the genotype can be formally expressed as follows:

female X-m X-m nn male X-m Y-M nn

Because Jp163 is homozygous recessive for n, one cannot demonstrate its presence (but Belize fish introduced the N allele and the N locus could be demonstrated). This strain is also homozygous for dot (D), a spot displayed on the tail.

Two P-alleles exist in this stock. P-1, the earliest p-gene documented in *X. maculatus*, is linked to the X-chromosome. P-2, the second earliest, is linked to the Y-chromosome.

Sex determination / sexing:

Sex determination in this stock is XX / XY (Gordon, 1954). Fish are sexed at 1 to 1.5 months of age and become sexually mature at about 4 months of age.

Scoring:

Fish are scored for the traits: dorsal-red, spotted-dorsal, stripe-sided, anal-red, shoulder spot, and dot. In this stock, the shoulder spot is expressed in females only, but a gene linked to Sr suppresses the expression of shoulder spot in males.

Maintenance:

This stock is maintained with reciprocal crosses between the different lines. The offspring of each mating are assigned a pedigree letter based on order of production among all crosses established within that generation. For example, the first mating to produce a brood in the 91st generation is assigned the pedigree A, along with the generation number [e.g., Jp 163 A^{91}(A)], the second mating to produce a brood is assigned the pedigree B [e.g., Jp 163 A^{91}(B)], and so on. The number of matings set up each generation depends on the demand for this stock, e.g., six or more matings. A sufficient number of matings must be set up to produce extra fish for line maintenance and other stocks, such as YSp, SpSr, JpYIrBr, JpYIr, JpYBr, and hybrids.

Stock source:

Prof. Klaus Kallman, the New York Aquarium, 9/10/92.

Xiphophorus maculatus, Jp 163 B

Female

Male

Strain code: Jp 163 B

Phenotypes scored: Spotted side (Sp), anal red (Ar)
Striped side (Sr)
Shoulder spot (ss), dot (D).

Introduction:

The Stock Center at San Marcos received Jp 163 B fish from the New York Aquarium in February 1993. The fish originally shipped were from the 76th and 77th inbred generations. Currently, the stock housed at the XGSC is in the 94th generation of inbreeding.

This stock of *Xiphophorus maculatus* is maintained for one X-linked gene, spotted side (Sp), and two Y-linked genes, anal red (Ar) and striped side (Sr). In addition, two autosomally inherited traits, one for shoulder spot (ss) and one for dot (D), are maintained in this stock (Kallman and Atz, 1966). The shoulder spot is expressed only in females, as a gene linked to Sr inhibits the expression of shoulder spot in males. These traits are fixed in this captive stock.

Two P-alleles exist in this stock, the same as with Jp 163 A. P-1, the earliest p-gene documented in *X. maculatus*, is linked to the X-chromosome. P-2, the second earliest, is linked to the Y-chromosome.

Sex determination / sexing:

The chromosomal sex determination in this stock is XX / XY. Fish are sexed at 1.5 to 2 months of age and reach sexual maturity at about 5 months of age.

Scoring:

Jamapa Jp 163 B fish are scored for three sex-linked traits at about 4 months of age, i.e., when sexual maturity is reached, spotted side (Sp), striped side (Sr), and anal red (Ar). Fish should also be scored for 2 autosomally inherited color patterns, ss and D.

Maintenance:

Six or more matings are initially set up to produce each new generation, depending on the demand for this stock. Previous generation matings are maintained until broods are produced by the following generation. The younger offspring of the previous generation are also maintained as backups in case the subsequent generation fails to produce sufficient offspring to maintain the line. Once the successive generation is producing sufficient numbers of offspring, the previous generation matings can be fixed. The fish from the previous generation matings are generally retained for research or used in other matings for strain production. For example, extra fish should be kept for maintaining such stocks as YSdSr, YSdDr, XSrAr, Nigra, and hybrid production.

The first mating to produce a brood in each generation is assigned a pedigree of A, the second mating to produce a brood is designated by pedigree B, and the third mating to produce is designated C. This pedigree assignment system continues for all matings producing within a single generation. For example, the first mating to produce a brood in the 58^{th} generation for Jp 163 B will have the pedigree number of Jp 163 B^{58}(A), the second will have the pedigree number of Jp 163 B^{58}(B), and so on.

The matings for the each generation are reciprocal crosses between the A, B, and C lines. For example, in setting up matings to produce the 59^{th} generation, a female of pedigree Jp 163 B^{58}(A) is mated to a male of pedigree Jp 163 B^{58}(B) and vice versa; a female of the Jp 163 B^{58}(C) pedigree is mated to a male of the Jp 163 B^{58}(A) pedigree, along with the reciprocal cross. This scheme is followed until the desired number of matings is established.

Stock source:

Prof. Klaus. Kallman, the New York Aquarium, 1993.

Xiphophorus maculatus, Belize Platy II

Female(++)

Male (blue, M, pgs)

Strain Code: BpII

Phenotypes scored: Body colors, Body red (Br), Spotted Dorsal (Sd), iridescent blue (blue), wild type body (+)
Tail-spot patterns, moon (M), wild type (+)
Other patterns, shoulder spot (ss), pseudo-gravidity spot (pgs).

Introduction:

About 60 *Xiphophorus maculatus* BpII arrived at the Stock Center on August 13, 2003 from Dr. Harry Grier. The fish were originally collected at Kate's Lagoon in Belize under a collecting permit issued to Craig Watson. A handful of the many varying color patterns represented in the population were chosen to perpetuate the stock. The four main body colorations include Body red (Br), Spotted Dorsal (Sd), iridescent blue (blue), and wild type body (+). Segregation of these color patterns indicates that Br, Sd and + are linked to Y chromosomes in a WY/YY sex determining mechanism. The segregation of the blue phenotype and the Moon (M) tail-spot pattern indicates that these two patterns are inherited autosomally.

Sex determination / sexing:

The chromosomal mechanism for sex determination in this stock is WY / YY. Sex should be determined early in these fish, at about one month of age, because early maturation rates may exist in this stock. However, because the later maturing alleles may also exist in this stock, all fish should be closely observed for any late developing males. Fish are generally mature and ready to mate at about 4 to 5 months of age.

Scoring:

Fish are scored with the unaided eye for Br, Sd, blue, +, and M. Also, presence or absence of shoulder spot (ss) and pseudo-gravidity spot (pgs) is also noted, although the inheritance of these patterns has not yet been worked out. All mature fish are measured upon fixing or discarding to collect data on maturation rates.

Maintenance:

Currently, this stock is maintained by setting up two crosses each for the following color patterns; Br, Sd and blue; in which each mating has one fish with the given color and one wild-

type fish. Moon is maintained within these matings in a heterozygous state by mating one fish expressing M to one that is not. The mating plan will look as follows;

Two matings to maintain Br:
Br, M, ss female (x) +, pgs male

+, M female (x) Br, + male

Two matings to maintain Sd:
+, ss female (x) Sd, M, pgs male

Sd, M female (x) +, ss, pgs male

Two matings to maintain blue:
Blue, M, ss female (x) +, pgs male

+ female (x) blue, M male

Note that ss and pgs may not be in all of the matings, but these patterns should be scored and recorded.

Stock source:
Dr. Harry Grier, Florida, received 8/13/03.

Xiphophorus maculatus, Coatzacoalcos Platy

Female (Sp-9)

Male (Sp-9, ArP-6)

Strain Code: Cp

Phenotypes scored: Anal fin color, anal red (Ar)
Spotting pattern #9 (Sp9)
Wild type body color (+)
Tail spot pattern, complete crescent (Cc)
Age of maturation, early (P-1), late (P-6).

Introduction:

Cp is a strain of *Xiphophorus maculatus* collected from the drainage basin in Coatzacoalcos Veracruz, off highway 185 in 1970. Three distinct Y-chromosomes are maintained in this stock (Kallman and Borkoski, 1978; Bao and Kallman, 1982). One Y-chromosome carries an allele for a specific spotting pattern, Sp9 (Y^{Sp9}), which results in a concentration of black pigment spots on the operculum and along the midline region of the body. This phenotype is limited to platyfish from this drainage. The Sp9 fish are small in size, as this spotting allele is linked to a P-allele (P-1), that results in early maturation and thus, small size. A second Y-chromosome carries an allele for red anal fin, Ar. In females, this pattern is faintly expressed at 6 to 9 months of age as sparsely arranged red spots on the proximal portion of the anal fin rays. This phenotype must be scored under a microscope at 10x magnification. The gonopodium appears yellowish in males carrying an Ar allele. Males must also be scored under 10X magnification for Ar. A P-allele for late maturation, P-6, is linked to the Ar allele (Y^{ArP-6}). Fish carrying this phenotype should be larger in size. The third Y-chromosome carries only the wild type allele for color, i.e., no pigment pattern (Y+). The Cp stock is fixed for the tail-spotting pattern, complete crescent (Cc).

Sex determination / sexing:

The sex determination mechanism in this stock is WY / YY. Immature fish should be identified as to sex at 6-8 weeks of age, because the fish with the P-1 allele (linked to Sp9) mature early. However, because the later maturing P-6 allele is carried on the Y^{ArP-6}

chromosome, all fish should be closely observed for any late developing males. Fish are generally mature and ready to mate at about 4 to 5 months of age.

Scoring:

Fish are scored with a dissecting microscope, particularly when scoring Ar. Ar females and males can be scored using at least 10x magnification and display single orange-red pigment cells along the proximal portion of the anal fin rays. Sp9 and Cc patterns are generally scored using the unaided eye.

Maintenance:

This stock is maintained by establishing reciprocal crosses between parallel lines. Each mating should include all three Y-chromosomes (including Y^{+}), but any particular Y-chromosome should be represented in a mating only once. Matings that result in both WY^{+} females and $WY^{ArP\text{-}6}$ females should be avoided when possible, as it is most difficult to distinguish between these two phenotypes. It is a good rule to score this stock when mating the fish and also rescore the fish when the matings are being fixed or discarded, if not earlier.

Stock source:

Prof. Klaus Kallman, the New York Aquarium, received 2/28/93.

Xiphophorus maculatus, JpY^{IrBr}

Male

Strain code: JpYIrBr

Phenotypes scored: Pigment patterns, Eye color, iris red (Ir)
Body color, body-red (Br)
Spotted dorsal (Sd), dorsal red (Dr)
Shoulder spot (ss) and dot (D).

Introduction:

This stock has been maintained as a stock separate from the other *Xiphophorus maculatus* since a crossover event occurred in the Belize platyfish. The crossover resulted in the linkage of the genes for iris red (Ir) and body red (Br) on the Y chromosome and consequently, males of this stock show both patterns. In addition, the ventral margin of the caudal fin is often black in males. This stock also exhibits dot (D), shoulder spot (ss), spotted dorsal (Sd) and dorsal red (Dr), all characters derived from *X. maculatus* Jp 163 A.

The X-chromosome of this stock carries the early maturing P-1 factor. Preliminary data also indicates that after the Br gene crossed over, the late maturing P-4 factor remains linked.

Sex determination / sexing:

The chromosomal sex determination in JpYIrBr is XX / XY. The fish are sexed at 2 to 3 months of age and males mature quite late at about 6 or 7 months of age.

Scoring:

Fish in this stock are scored for the traits Sd, Dr, Ir, Br, D, and ss. Since the genes for IrBr are Y-linked, females do not inherit these traits and are scored and subsequently discarded. Expression of Sd and ss may vary in males. An occasional female expressing IrBr has been noted and it is possible that these are XY females. These females are also scored and discarded.

Maintenance:

This stock is maintained by breeding JpYIrBr males with the genotype X^{SdDr} Y^{IrBr} ss D to Jp 163 A females. The Jp 163 A females have the genotype X^{SdDr} X^{SdDr} ss D. The mating scheme for maintaining this stock is:

X^{SdDr} X^{SdDr} ss D (x) X^{SdDr} Y^{IrBr} ss D
Jp 163 A JpYIrBr

At least 2 matings are set up each generation to ensure production of one new pedigree in that generation. All male progeny should possess X^{SdDr} and Y^{IrBr} and be used in matings to produce the successive generation.

Stock source:

Prof. Klaus Kallman, the New York Aquarium, 5/12/93.

Xiphophorus maculatus, Nigra

Female

Male

Strain code: Nigra

Phenotypes scored: Pigment pattern, nigra (N), striped side, (Sr)
Orange caudal peduncle (Cpo), spotted side (Sp)
Anal fin color, anal red (Ar)
Shoulder spot (ss)
Tail spot pattern, dot (D).

Introduction:

Nigra is a strain of *Xiphophorus maculatus* that carries alleles at two X-linked genes that give rise to the nigra pigment pattern (N = big black spots) and orange caudal peduncle (Cpo). This chromosome is designated X^{NCpo}. The gene encoding the Nigra pattern is linked to a maturation allele, P-5, which results in the "latest" maturation documented in *X. maculatus*. Therefore, this strain has very late maturing, large fish (Kallman and Borkoski, 1978). This X chromosome, X^{NCpo}, originated from a collection of *X. maculatus* from Belize. The X chromosome was crossed into Jp stock in order to determine maturation rates and inheritance.

Sex determination / sexing:

The chromosomal sex determination mechanism is XX / XY. Although the Nigra fish are late-maturing, they still must be sexed at about 2 months of age. This is due to early maturing segregants that are not required to maintain the stock. Males and females are separated after sexing and the results are checked after 2 to 4 weeks.

Scoring:

The Cpo pattern can be scored at sexing, at around 2 months of age. This allows for early disposal of the segregants. Nigra spots may easily visible and can appear as large, black, randomly arranged spots. However, expression of the nigra pattern does not exhibit complete penetrance and occasionally a fish carrying the nigra gene may not display any spots. The

orange caudal peduncle gives the appearance of an orange wash of color over the caudal peduncle. Fish without the NCpo are discarded.

Maintenance:

The stock is maintained by mating Nigra females to Jp 163 B males thus producing both male and female Nigra progeny. This is done because Nigra females tend to mature earlier than the males. To ensure a sufficient number of Nigra fish are produced, four matings are set up for each generation. The P_1 mating scheme is:

$X^{NCpo}X^{Sp}$ ss D	x	$X^{Sp}Y^{SrAr}$ D
(Nigra)		(Jp 163 B)

Four genotypes are expected in the F_1 progeny:

Females:	$X^{NCpo}X^{Sp}$ ss D, $X^{Sp}X^{Sp}$ ss D,
Males:	$X^{NCpo}Y^{SrAr}$ D, $X^{Sp}Y^{SrAr}$ D.

Once two of the four original P_1 matings have produced three broods each, the other two P_1 matings are either fixed (if they produced offspring), or discarded (if no offspring were produced).

When the NCpo female offspring reach sexual maturity (about 5 months of age), the next generation of matings is set up. These matings (P_2) are constructed as described above, a female carrying the Nigra allele ($X^{NCpo}X^{Sp}$ ss D, showing nigra spots and orange caudal peduncle) is mated to a Jp 163 B male. Sufficient back-up fish should be maintained until the P_2 matings have produced a sufficient number of offspring. Then any original P_1 matings, as well as any back-up fish, can be fixed.

If only male fish carrying the Nigra allele are available, these can be mated to Jp 163 B females. Only female Nigra progeny will be obtained from this mating and these are then used to establish the original mating scheme. Male offspring can be discarded.

Stock source:

Prof. Klaus Kallman, the New York Aquarium, 11/20/92 and 5/12/93.

Xiphophorus maculatus, Papaloapan Platy

Female (DrSp Iy, OT)

Male (Iy ArSp, TT)

Strain code: Pp

Phenotypes scored: Dorsal fin color, dorsal red (Dr)
Spotted side (Sp)
Eye color, iris yellow (Iy)
Red spot over the anal fin (Arsp)
Tail spot patterns, one-spot (O) and twin-spot (T).

Introduction:

This strain originated from the Rio Papaloapan drainage ditch along highway 145, 14 Km south of LaTinaja, Veracruz, Mexico, collected by Dr. Klaus Kallman in 1970. A Y-linked gene causes the expression of a red spot over the anal fin (Arsp) in adult males; this is also known as ruby throat. Genes encoding additional pigment patterns mark two X-chromosomes segregating in this population. One X-chromosome carries an allele at a gene that encodes a yellow iris (Iy). The other X-chromosome carries two linked pigment genes, one affecting dorsal fin color, dorsal red (Dr), and the other spotting on the flank (Sp). This stock also has two alleles segregating at a gene for an autosomal tail-spot pattern, the allelic designation reflecting the number of spots expressed, one spot (O) and twin spot (T).

Sex determination / sexing:

Chromosomal sex determination is XX / XY. Fish are sexed at 1.5 to 2 months of age and should be monitored closely for about a month after sexing to confirm results. They become sexually mature at about 4 months of age.

Scoring:

The above phenotypes are scored when the fish reach maturity, at about 4 months of age. This approximately coincides with the timing to set up matings. All phenotypic color patterns in younger fish should always be scored using a dissecting scope.

Maintenance:

This stock is maintained with reciprocal crosses between fish of two different pedigrees. Ideally, two to three matings are set up each generation to maintain the stock. All three X-chromosomes should be present in each mating. The mating scheme currently followed at the Stock Center involves a spotted, iris yellow female with a non-spotted, ruby throat, iris yellow male:

$X^{Iy}X^{DrSp}$ OT (x) $X^{Iy}Y^{Arsp}$ TT

Or

$X^{Iy}X^{DrSp}$ TT (x) $X^{Iy}Y^{Arsp}$ OT

In each mating, one parent is heterozygous for one-spot (OT) and homozygous for twin-spot (TT). Segregation of these alleles produces 1/2 TT and 1/2 OT offspring. The female offspring will be either $X^{Iy}X^{DrSp}$ or $X^{Iy}X^{Iy}$. Only the spotted females (which are also iris-yellow) are used in subsequent matings. The male offspring will be either $X^{DrSp}Y^{Arsp}$ or $X^{Iy}Y^{Arsp}$. Only non-spotted, iris-yellow males are used in matings. The iris of fish with the Iy genotype turns red in older males.

Stock source:

Prof. Klaus Kallman, the New York Aquarium, 4/15/93.

Xiphophorus maculatus, Sex Reversal

Female

Male

Strain code: SR

Phenotypes scored: Anal red (Ar)
Shoulder spot (ss), dot (D).

Introduction:

Both males and females in this stock are homogametic, i.e., the sex chromosomes in both sexes are YY. The Y-chromosome of this stock, which carries the gene for anal red (Ar) and shoulder spot, was derived from Belize platyfish (Bp) and introgressed into the Jp 163 stock. The introduction of the Bp Ar gene into the Jamapa genetic background resulted in enhanced expression of Ar. The fish, particularly males, may show expression levels of Ar ranging from the appearance of a red dorsal fin to an entirely red fish. The Ar allele on the Y chromosome is linked to an unidentified P allele, which appears to delay maturation even later than the P-3 allele (about intermediate).

Sex determination / sexing:

Both males and females have the sex chromosomes YY. These fish can be sexed at about 2 months of age. Fish are carefully watched for sexual development for 1 or 2 months to ensure there are no males with the females. Sex ratios are sometimes biased; therefore, sufficient numbers of offspring must be raised to insure both sexes are adequately represented. Fish mature at about 4 to 6 months of age.

Scoring:

SR fish are scored for Ar, the tail spot pattern, dot (D), and shoulder spot (ss). The tail spot trait is derived from the Jamapa fish.

Stock maintenance:

These fish are homozygous for all scoreable traits. Three to four matings are set up for each generation, i.e., reciprocal crosses between parallel lines. One or two females can be used in each mating. If only one or two pedigrees are available, the fish can be inbred. This stock is

known for biased sex ratios. Always retain at least one tank each of females and males from the previous generation until males and females of the current generation have been identified.

Stock source:

Prof. Klaus Kallman, the New York Aquarium, 4/15/93.

Xiphophorus maculatus, SpSr

Female

Male

Strain code: SpSr

Phenotypes scored: Striped side (Sr), spotted side (Sp)
Dorsal red (Dr)
Anal red (Ar)
Spotted dorsal (Sd)
Shoulder spot (ss) and dot (D).

Introduction:

The Jp 163 B stock of *Xiphophorus maculatus* maintained at the New York Aquarium experienced a number of crossover events in the late 1980's as a result of which two genes, spotted side (Sp) and striped side (Sr), became linked on the X-chromosome.

Sex determination / sexing:

The chromosomal sex-determination mechanism is XX / XY. SpSr fish are sexed at 8 weeks of age. Fish reach sexual maturity at about 4 months of age.

Maintenance:

The SpSr stock is maintained by crossing either a male or female SpSr fish with a Jp 163 A fish of the opposite sex. If a SpSr female is crossed with a Jp 163 A male, then some of the offspring with the SpSr trait will be both male and female. With either direction of the mating, one-half of the progeny are expected to be of the SpSr phenotype. Fish are scored for phenotype (SpSr, etc.) and any possible crossovers. Fish not displaying the SpSr phenotype are discarded. This can be done prior to sexing when the fish are 2 to 3 weeks old. As in the Jp 163 stocks, females of this stock also display shoulder spot (ss) which is suppressed by a gene linked to Sr in males. Fish are also scored for the tail spot pattern dot (D).

A minimum of two matings are set up as soon as mature SpSr fish and Jp 163 A fish are available to produce the next generation:

X-SpSr X-SdDr ss D (x) X-SdDr Y-SrAr D
(SpSr) (Jp163A)

or

X-SdDr X-SdDr ss D (x) X-SpSr Y-SrAr D .
(Jp 163 A) (SpSr)

Stock source:

The Stock Center received both males and females with this crossover chromosome in September, 1992. Two additional mating pairs were received in May, 1993.

Xiphophorus maculatus, Usumacinta Platy

Female

Male

Strain code: Up-2

Phenotypes scored: Pigment patterns, spotting pattern # 4 (Sp-4)
Iris yellow (Iy).

Introduction:

The Up-2 stock was collected in 1963 from the Rio de la Pasion (Rio Usumacinta system), Alta Verapaz, Guatemala. The sex chromosomes, W and Y, segregate in this stock; the W chromosome carries no color pattern alleles. The Y-chromosomes carry two different pigment genes, for iris yellow (Iy) or the macromelanophore spotting pattern #4 (Sp-4). Both color patterns are displayed in males and females. No tail patterns occur in this stock.

Females have been documented to display more than one phenotypic color pattern and dissection revealed that these females contained undifferentiated gonads. This indicates that the females were YY with some secondary sex determining mechanism in effect.

Sex determination / sexing:

Sex determination in this stock is WY / YY. Up-2 broods are sexed at about 2 months of age.

Scoring:

When fish are mature they are scored for Iy and Sp-4.

Maintenance:

The Up-2 stock is maintained through reciprocal crosses among lines, ensuring that the 2 Y chromosomes are present in each mating. The typical mating scheme may resemble the following:

WY^{Sp-4} (x) $Y^{Iy}Y^{Sp-4}$
or
WY^{Iy} (x) $Y^{Iy}Y^{Sp-4}$
or
WY^{Sp4}(x) $Y^{Iy}Y^{Iy}$.

This scheme ensures that the two Y-chromosomes are maintained in the following generations.

Stock source:

Prof. Klaus Kallman, the New York Aquarium, 2/18/93.

Xiphophorus maculatus, Wild Jamapa Platy

Female

Male

Strain code: JpWild

Phenotypes scored: Tail pattern, twin spot (T), and crescent (C).

Introduction:

This stock of *Xiphophorus maculatus* also originated from the Rio Jamapa drainage, Veracruz, Mexico, and has been under cultivation since 1971. This stock shows two phenotypes for a tail spot pigment pattern, twin spot (T), and crescent (C). Both of these phenotypes can vary in expression and may be difficult to score. The gene(s) encoding these is presumed to be autosomal, but the inheritance of this pattern remains uncertain. This fish also has blue-green iridescence in the shoulder region. No P-genes have been identified in this stock.

Sexing:

Fish are sexed at 1.5 to 2 months of age. JpWild fish reach sexual maturity at about four months of age.

Scoring:

Fish are scored for the tail spot patterns T and C once they are sexually mature.

Stock maintenance:

This stock can be maintained by reciprocal crosses between at least 2 pedigrees. The primary objective in setting up matings is to include both tail spot patterns in the mating. This can be accomplished by several different crosses such as a TC fish mated to a TC fish, or a T fish mated to a C fish, as long as both patterns are present in each mating.

Stock source:

The stock currently maintained at the Stock Center was obtained from Klaus Kallman in 1993, with a shipment containing one pedigree of fifteen females and twelve males and two matings. One mating was a female homozygous for C with a male homozygous for T and the second mating was the reverse of these parental genotypes.

Xiphophorus maculatus, XSrAr

Female

Male

Strain code: XSrAr

Phenotypes scored: Striped side (Sr)
Spotted side (Sp)
Anal red (Ar)
Shoulder spot (ss) and dot (D).

Introduction:

The Jp 163 A stock of *Xiphophorus maculatus* maintained at the XGSC in San Marcos produced a crossover female in 1999 who expressed the male phenotype of striped side and anal red (SrAr). This female was mated to create a new strain with the SrAr genes linked to the X-chromosome. This stock is now perpetuated by crossing to the Jp 163 B stock.

Sex determination / sexing:

The chromosomal sex-determination mechanism is XX / XY. XSrAr fish are sexed at 8 weeks of age. Fish reach sexual maturity at about 4 months of age.

Scoring:

Both males and females in this stock are all scored for Sr, Ar, Sp, ss and D.

Maintenance:

The XSrAr stock is maintained by crossing either a male or female XSrAr fish with a Jp 163 B fish of the opposite sex. If an XSrAr female is crossed with a Jp 163 B male, then the resulting offspring expressing the XSrAr trait will be both male and female. If a male carrying the X-linked SrAr pattern is mated to a Jp 163 B female, then all of the female offspring will carry the XSrAr pattern while no males will.

A minimum of two matings are set up as soon as mature XSrAr fish and Jp 163 B fish are available to maintain the next generation:

X-SrAr X-Sp ss D (XSrAr) (x) X-Sp Y-SrAr D (Jp 163 B)

or

X-Sp X-Sp ss D (Jp 163 B) (x) X-SrAr Y-SrAr D . (XSrAr)

One half of the resulting progeny will be segregants that are not required to maintain the stock. These animals can be discarded upon scoring.

Stock source:

XGSC, Texas State University – San Marcos, 10/19/99.

Xiphophorus maculatus, YSdDr

Male

Strain code: YSdDr

Phenotypes scored
Pigment pattern, spotted dorsal (Sd)
Dorsal red (Dr)
Spotted side (Sp)
Shoulder spot (ss), dot (D).

Introduction:

This stock is a product of a crossover event in Jp 163 A which resulted in linkage of the spotted dorsal (Sd), the dorsal red (Dr), and the shoulder spot factors to the Y chromosome. This line is maintained by mating a YSdDr male with a Jp 163 B female; therefore, these males also inherit the X linked gene for spotting, X^{Sp}.

Sex determination / sexing:

Chromosomal sex determination is XX / XY. The fish are sexed at 1.5 to 2 months of age. At this time, the females can be discarded.

Scoring:

This stock cannot be scored for all the above patterns at the time of sexing because they do not develop until the fish are older. Only Sp may be apparent. Nevertheless, females are scored and then discarded because they are not needed for further crosses. At maturity the fish are scored for Sp, Sd, Dr, ss, and dot.

Stock maintenance:

Two to three matings are set up to propagate each generation; however, the male offspring of only one or two new pedigrees are required for the successive generation's matings to Jp 163 B females. The mating scheme and expected offspring are:

$X^{Sp}\ X^{Sp}$ ss D (x) $X^{Sp}\ Y^{SdDr}$ ss D (P_1)
(Jp 163 B) (YSdDr male)

$X^{Sp}\ X^{Sp}$ ss D (females), $X^{Sp}\ Y^{SdDr}$ ss D (males) (F_1)

The progeny are scored for possible crossovers in males and crossovers in the females cannot be detected until they are raised to maturity and the patterns have developed.

Stock source:

Prof. Klaus Kallman, the New York Aquarium, 9/10/92.

Xiphophorus maculatus, YSdSr

Male

Strain code: YSdSr

Phenotypes scored: Pigment pattern, spotted side (Sp)
Striped side (Sr)
Spotted dorsal (Sd)
Shoulder spot (ss), dot (D).

Introduction:

This stock of *Xiphophorus maculatus* arose from a crossover in Jp 163 A, resulting in spotted dorsal (Sd) becoming linked to striped side (Sr) on the Y-chromosome. Mating the YSdSr males with Jp 163 B females allows for maintenance and easy detection of cross-overs.

Sex determination / sexing:

Sex determination is XX / XY. These fish can be sexed when they are about 8 weeks old. Females can be discarded at this time because they are not required to maintain the stock.

Scoring:

The males are scored after they have attained sexual maturity. Sd may not be expressed until a fish is mature. The fish are also scored at this time for spotted side (Sp), Sd, Sr, shoulder spot (ss) and dot (D). Some males display a red dot in their mid-upper flank, almost like ss, but located in a slightly more posterior position. Presence of this spot should be noted.

Maintenance:

This stock is maintained by crossing the males to Jp 163 B females. Two or three matings are set up for each generation. Offspring of only one or two matings are needed for the next generation as they are out-crossed. Fish are set up in matings as soon as they mature and have been scored. If there is no production in about 3 months, artificial insemination should be performed. Following is a diagram of the mating scheme:

$X^{Sp}X^{Sp}$ ss D (x) $X^{Sp}Y^{SdSr}$ D (P_1)
Jp 163 B female YSdSr male

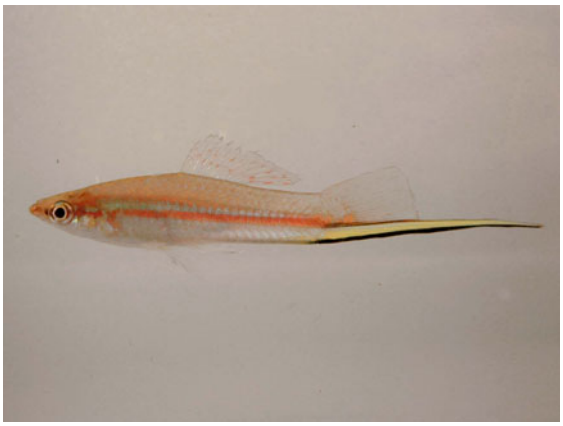
$X^{Sp}X^{Sp}$ ss D , $X^{Sp}Y^{SdSr}$ D (F_1)
Jp 163 B female YSdSr male.

Stock source:

Prof. Klaus Kallman, the New York Aquarium, 10/21/92.

Xiphophorus helleri, Sarabia

Female



Male

Strain code: Sara-Or
Sara-Gr

Phenotypes scored: Sword color, orange (Or) and green (Gr)

Introduction:

The Sarabia strain of *Xiphophorus helleri* was collected in 1963 from the Rio Sarabia near Oaxaca, Mexico (Kallman, 1975). A subset of this stock was given to Drs. Siciliano and Morizot in Texas and Dr. Vielkind in Vancouver. The stock was shipped to the Stock Center in early 1993. This strain is marked by a bright red stripe, extending from the eye to the caudal peduncle and one to two horizontal rows of red spots on the dorsal fin. In the female, yellow spots are located on the ventral portion of the caudal fin and yellow cells also appear in the anal and pectoral fins. This stock is polymorphic for sword color. Two alleles, orange and green, segregate in the stock and the green allele is recessive to orange. The gene for sword color is Y-linked (Kazianis, 2005).

Sex determination / sexing:

The chromosomal sex determination for this stock is WY / YY. Fish are sexed at 2 to 3 months of age and mature around 6 months.

Scoring:

Expression for sword color is observed only in males from this strain and a male will express either an orange or green colored sword. As the green allele is recessive, the green phenotype is seen only in males homozygous for this allele.

Stock maintenance:

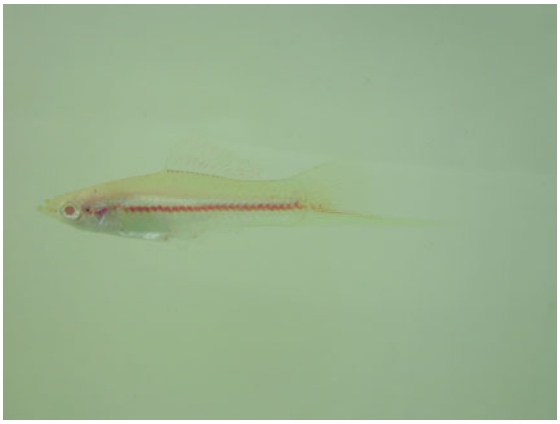
Two strains are maintained for management of Sarabia. One strain is used to preserve the homozygous green phenotype (Sara-Gr) and the other to maintain the orange allele in a homozygous state (Sara-Or). Reciprocal crosses between pedigrees in the Sara-Gr strain are used for maintenance and to minimize inbreeding. The Sara-Or strain is also maintained with reciprocal crosses.

Stock source:

The Stock Center received 3 males and 3 females from Dr. J. Vielkind, Vancouver, Canada, on 10/28/99 establishing the Sara-Or strain. 4 males and 4 females were acquired from University of Texas, Science Park Research Division, on 1/24/00 establishing a separate Sara-Gr strain.

Xiphophorus helleri, Albino

Female



Male

Strain Code: HeAlb

Phenotypes scored: Albinism (i)
Sword color, green (Gr).

Introduction:

The albino *Xiphophorus helleri* (HeAlb) was created by Dr. Manfred Schartl. He used an albino platyfish and continually crossed it with his *X. helleri* Lancetilla stock while selecting for the albinism, thus generating an essentially pure stock of *X. helleri* Lancetilla that is albino. This stock arrived at the Stock Center on July 3, 2001 from, Dr. Schartl at the University of Wuerzburg, Germany, via Dr. Ingo Schlupp.

Sex determination / sexing:

Sex determination is currently undetermined. Fish are sexed at 1.5 to 2 months of age and mature at about at 3 to 4 months of age.

Scoring:

Sword color is scored in males when they reach sexual maturity. For the most accurate assessment, fish must be anesthetized and scored with a dissection microscope on a black background. Albinism (i) is scored in both males and females.

Stock Maintenance:

Matings are established to produce enough offspring for stock maintenance and for research purposes. At least six matings are set up for each generation using reciprocal crosses from parallel lines. This helps ensure appropriate quantities of fish are generated.

Stock Source:

Dr. Manfred Schartl, University of Wuerzburg, Germany, 7/3/01.

Xiphophorus helleri, Belize

Female

Male

Strain code: Bel

Phenotypes scored: Sword color, orange (Or) and green (Gr).

Introduction:

The Belize stock was originally collected from the Belize River in Belize in 1989. The stock has been in the Stock Center since 1993. Males in this stock possess either a bi-colored orange / green sword, or an all green sword. The gene for sword color is Y-linked, with two co-dominant alleles, orange and green, segregating at the locus. The sex determination mechanism for this stock is WY / YY; therefore, both sexes possess a Y chromosome(s) with the gene for sword color, but expression is sex-limited to males.

Sex determination / sexing:

Females are heterogametic (WY), and males are homogametic (YY) in this species. Belize swordtails are sexed at 3 to 4 months of age, and can reach maturity at about 6 months of age.

Scoring:

Sword color of fully mature males should be scored with a dissecting microscope on a black background. The two sword colors are co-dominant in this strain, so you will see orange and green if the male carries one of each allele.

Maintenance:

This stock is maintained by reciprocal crosses. Two lines are maintained and at least two matings should be set up for each line. To maintain a green line, females from this line (with the Y^{Gr} allele) are mated to males homozygous for a green sword (Y^{Gr} Y^{Gr}). Two matings are also set up for an orange / green line (females from the greenline with a male with an orange / green sword). The following mating schemes are used for stock production:

Phenotype of
male offspring

WY^{Gr} (x) $Y^{Gr}Y^{Gr}$ → All Green swords **Green line**

WY^{Gr} (x) $Y^{Or}Y^{Gr}$ → ½ Orange & Green swords, ½ Green swords **Orange / green line**

Females produced from the orange / green matings are generally discarded.

Stock source:

Prof. Klaus Kallman, the New York Aquarium, 5/12/93.

Xiphophorus helleri, BXII

Female

Male

Strain code: BXII

Phenotypes scored: Pigment pattern, dabbed-1 (Db-1).

Introduction:

The BXII strain of *Xiphophorus helleri* is one of the southern swordtails and was originally obtained from the Belize River, Belize, in 1949 by Dr. Myron Gordon and G. Fairweather. Since 1955 this stock was maintained by Curt Kosswig until 1962 where it was then bred in Dr. Dierk Franck's laboratory at the Zoologishes Institut, Universitat Hamburg, Germany. Under laboratory conditions, inbreeding has been avoided during the perpetuation of this stock. Fish representing the BXII strain arrived at the Stock Center in San Marcos on July 3, 2001. This stock appears to be fixed for an orange sword color and the macromelanophore spotting pattern, dabbed-1 (Db-1) pattern described in 1966 by Kallman & Atz (Zoologica N.Y., 51, 107-135). This pattern Db-1 is coded by an autosomal dominant allele and appears to show 100% penetrance (Kallman, 1975).

The gene for dabbed-1 (Db-1) is distinct from the gene encoding the dabbed-2 (Db-2) pigment pattern found in the HX strain of *X. helleri*. This was demonstrated with crosses between these two stocks. The spots produced with the Db-2 allele in HX form apparent rows, while the spots produced with the Db-1 allele of BXII are more randomly distributed (Kallman & Atz, 1966).

Sex determination / sexing:

Little is known of the sex determining mechanism in BXII, but this stock has exhibited biased sex ratios. BXII fish are sexed at two to three months of age. This should be done as early as possible to determine if sex ratios are unacceptably biased.

Scoring:

The Db-1 spotting pattern is the major phenotype scored in this fish, and this character is relatively unambiguous as the stock is homozygous for this allele. Sword color is also scored in males with a dissecting microscope on a black background.

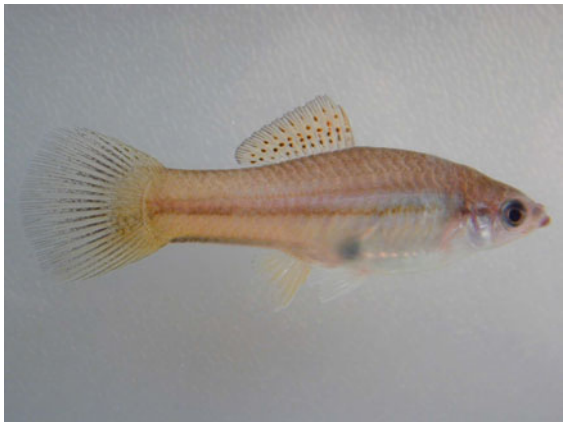
Maintenance:

The BXII stock is maintained by reciprocal crosses of parallel lines within a generation. Because these fish are prone to biased sex ratios, it is recommended that at least three matings are set up for each generation to ensure adequate numbers of both males and females for the next generation. An extra tank, each of males and females, should be retained from the previous generation until males and females have been verified to exist in the subsequent generation.

Stock source:

Prof. Dierk Franck, Zoologishes Institüt, Universität Hamburg, Germany, 7/3/01.

Xiphophorus helleri, Cd



Female

Male

Strain code: Cd

Phenotypes scored: None.

Introduction:

The ancestors of this Cd stock were originally collected in the Rio Jamapa near Cordoba, Veracruz, Mexico in the early 1930's by Dr. Clarence L. Turner. Cd stands for Cordoba, Veracruz. The Stock Center obtained its stock in 1949 from Dr. Reeve M. Bailey, University of Michigan, where it was maintained from 1930 until 1949. This stock has been maintained in the Stock Center by inbreeding for over 50 generations. Individuals of this strain are characterized by the presence of a dark brown lateral stripe, which extends from the eye to the base of the caudal fin. Cd males possess a yellow / green sword and also display a brilliantly colored yellow dorsal fin. The gonopodium in males is also yellow.

Sexing:

The Cd stock differs from any other *Xiphophorus helleri* stocks in the unusually long time required to reach sexual maturity. Individuals in this stock may take anywhere from 6 months to one year to reach maturity. Males should be kept at low densities to expedite maturation. Individuals can be sexed at around 4 to 5 months of age.

Scoring:

No phenotypically variable traits are scored in this stock.

Maintenance:

The Cd stock has been maintained by using reciprocal crosses between pedigrees of the same generation. At least six matings should be set up for each generation since this stock has a history of low production. Fish are to be mated as soon as they become mature because they may already be one year old by this time. This stock is infamous for producing biased sex ratios. Matings should be maintained long enough to ensure production of sufficient numbers of both sexes. Also, at least one tank of males and one tank of females should be retained from the

previous generation until males and females in the new generation have been positively identified.

Stock source:

Prof. Klaus Kallman, the New York Aquarium, 2/18/93.

Xiphophorus helleri, Doce Millas

Female

Male

Strain code: Doce

Phenotypes scored: Sword color, orange (Or) and green (Gr)
rubra litoralis (Rl).

Introduction:

This new strain of *Xiphophorus helleri* was collected from a small stream named Doce Millas, Rio Junapan drainage, Oaxaca, Mexico on March 23, 2001. Males express sword coloration as either orange (Or) or green (Gr). Some males also express an additional red line above the mid-lateral line, which can become more enhanced in older, dominant males. This coloration has been termed rubra litoralis (Rl) and appears to be linked to the green sword color. Preliminary data also indicates that the orange sword color shows complete dominance over the green sword color. Sex determination and pigment inheritance is still being studied in this strain and is yet to be conclusively determined.

Sex determination / sexing:

These swordtails are sexed at 3 to 4 months of age and can reach full sexual maturity at about 6 months of age.

Scoring:

Sword color of fully mature males should be scored with a dissecting microscope on a black background. The orange sword colors appear to exhibit complete dominance, so you will see either an orange or a green sword in males. Rl is scored by observing the fish in an observation dish with the naked eye and this pattern appears as a second orange to red stripe above the mid-lateral line.

Maintenance:

Matings are set up to ensure perpetuation of the three color patterns found in this strain. Two matings are required for each coloration, resulting in a total of six matings for each generation. Females resulting from pedigrees fathered by a male with a green sword (i.e. green

line) are used in all of the matings. The three mating types, set up in duplicate, will look as follows:

green line female	(x)	green male
green line female	(x)	green male with rubra litoralis
green line female	(x)	orange male.

Females produced from the green with rubra litoralis and orange matings are generally discarded and not required for stock maintenance.

Stock source:

Dr. Klaus Kallman, Dr. Steven Kazianis, and Anthony Pedroza, collected in Oaxaca, 3/23/01.

Xiphophorus helleri, HX

Female

Male

Strain code: HX

Phenotypes scored: Pigment pattern, dabbed-2 (Db-2) and wild-type (++)
Sword color: orange (Or) and green (Gr).

Introduction:

Specimens of the HX strain of *Xiphophorus helleri* were collected by Myron Gordon in 1951, from the Rio Lancetilla in Honduras. This stock, which was originally bred in the laboratory of Dr. Gordon, exhibited macromelanophore spotting in the flank. This pattern, named dabbed-2 (Db-2), consisted of spots of irregular size and shape that "show a tendency to be arranged in rows". This arrangement of spots gives the appearance of stripes and this striping pattern becomes more pronounced with age. Each 'stripe' consists of approximately 8-10 spots (Kallman and Atz, 1966). (Such striping is rare in the BXII strain, where any similar row consists of at most 4 spots.) Results of breeding experiments between HX and BXII strains suggest that these two spotting patterns are controlled by different genes. Further breeding experiments showed that the Db-2 phenotype is controlled by an autosomal dominant gene that shows 100% penetrance.

Another segregating color pattern observed in the HX strain is sword color. According to preliminary Center data, this trait appears to be Y-linked and sex limited to males. Two alleles are seen at this locus in this population; one expresses an orange color and the other a green color in the sword. These two alleles are co-dominant. In heterozygotes, orange color appears on the top half of the sword and green appears on the lower portion of the sword.

Sex determination / sexing:

Chromosomal sex determination is WY / YY, females and males, respectively. HX fish are sexed at 3 to 6 months of age and may take up to a year to reach maturity.

Scoring:

Both male and female fish are scored for the Db-2 and (+) pigment patterns. A mature male's sword color can be examined with a dissecting microscope against a black background. The coloration should be recorded as green (Gr) or orange (Or), or a combination of both. Fish

should always be scored when being set up in matings and also at a later date such as when the mating is being taken down or fixed.

Maintenance:

Stocks are managed for retention of both the macromelanophore spotting pattern, Db-2 and the wild-type (+) alleles, and both sword colors. Db-2 is maintained through reciprocal matings of Db-2 heterozygotes to wild-type (++) fish, producing a 1:1 ratio of Db-2 to wild-type phenotypes in offspring. Both the orange and green sword color alleles are maintained in two lines, a 'green' line and an 'orange-green' line. All females used in matings are from the 'green' line and presumed to carry only the 'green' allele. Both alleles are then maintained by crossing 'green' females to a 'green' male (green-line) and to an 'orange-green' heterozygous male (orange-green line).

An example of crosses that would be set up to manage one generation:

Orange / green line:
WY^{Gr} Db-2 (x) $Y^{Or}Y^{Gr}$ ++,
WY^{Gr} ++ (x) $Y^{Or}Y^{Gr}$ Db-2+,

green line:
WY^{Gr} ++ (x) $Y^{Gr}Y^{Gr}$ Db-2 +,
WY^{Gr} Db-2+ (x) $Y^{Gr}Y^{Gr}$ ++.

Stock source:

Prof. Klaus Kallman, the New York Aquarium, 7/23/93.

Xiphophorus helleri, Jalapa

Female

Male (Rr)

Male (rr)

Strain code: Jalapa

Phenotypes scored: Male coloration, red (Rr) or black (rr).

Introduction:

Specimens of this stock were first collected in the spring of 1963 from a tributary of the Rio Chachalaca, near the town of Jalapa, Veracruz, Mexico. These fish were maintained as a stock at the Zoological Institute in Hamburg, Germany (Zander, 1967). In June of 1993, the Stock Center received five females and five males, estimated to be about one-year of age, from two different pedigrees.

Two phenotypes are represented in males of this stock, black and red. Although sex determination has yet to be established, heritability studies are underway at the Center using these two phenotypes of males.

Sexing:

Jalapa fish must be sexed at 2 months of age because of the early maturing black males. Sexing should be reevaluated again 2- 4 weeks later to ensure there are no later maturing red

males in with the females. The chromosomal mechanism for sex determination is undetermined at this date.

Scoring:

The black males develop a dark reddish-brown midlateral line and a dark axillary stripe that extends anteriorly beyond the pectoral fin. A yellow line is located immediately above the midlateral line. The flank above the axillary stripe shows a metallic bluish-green color. These males are smaller and mature at an earlier age. In the red male, the mid-lateral line is red with a more extensive red flush, i.e., the area below (and somewhat above) is suffused with red. Also, the axillary stripe is not present in the red males. Red males mature later and thus, reach a larger size (Zander, 1967).

Only males in this stock are scored for the red or black phenotype. MS-222 anesthetized males are scored for this phenotype at 10X magnification. Determination of phenotype is based on the anterior extension of the axillary stripe. In black fish, complete extension or scattered black pigment cells are present anterior to the pectoral fin; in red males, this area is free of black pigment.

Maintenance:

The stock is maintained as two lines, a black line and a red line. In the black line, males expressing the black phenotype are mated to females from the black line, preferably from different pedigrees. Consequently, all the offspring carry only the black allele. The red allele is maintained in a heterozygous state by always mating phenotypically red males to black line females.

Stock source:

Prof. Klaus Kallman, the New York Aquarium, 6/11/93.

Xiphophorus helleri, Lancetilla

Female

Male

Strain Code: Lance

Phenotypes scored: Sword color, orange (Or) and green (Gr)

Introduction:

Dr. Gordon collected the progenitors of the strain(s) in the Rio Lancetilla, Honduras, 1951. He called this strain "Hx". "H" stands for Honduras and "x" for *Xiphophorus*, to wit "Honduras *Xiphophorus*". The stock was heterozygous for the macromelanophore pattern Db^2. Soon after the fish arrived in his laboratory in New York, he sent a subset of Hx fish to Dr. Curt Kosswig in Istanbul. In Turkey, he designated the stock "Lancetilla." Eventually the Hx strain died out in New York.

Dr. Kosswig returned to Hamburg, Germany, in 1957 and brought the Lancetilla fish with him. During the next 40 years, a subset of the Lancetilla stock was disseminated to other laboratories: Dr. Anders, Dr. Schartl, and Dr. Schroder. When Dr. Kallman visited Dr. Schroder's laboratory in 1993, he returned the Lancetilla fish to New York and reverted the stock back to the "Hx" designation. While the Hx stock in the Stock Center was breeding poorly in the mid 1990's, more of the Lancetilla fish were obtained from Dr. Schartl's Laboratory at the University of Wuerzburg, Germany in 1996. This stock continues to be termed Lancetilla. While the Lancetilla stock expresses bi-colored swords, these fish do not possess the Db-2 pattern.

Sex determination / sexing:

Sex determination is presumed to be WY / YY. Fish are sexed at 1.5 to 2 months of age and mature at about at 3 to 4 months of age.

Scoring:

Sword color is scored in males when they reach sexual maturity. However, the sword color differences are not as pronounced as observed in the Hx strain, making scoring more difficult. For the most accurate assessment, fish must be anesthetized and scored with a dissection microscope on a black background.

Stock Maintenance:

Matings are designed to preserve both the orange and green alleles for sword color. Two lines, defined by sword color, are maintained, a green line and an orange / green line. Maintenance of the green line requires mating a female from the green line (W Y-Gr) with a male expressing green sword color (Y-Gr Y-Gr):

WY^{Gr} (x) Y^{Gr} Y^{Gr}.

The orange/green line requires mating a female from the green line (W Y-Gr) to a male exhibiting an orange / green sword (Y-or Y-Gr):

WY^{Gr} (x) $Y^{Or}Y^{Gr}$.

Females from the orange / green line are generally not used in stock maintenance and can be discarded if they are not needed.

Stock Source:

Dr. Manfred Schartl, University of Wuerzburg, Germany, 9/6/96.

Xiphophorus helleri, Lineatus

Female

Male

Strain code: HeLi

Phenotypes scored: Pigment pattern, Lineatus (Li) and wild-type (+)

Introduction:

The HeLi stock was constructed in Dr. Anders' laboratory by crossing *Xiphophorus variatus* Lineatus with *X. helleri* Lancetilla (Anders, et al., 1973). This mating produced hybrid progeny displaying the Lineatus (Li) pigment pattern. These fish were then crossed repeatedly into pure Lancetilla fish, until the resulting stock was relatively pure *X. helleri* expressing the Li pattern. HeLi is now maintained as a separate *X. helleri* stock in the Stock Center. The gene for the Li pattern maps to the X-chromosome and two alleles segregate at this locus in this captive stock, wild-type (++) and Li.

Sex determination / sexing:

The two parental species have different chromosomal sex determination mechanisms. The original *X. variatus* Lineatus and *X. helleri* Lancetilla strains were $X^{+}X^{Li}$ / $X^{Li}Y$ and WY / YY, respectively. This cross results in WX^{Li} females and X^{Li} Y males displaying the Lineatus pattern. It is therefore important to identify the parental chromosomal makeup to accurately predict the genotypes of the progeny.

Non-pigmented fish mature much earlier than animals carrying the Li-pattern allele. Therefore, non-pigmented fish should be discarded at around 1 month. The pigmented (Li) animals generally show sexual differentiation at around 4 to 5 months of age and reach sexual maturity at about 6 months to one year of age.

Scoring:

(Li) and (+) can be scored at about 1 month of age, at which time the (+) animals can be discarded. Sword color can be scored in fully mature males using the dissecting scope and a black background. Like the Lancetilla stock, sword colorations include orange (Or) and green (Gr)

Maintenance:

This stock is propagated by out-crossing HeLi animals to *Xiphophorus helleri* Lance. About three matings are set up for each generation to ensure offspring production. Only one highly productive mating is required to maintain the line. Mating record data indicates a better production rate resulting from matings that contain a Lance female and a HeLi male.

Stock source:

Prof. Manfred Schartl, The University of Wuerzburg, Germany, 10/15/96.

Xiphophorus montezumae, Capuchin

Female

Male

Strain code: Capuchin

Phenotypes scored: None

Introduction:

Representatives of this strain were collected in the Cienega Grande, San Luis Potosi, Mexico in 1984 by Drs. Kallman and Morizot. A trait showing some variation in the population is the presence or absence of a dark stripe along the lateral line. This stripe is seen in both males and females, but the genetic basis of this is not understood. Males show a basal row of melanophores on the dorsal fin and random, but numerous melanophores scattered on the dorsal fin. No other melanophore patterns are observed in this population. The stock was initiated in 1984 with a shipment of 29 fish, 12 males with a red stripe (A line) and 17 wild type males (B line). The red stripe is possibly sex-linked (K. Kallman, pers. comm.), although this trait may have been lost in the XGSC population. These fish require large tanks and large amounts of food for healthy development. When more than one male is combined in a single tank, only one will develop a sword, i.e., production of the sword is suppressed in the other males.

Sexing:

Fish are sexed at 2 to 3 months of age and become sexually mature at 4 to 5 months of age.

Scoring:

No phenotypic traits are scored in this stock.

Maintenance:

4 to 6 matings should be established for each generation to ensure production of at least 2 pedigrees. This stock has a history of limited production and biased sex ratios have been observed. Be sure to keep backup males and females until it is certain that enough males and females are produced to continue into the next generation.

Stock source:

Prof. Klaus Kallman, the New York Aquarium, 6/24/93.

Xiphophorus montezumae, Ojo Caliente

Female

Male

Strain Code: OjoCal

Phenotypes scored: None

Introduction:

Specimens of this strain were collected in 1983 from the Rio Ojo Caliente, San Luis Potosi, Mexico, by Drs. Kallman and Morizot. This is a small, short tributary of the Rio Santa Maria that is just north of the Rio Verde, where *Xiphophorus* is apparently absent (Rauchenberger et al., 1990).

Two rows of melanophores are seen in the dorsal fin of males, a row along the base and a more irregular row through the center of the fin. However, this pattern may be weakly expressed to absent in some males. No polymorphic pigment patterns are known in this stock.

Sexing:

Fish are sexed at 2 to 3 months of age and become sexually mature at 3 to 5 months of age.

Scoring:

No traits are scored in this stock.

Maintenance:

Four to six matings should be established for each generation to ensure production of at least two pedigrees. This stock has a history of limited production and biased sex ratios have been observed. Be sure to keep backup males and females until it is certain that enough males and females are produced to continue into the next generation.

Stock source:

Prof. Klaus Kallman, the New York Aquarium, 4/13/93.

Xiphophorus montezumae, Rascon

Female

Male (++)

Male (M+)

Strain code: Rascon

Phenotypes scored: Macromelanophore pattern (M) and wild type (+)

Introduction:

This strain descended from fish collected near Damian Carmona, north of Rascon, San Luis Potosi, Mexico. These fish were collected from the Rio Ojo Frio in the Rio Gallinas system by Drs. Kallman and Morizot in 1983. Representatives of this stock were first shipped to the Stock Center in two stages, in 1992 and 1993. This strain is polymorphic for a macromelanophore pigment pattern, which is controlled by a dominant autosomal allele, M. Unlike other *Xiphophorus montezumae* populations, males from the Rascon population do not show distinctive dorsal fin patterns.

The first shipment of Rascon fish to the Stock Center arrived on October 21, 1992, and was comprised of two pedigrees with six spotted (heterozygous, M+) females and six wild type males. The next shipment came on May 12, 1993, and contained two pedigrees with a total of 21 females and 22 males. This shipment contained some fish of each sex that were heterozygous for the M/+ alleles.

Sexing:

Fish are sexed at 2 to 3 months of age. The chromosomal mechanism for sex determination is unknown in this species, but sex ratios are close to 1:1.

Scoring:

Expression of the macromelanophore pattern is highly variable. Phenotypes can range from numerous, irregular, and variably sized spots on the flank to a single, small spot extending from the mid-lateral line. Often irregular spot(s) may be observed on the dorsal fin, especially in males. A single spot on the fish is scored as positive expression of this gene. The pattern is more prominently expressed in males than in females, making scoring of females particularly difficult. Therefore, it is best to score the fish when they are mature, just prior to mating. Look carefully for small, black (not brown) spots that may be hiding in the mid lateral line. A deep bronze to red coloration is produced by rows of pterinophores in a few males in all populations, perhaps a sign of dominance.

Stock Maintenance:

Five to eight matings should be established for each generation to ensure production of enough pedigrees and fish. Typically three to five matings contain one fish that is M+ and one that is ++. Also, in the same generation two to three matings should be set up with fish that are all presumed to be ++. This will help ensure the + allele is not lost in the event a fish is improperly scored. All matings should be re-scored upon fixing and all offspring should be scored and tallied.

Stock source:

Prof. Klaus Kallman, the New York Aquarium, 10/21/92 and 5/12/93.

Xiphophorus alvarezi, Dolores

Dolores, female

Dolores, male

Strain code: DL

Phenotypes scored: Sword color orange (Or).

Introduction:

This stock of *Xiphophorus alvarezi*, Dolores was derived from fish collected by Don Rosen in the late 1960's and early 1970's. This stock is maintained as an inbred line.

The Dolores stock is found naturally in the Rio Dolores system. The males are characterized by a bright red flush below the lateral line, extending from the operculum to the caudal fin. Males carry a Y-linked gene for orange sword color. Females also carry this gene, but do not express the trait.

Sex determination / sexing:

Sex determination in DL is WY / YY (Kallman and Bao, 1987). This species can be sexed at about 3 months of age. Maturation can be expedited in males by growing them at low densities.

Scoring:

Sword color is scored on a black background with a microscope. Males can be scored when sexually mature and color may become more apparent with age.

Stock maintenance:

At least 4 to 6 matings should be established for each generation. A larger number of matings is required to compensate for low production numbers and biased sex ratios. Reciprocal crosses or brother/sister matings can be used, whichever is available.

Stock source:

Prof. Klaus Kallman, the New York Aquarium, 5/12/93.

Xiphophorus andersi

Female

Male

Strain code: andB, andC

Phenotypes scored: Presence or absence of a pseudogravidity spot

Introduction:

Xiphophorus andersi was first described by two German collectors in 1980. The progenitors of the *X. andersi* maintained at the Stock Center were collected in 1981 from the Rio Atoyac in Veracruz, Mexico. This species has a limited distribution and is restricted to the headwaters of the Rio Atoyac, making it a species of special concern.

Dr. Klaus Kallman established two strains for size differences in this species. One strain was established using parents with the genotype for small size (andB) and the other from parents with a genotype for large size (andC). This size difference has been attributed to a gene that controls the age of sexual maturation, a Y-linked pituitary factor (P-factor). Two alleles segregate at this locus in the stock: one allele for early maturation, hence, small size, and a second for late maturation and large size. Males of the andB strain mature at an earlier age than males of the andC strain and therefore, are smaller than males of the andC stock. A second difference between the two stocks is the presence or absence of a pseudogravidity spot. Males of the andC strain display this spot, while males of the andB strain have a dominant, Y-linked suppressor gene that prevents expression of the pseudogravidity spot.

Sex determination / sexing:

Sex determination is XX / XY in this species. The early maturing andB males must be sexed early, at 1.5 to 2 months of age and sex should be rechecked after 2 weeks. The slower maturing andC stock is sexed at a later age, 3 to 4 months old. This sex designation is also rechecked after 2 to 4 weeks.

Scoring:

The andB and andC fish are scored when mature for the presence or absence of the gravidity spot in females and pseudogravidity spot in males.

Maintenance:

The stocks are maintained through reciprocal crosses between males and females of the two strains. Strain designation (B or C) follows the male line. Twelve matings are set up for each generation; six to maintain andB stock (andC female x andB male) and six to maintain andC stock (andB female x andC male):

6 matings: andC female (x) andB male------→ andB strain production

6 matings: andB female (x) andC male------→ andC strain production

Because the two stocks differ in rates of maturation in males, the andC males are raised at low densities so they will mature faster and can be mated on schedule.

Source:

Prof. Klaus Kallman, the New York Aquarium, 10/21/92.

Xiphophorus birchmanni

Female(Cam)

Male (++)

Strain code: birchII

Phenotypes scored: Macromelanophore pattern, carbomaculatus (Cam) and wild-type (+).

Introduction:

This stock arrived at the Stock Center from Dr. Manfred Schartl's laboratory in Wuerzburg, Germany, on July 3, 2001. The line was originally collected from Rio Axtlapezco, Mexico, in March of 2000. The fish express the macromelanophore pattern termed carbomaculatus (Cam) which is also seen in *Xiphophorus cortezi*. Cam consists of large, black pigment cells generally located on the body and may extend into the dorsal fin.

Sexing:

Fish in this stock should be sexed at about 1.5 to 2 months of age and reach full sexual maturity by 5 to 6 months of age.

Scoring:

Males and females should be scored for Cam and + in this stock. This is generally done by placing mature fish in an observation dish and observing these patterns with the unaided eye.

Maintenance:

Six matings using reciprocal crosses are set up for each generation in this stock. Four of these matings are designed to maintain Cam in a heterozygous state by crossing a fish that is expressing Cam to a fish that is not. Two of the matings are designed to ensure the perpetuation of wild-type fish in the event of a miss-scored fish in a mating, by mating two + fish together. The mating scheme will look as follows.

Four Cam/+ Matings:

	Cam female	(x)	+ male
Or			
	+ female	(x)	Cam male

Two +/+ Matings:

+ female (x) + male

Stock source:

Dr. Manfred Schartl, University of Wuerzburg, Germany, 7/3/01.

Xiphophorus clemenciae

Xiphophorus clemenciae was described by Alvarez (1959) from the Rio Sarabia, Oaxaca, Mexico. Specimens were originally classified as *X. helleri* because of similarities in size, shape, and coloration. In addition, it was first observed in habitats characteristic of *Xiphophorus helleri* and other swordtails, clear water with little to no vegetation, and a bottom of mud, silt, and brush. The current in streams where *X. clemenciae* was found was quite variable, ranging from moderate or almost no flow to fast flowing at other times of year.

Members of *X. clemenciae* are described in Rosen (1960, 1979) as small to moderate in size, with a slender body. The males have a well-developed, yellowish sword, with the upper and lower margins lined in black. The caudal fin above the sword is convex, with no marginal pigmentation. Two solid red stripes extend from the opercle to the base of the caudal fin. Males of this species show an axillary stripe originating at the base of the pectoral fin extending posteriorly along the belly up to and past the gonopodium. A small black line connects the front of the eye with the lips. A ridge of deep-lying black pigment is found in the midventral area of the caudal peduncle, and at the base of the caudal fin are 3 to 6 red to orange spots. The dorsal fin is more square-shaped in males and rounded in females and young. There is a row of dark, triangular markings in the interradial membrane of proximal dorsal fin, while a diffuse band of pigment is seen on the distal portion. Kallman and Atz (1966) noted that there are no macromelanophore patterns or tail spot patterns observed in *X. clemenciae* from the Rio Sarabia.

X. clemenciae is sympatric with *X. helleri* in some areas. As noted above, there are many phenotypic similarities between the two species. However, there are several consistent differences. *X. clemenciae* differs from *X. helleri* in the dorsal fin markings, as well as a number of gonopodial characteristics. Representatives of two distinct populations of *X. clemenciae* fish are maintained at the Stock Center, Grande and FincaII.

Xiphophorus clemenciae, FincaII

Female

Male

Strain Code: FincaII

Phenotypes scored: Sword color, orange (Or)

Introduction:

Representatives of the FincaII stock of *Xiphophorus clemenciae* were originally collected from the San Carlos type-location in Oaxaca, Mexico by Dominic Isla and his wife, Janet Ruli. These wild caught fish were sent to the Stock Center on November 15, 2000. The fish currently in the Stock Center are descended from these wild-caught females.

Sex determination / sexing:

FincaII fish should be sexed at 2 to 3 months of age. Once matings are set up, the remaining back-up fish can be combined with their siblings.

Scoring:

Sword color is scored with the aid of the black background of a dissection microscope, on sexually mature males. This stock appears to be fixed for orange sword color.

Stock maintenance:

About 6 matings are set up for each generation. This is done to ensure enough progeny to continue on into the next generation.

Stock source:

Dominic Isla and Janet Ruli, 11/15/00.

Xiphophorus clemenciae, Grande

Female

Male

Strain Code: Grande

Phenotypes scored: Sword color, orange (Or) and green (Gr)

Introduction:

Representatives of the Grande stock of *Xiphophorus clemenciae* were collected from the Rio Grande in Veracruz, Mexico, by Klaus Kallman in 1993. The fish currently in the Stock Center are descended from wild caught females.

Sex determination / sexing:

Grande fish should be sexed at 2 to 3 months of age. Once matings are set up, the remaining back-up fish can be combined with their siblings. The chromosomal mechanism for sex determination for this stock is likely XX/XY although it has not been conclusively worked out.

Scoring:

Sword color is scored with the aid of the black background of a dissection microscope on sexually mature males. It is presumed that alleles for orange and green sword color are present, but this is yet to be determined.

Stock maintenance:

About 6 matings are set up for each generation. This is done to ensure enough progeny to continue on into the next generation.

Stock source:

Prof. Klaus Kallman, the New York Aquarium, 1993.

Xiphophorus continens

Female

Male

Strain code: contiIII

Phenotypes scored: No phenotypes scored in this stock.

Introduction:

Six females and five males of the contiIII stock arrived at the Stock Center on January 16, 2003. These fish originated from Dr. Mike Ryan's Laboratory at the University of Texas in Austin. There are no segregating phenotypes scored in this stock.

Sexing:

Fish are sexed at about 2 months of age. They mature at about 6 months of age.

Maintenance:

About six matings using reciprocal crosses are set up for each generation in this stock. Although there are not any varying color patterns associated with contiIII, production trouble has been noted and this high number of matings will help ensure enough offspring are produced to perpetuate the stock.

Stock source:

Dr. Michael J. Ryan, University of Texas, 1/16/03.

Xiphophorus cortezi

Female (++)

Male (Sc, Cam)

Female (Cam/Cb)

Male (++)

Strain code: cortezi

Phenotypes scored: Macromelanophore patterns, carbomaculatus (Cam), atromaculatus (At), or wild type (+), and spotted caudal (Sc)
Tail spot patterns, tail yellow (Ty), caudal blotch (Cb), and wild type (+).

Introduction:

This species displays a single zigzag, horizontal stripe and especially in some males, long, narrow, vertical bars appear along the flanks. The sword is upturned with dark melanophores along the ventral margin. The grave spot is well developed and anterior to the caudal fin (Rauchenberger et al., 1990).

The *Xiphophorus cortezi* stock was introduced into the Stock Center in 1982. Several phenotypes are segregating in this stock. The different phenotypes include two autosomal macromel-anophore patterns, atromaculatus (At) and carbomaculatus (Cam). The At pattern is disting-uished from Cam by the number, size and general location of the macromelanophores. The At pattern comprises smaller and more numerous spots located on the dorsal fin and dorsal flank of the body, just below the dorsal fin. The spots in the Cam phenotype are less numerous and larger (usually no more than two or three) and are more predominant on parts of the body,

but may be present on the dorsal fin as well. The Cam phenotype masks At. Therefore, it is important to never mate At fish with Cam fish, (Rauchenberger et al., 1990; Kallman, 1971).

Along with the two autosomal macromelanophore patterns, there is also an autosomal tail pigment pattern, caudal blotch (Cb). This pattern is maintained in a heterozygous state by crossing Cb fish with wild type individuals. There are also two Y-linked pigment patterns, tail-yellow (Ty) and spot-caudal (Sc). Some Sc males have developed melanosis and small melanomas. A P-factor for early maturation is linked to the X-chromosome and one for late maturation on the Y. In natural populations there are also Y-chromosomes with an early P-factor.

Sex determination / sexing:

Sex determination for this stock is XX / XY (Kallman, 1971). The fish are sexed at around 2-3 months of age. Males begin sexual maturation at around 6 months. If raised at low densities, males can mature as early as four months of age. *X. cortezi* fish are set up in matings as early as possible, depending on time of sexual maturation.

Scoring:

All phenotypes are scored on mature fish. Cam, At, and + fish can be distinguished with the naked eye. Cb should always be scored using the dissecting microscope using a white background. Ty is fixed in this stock and can be scored under the scope with a white background. The pattern develops with age in mature males. Sc males do not always express the pattern because of the varying degrees of penetrance. Penetrance does appear to increase with age.

Stock maintenance:

A mating scheme has been established that preserves all alleles of this stock. Typically 6 matings are set up for each generation, 2 for each of the macromelanophore patterns, Cam, At, and +. In these matings one fish should be Cam, At, or + and the other fish should be +. For each of these matings, one fish should express Cb and the other should be +. This generates either + fish or heterozygous fish, Cb/+. All males will inherit Ty. Finally, at least 2 of the matings should use males that express Sc. A typical mating plan may look as follows;

Cam/+, Cb/+ female	(x)	+/+, +/+, Ty, Sc male
+/+, Cb/+ female	(x)	Cam/+, +/+, Ty male
At/+, +/+ female	(x)	+/+, Cb/+, Ty, Sc male
At/+, Cb/+ female	(x)	+/+, +/+, Ty male
+/+, Cb/+ female	(x)	+/+, +/+, Ty male
+/+, +/+ female	(x)	+/+, Cb/+, Ty, Sc male.

Notice that only three of the matings contain Sc males. Duplicate matings for each phenotype ensures there will be enough fish with all the patterns and none of the alleles will be lost in the successive generation. If matings do not produce offspring after three months, the matings must be artificially inseminated and males checked for sperm, since timing is essential in maintaining this difficult stock.

Stock source:

Prof. Klaus Kallman, the New York Aquarium, 11/92, 6/93, 7/93.

Xiphophorus couchianus

Female

Male

Strain Code: Xc

Phenotypes scored: None

Introduction:

Representatives of *Xiphophorus couchianus* maintained in the Stock Center were collected in 1961, from Huasteca Canyon, Nuevo Leon, Mexico (Kallman, 1975). As of 1975, this stock had been maintained by inbreeding for 22 generations. This species is extinct in its natural habitat. Currently, the *X. couchianus* stock is in the 71st generation of inbreeding. Many generations of inbreeding have produced individuals homozyogus at most, if not all, loci.

Sex determination / sexing:

Sex determination is XX / XY. *X. couchianus* fish are sexed at 1.5 months of age and reach sexual maturity at about 4 months of age.

Maintenance:

As *X. couchianus* is used as a parent in a number of hybrid crosses involving cancer research, production of sufficient numbers of offspring is a continual goal, for both line-maintenance and research investigations. Therefore, a minimum of six crosses between males and females originating from different matings (i.e., pedigrees) are set up each generation. Currently, reciprocal crosses are set up between males and females within the same generation, but from different pedigrees (e.g., B and C). The new pedigree is designated by generation number and assigned a letter (A, B, or C), according to which mating produced offspring first. For example, the first mating to produce offspring in the 67th generation would be generating pedigree # Xc67(A), the second would have pedigree # Xc67(B) and so on.

Stock source:

The Stock Center received one shipment of *X. couchianus* from Dr. Kallman in 1992. This shipment contained 9 males and 8 females from the 48th generation of inbreeding. A second shipment of six females was received in October, 1993; these females were from the 51st inbred generation.

Xiphophorus evelynae

Female (se-1)

Male (se-2)

Strain code: eve

Phenotypes scored: Spotting (or speckling pattern), speckled-1 (Se-1), speckled-2 (Se-2) and wild type (+).

Introduction:

The *Xiphophorus evelynae* arrived from Dr. Manfred Schartl (Germany) in 1998. Progenitors of this stock were collected from a lake near Necaxa, Hidalgo, Mexico. This stock displays two spotting patterns, an X-linked speckled-1 (Se-1) and a Y-linked speckled-2 (Se-2). There is also an X-linked wild-type (+) allele. Se-1 fish display a few larger spots and Se-2 fish display many smaller spots, such that the pattern is often described as 'salt and pepper'. There is also vertical barring seen predominantly in males and concentrated toward the middle of fish. Sex determination in this stock is XX /XY.

Sexing:

Fish are sexed at about 2 months of age. The results should be rechecked and confirmed over the month following the initial sexing to identify any late-maturing males.

Scoring:

Females may display the Se-1 or + pattern. Males display the Se-2 spotting pattern; however, males may also carry the Se-1 or + pattern allele, but these phenotypes are masked by Se-2. Fish should be scored for Se-1, Se-2, and +.

Maintenance:

Six matings are set up for each generation. Reciprocal crosses are established between the available pedigrees for the purpose of minimizing inbreeding. Until the genetics are better understood, matings should use only one female and one male per mating, i.e., an Se-1 female is mated to a Se-2 male. Depending on how matings were designed for the previous generation, exact genotypes may not be known for all of the fish. Matings can be designed to determine

these genotypes from the offspring produced. The following is an example of a mating plan for one generation;

X-+ X-+ female	(x)	Se-2 male
X-Se-1 X-+ female	(x)	X-+ Y-Se-2 male
X-Se-1 X-+ female	(x)	Se-2 male
X-+ X-+ female	(x)	X-+ Y-Se-2 male
X-+ X-+ female	(x)	X-+ Y-Se-2 male
X-+ X-+ female	(x)	Se-2 male

Stock source:

Prof. Manfred Schartl, Wuerzburg, Germany, 11/9/98.

Xiphophorus gordoni

Female

Male

Strain code: gordoni

Phenotypes scored: None.

Introduction:

This stock is one of the three species of northern platyfish maintained at the *Xiphophorus* Genetic Stock Center which were collected February 18, 1990 by Dr. Klaus Kallman at Laguna Santa Tecla, Coahuila, Mexico. *Xiphophorus gordoni* has the deep lying body spots (albeit poorly developed) characteristic for the northern platyfish. These deep lying body spots are found in the septae of muscles and in the connective tissue lining the blood vessels, especially in the blood vessel along the lateral line just below the vertebral column (Atz, 1962). The other two species with this pattern currently housed at the Stock Center are *Xiphophorus couchianus* and *X. meyeri*.

X. gordoni is considered endangered due to a narrow and very limited habitat range, in Cuatro Cienegas, Coahuila, Mexico. Therefore, it would be difficult to recollect these from the wild, thus increasing the importance of the stock currently under cultivation.

Sexing:

These fish are sexed at about 2 to 3 months of age. The sex should be confirmed after about two weeks from the date of sexing.

Scoring:

There are no variable traits identified in these fish at this time; however, all fish should exhibit the deep lying body spots.

Maintenance:

A minimum of four matings should be set up to maintain each generation, therefore, at least two pedigrees should be produced from each generation. If, after about three months no mating produces offspring, the males should be checked for sperm and artificial insemination attempted. In addition, more matings can be established. Using the two or more pedigrees

obtained from each generation, reciprocal crosses can be set up to produce the subsequent generation.

Stock source:

Prof. Klaus Kallman, the New York Aquarium, 4/15/93.

Xiphophorus meyeri, Muzquiz platyfish

Female(sp/+)

Male (+/+)

Male (sp/+)

Strain code: meyeri

Phenotypes scored: Pigment pattern, spotted (sp) or wild type (+).

Introduction:

The Muzquiz platyfish was collected from Melchor Muzquiz, Coahuila, Mexico in 1983. Dr. Schartl shipped the progeny of wild-caught fish to the New York Aquarium in September, 1983. Two lines displaying a difference in melanophore pigmentation were identified and have been maintained since that time. The spotting pattern is a polygenic species-specific trait. Variation at an autosomal locus controls its presence or absence. A recessive allele acts as a suppressor for spotting. Evidence for this view is provided by the observation that when unspotted *Xiphophorus meyeri* are hybridized with unspotted *X. maculatus* all F_1 progeny develop deep lying spots at a somewhat reduced rate. In the back-cross generation to *X. maculatus*, the spotting is highly variable and becomes significantly reduced (Kallman unpublished). The pattern is caused by deep lying macromelanophores arranged around blood vessels and myoseptae along the flank.

Sex determination / sexing:

The male of this species is heterogametic, XY; the female is homogametic, XX. Fish are sexed at about 1.5 months of age and become mature at 3 months of age.

Scoring:

Fish are scored for pigment pattern, spotted (sp) or wild type (++), when being sexed. Only score this stock with the naked eye, do not use the dissection microscope. Upon close inspection with the dissection scope, one may notice very small, deep lying body spots on a + fish since the fish actually carries the spotting gene and it is only suppressed.

Maintenance:

Each generation is propagated setting up 3 to 6 matings. Occasionally generations have seen a high number of deformed offspring termed sinkers due to their inability to swim properly. If this situation should arise, it is good to set up the additional matings to ensure viable offspring. In the mating scheme at least 2 matings should include an sp fish and a + fish, and at least one mating should be a cross between 2 + fish:

sp female (x) + male

+ female (x) sp male

+ female (x) + male

Stock source:

On Jan. 6, 1993, seventeen *X. meyeri* were shipped to the *Xiphophorus* Stock Center. Six fish were wild type, or unspotted (4 females, 2 males), and eleven were spotted (4 females, 7 males). Another shipment of *X. meyeri* was received on Jan. 21, 1993, containing 10 fish (5 females, 5 males). All fish were wild type (+).

Xiphophorus milleri

Female (++)

Male (TyS)

Strain Code: mil82

Phenotypes scored: Pigment pattern, spotting, striped side (Sr) / faint striped side (Srf) / wild type (+)
Black gonopodium (Gn)
Maturation time, early (small, S) / late (large, L)
Autosomal tail pattern, point (pt) / wild type (+)
Sex-linked tail color, tail yellow (Ty) / wild-type (+).

Introduction:

Xiphophorus milleri was described by Rosen (1960) in collections from Catemaco, Veracruz, Mexico. The fish in early collections displayed two macromelanophore patterns, one which showed strictly paternal inheritance, leading Kallman and Atz (1966) to conclude that this species has an XX / XY mechanism of sex determination. In addition, three micromelanophore tail-spot patterns were evident in this species.

The X. *milleri* stock maintained in the Stock Center was collected in 1982. These fish showed two types of striped side patterns, striped side (Sr), which looks similar to the Sr in *X. maculatus* and is more defined and faint striped side (Srf), which is more difficult to see. Sr expression masks the presence of Srf. The stock also has a Y-linked allele for black-gonopodium (Gn) and will only be seen in males. There are also two P-factors segregating in this stock, one for early maturation and small size (S) and one for late maturation and large size (L).

Sex determination / sexing:

Sex determination is XX / XY. Fish are sexed at 1 to 2 months of age ensuring that early maturing males and females are separated from each other. The late maturing fish reach maturity at about three or four months of age, while the early maturing fish are ready to be mated by 2 months of age. The results of sexing should always be checked after two weeks.

Maintenance:

Seven different sex-chromosomes are maintained in the stock. Four X-chromosomes and five Y-chromosomes segregate in this stock. They differ in the specific linkage of pigment-pattern and P-alleles. The X-linked alleles include faint-striped side ($X^{Srf\ S}$) and wild type (X^{+s}) Specific alleles and linkage groups defining the Y-chromosomes include faint striped side and early maturation ($Y^{Srf\ S}$), striped-side linked to an allele for late maturation ($Y^{Sr\ L}$), tail yellow linked with an early maturation allele ($Y^{Ty\ S}$), late maturation linked to the wild-type allele for color pattern ($Y^{+\ L}$), and late maturation linked to black gonopodiun ($Y^{+Gn\ L}$). Gn could be an allele of Sr and Srf. Two alleles segregate for an autosomal tail spot pattern, point (pt) and wild-type (+). This gene is maintained in the heterozygous state (pt/+) by ensuring one parent in a mating displays point (pt) and the other is wild-type (++).

In setting up matings for each generation, all chromosomes must be represented, including ones carrying wild-type alleles. In order to ensure this, progeny must be scored accurately and matings designed with great care. For example, Sr can mask Srf or it may be impossible to distinguish an $X^{Srf}Y^{+L}$ male from an $X^{+}Y^{Srf}$ male. Therefore, chromosomes must be carefully tracked through matings and offspring. These chromosomes, i.e., the stock, can be maintained with five matings each generation and each mating should have one fish heterozygous for the tail-spot pattern, pt, and one wild-type tail spot:

X-SrfS X-+S, pt/+ (x) X-+S / Y-+ L, +/+

X-SrfS / X-+S, +/+ (x) X-+S / Y-TyS, pt/+

X-+S / X-+S, +/+ (x) X-+S / Y-SrL, pt/+

X-+ S/ X-+S, pt/+ (x) X-+S / Y-SrfS, +/+

X-+S / X-+S, +/+ (x) X-+S / Y-+GnL, pt/+

All matings should be set up in duplicate to ensure that at least one will produce offspring.

Stock source:

Prof. Klaus Kallman, the New York Aquarium, 6/93 and 8/93.

Xiphophorus mixei, Sol

Female

Male

Strain Code: Sol

Phenotypes scored: None

Introduction:

The progenitors of this stock were originally collected from the Rio del Sol, on the Isthmus of Tehuantepec in Oaxaca, Mexico, in 1992 by Morizot, Kallman, Borowsky, and Isla. The stock of Sol fish at the XGSC were acquired from Dr. Don Morizot's laboratory at the University of Texas M. D. Anderson Cancer Center, Science Park Research Division on November 22, 2002. This species was originally described in Kallman, et al. (2004).

This newly described species, like *Xiphophorus clemenciae*, lacks a row of spots in the dorsal fin, as found in *X. helleri*. The *X. clemenciae* and *X. mixei* populations differ, however, in the color of the spots at the base of the caudal fin (red in *X. clemenciae*, and black in *X. mixei*), as well as in number (fewer spots are seen in Sol fish). In addition, the sword tapers more rapidly in Sol fish. Males show a reddish coloration, although it was noted that the father of one of these broods showed no trace of this red coloration (K. Kallman, pers. comm.).

Sexing:

Fish are sexed at about 3 months of age. Males should be kept in small numbers to allow them to mature quickly because they mature considerably slower than do the females. After completing sexual maturation, they can be combined with the other males of their pedigree. Sex determination has not been established; however, this stock is extremely prone to biased sex ratios.

Scoring:

There are no polymorphic pigment patterns currently scored in Sol fish maintained at the Stock Center. Initially, these fishes were scored for the presence or absence of 5 lateral stripes. However, the data did not yield results consistent with a simple genetic model of inheritance and scoring of this trait was discontinued. Sword color should be noted to ensure there is no variation in this trait.

Maintenance:

Because of biased sex ratios, a larger number of matings should be used to maintain this stock, about 6, if possible. Reciprocal matings are used. Also, at least one tank of females and one tank of males should be kept for the previous generation until males and females of the new generation have been positively identified.

Stock Source:

Dr. Don Morizot, the University of Texas M. D.Anderson Cancer Center, Science Park Research Division, 11/22/02.

Xiphophorus monticolus, El Tejon

Female

Male

Strain code: Tej

Phenotypes scored: Sword color (Or)

Introduction:

The recently described *Xiphophorus monticolus* resembles *X. clemenciae*. One primary character aligning this group with *X. clemenciae* is the shape of the dorsal fin. It is square in males of both *X. clemenciae* and *X. monticolus* fish, in contrast to the more rectangular shape seen in *X. helleri.* Like *X. clemenciae*, these fish are small to moderate in size. Also in common, there are no macromelanophore or tail-spot patterns, the caudal fin shows a convex shape immediately above the sword, and has no marginal pigmentation. The sword is orange-ish in color with black lining the upper and lower margins of the sword. The axillary stripe that extends from the base of the pectoral fin past the gonopodium is less distinct in El Tejon males, i.e., it is diffuse and faint, as is a black line between the eye and lips. Differentiating *X. monticolus* from *X. clemenciae* is one yellowish, faint stripe that runs from the opercle toward the caudal fin and the presence of black spots at the base of the caudal fin. This species was originally described in Kallman, et al. (2004).

Sexing:

Fish are sexed at 3 to 4 months of age. The chromosomal mechanism for sexual determination is unknown and this stock is prone to biased sex ratios.

Scoring:

Although no variation in phenotypes has been noted, sword color is scored in this stock using a black background and a dissecting microscope. The stock appears to be fixed for orange sword color (Or).

Maintenance:

The El Tejon stock is maintained with reciprocal crosses between two or more different pedigrees. Typically 5 or 6 matings are set up for each generation. Also because of the occurrence of biased sex ratios at least one tank of females and one tank of males should be kept

from the previous generation until males and females of the new generation have been positively identified.

Stock source:

The Stock Center received the first *X. monticolus* El Tejon fish from Klaus Kallman on April 4, 1995. This shipment comprised 5 wild caught fish, 4 females and 1 male. These fish gave rise to 4 different pedigrees of the El Tejon fish, which have been used to propagate this stock in the Stock Center since 1995. An additional shipment of fish that originated in the wild was received from Dr. Kallman in May, 1997.

Xiphophorus multilineatus

Female

Male

Strain code: multi

Phenotypes scored: Body color, blue and wild-type (+)
Maturation rate, large (L)
Tail pattern, caudal blotch (Cb).

Introduction:

This stock arrived at the Stock Center from Dr. Mike Ryan's Laboratory in Austin, Texas, on January 16, 2003. Males of this stock express a blue body coloration (blue) and mature late and at a large size (L). The tail pattern, caudal blotch (Cb), is segregating in this stock also.

Sexing:

Fish in this stock should be sexed at about 2 to 3 months of age. Males may take six months or more to become fully mature.

Scoring:

Males and females should be scored for body coloration in this stock. Because the blue coloration seems to be limited to the males, females should appear wild-type (+). Both males and females in this stock are scored for Cb. All mature fish should be measured upon discarding or fixing in order to collect data on maturation rates.

Maintenance:

About four to six matings using reciprocal crosses are set up for each generation in this stock. In each mating there should be one fish expressing Cb and one fish with no Cb expressed. This will maintain Cb in a heterozygous state as Cb/+.

Stock source:

Dr. Michael J. Ryan, University of Texas, 1/16/03.

Xiphophorus multilineatus, Rio Coy

Female

Male

Strain code: CoyIII

Phenotypes scored: Body color, blue and wild-type (+)
Maturation rate, small (s).

Introduction:

Originators of this stock arrived at the Stock Center from Dr. Manfred Schartl's laboratory in Wuerzburg, Germany, on July 3, 2001. Males of this stock express a blue body coloration (blue), mature early, and at a small size (s).

Sexing:

Fish in this stock should be sexed at about 1.5 to 2 months of age because of early maturing males.

Scoring:

Males and females should be scored for body coloration in this stock. Because the blue coloration seems to be limited to the males, females should appear wild-type (+). All mature fish are to be measured upon discarding or fixing in order to collect data on maturation rates.

Maintenance:

About six matings using reciprocal crosses are set up for each generation in this stock. Although there are not many varying color patterns associated with CoyIII, production trouble has been noted and this high number of matings will help ensure enough offspring are produced to perpetuate the stock.

Stock source:

Dr. Manfred Schartl, University of Wuerzburg, Germany, 7/3/01.

Xiphophorus nezahualcoyotl, General Introduction

The holotype for this species was collected from a drainage basin in Mexico, the Arroyo Gallitos, Tamaulipas, in 1985. However, representatives of this species have appeared in earlier collections (e.g., by Rosen in 1960) and have been classified as *X. montezumae*. This species is closely related to *X. montezumae*, showing similar markings including multiple zigzag lateral stripes and a well-developed reticulum. However, these two species differ in overall body shape (truss analysis shows significant differences) and in sword size and shape. The sword index (the ratio of sword length to standard length) averages 0.56 in *X. nezahualcoyotl*, as opposed to 1.0 in *X. montezumae*. The sword is upturned in *Xiphophorus nezahualcoyotl*, and straight in adult *X. montezumae*. *X. nezahualcoyotl* has a smaller adult maximum size (average standard length is 48 mm vs. 62 mm in *X. montezumae*), but a deeper caudal peduncle (Rauchenberger, et al., 1990). The grave spot is well developed in this species. Populations of *X. nezahualcoyotl* show polymorphisms for an autosomal micromelanophore pattern, caudal blotch, and for the yellow xanthophore pattern in the sword. Populations of *X. nezahualcoyotl* are also polymorphic for a macromelanophore spotting pattern on the flank.

X. nezahualcoyotl is restricted to two tributaries each of the Rio Tamesi (Tamaulipas) and the Rio Santa Maria (San Luis Potosi, Mexico) (Rauchenberger et. al 1990). All show a high degree of morphological, phenotypic, and allozymic variability. Two stocks of *X. nezahualcoyotl* are currently maintained at the Stock Center, El Salto and Ocampo.

Xiphophorus nezahualcoyotl, El Salto

Female (M/+)

Male (M/+)

Female (++)

Male (+/Cb)

Stock code: El Salto

Phenotypes scored: Macromelanophore pattern (M) and wild type (+)
Caudal blotch presence (Cb) or absence (+)
Size at maturity in males, i.e., early maturing and small (S) or late maturing and large (L).

Introduction:

The El Salto stock originated from a collection at Rio Salto de Agua, San Luis Potosi, Mexico, in 1965. Members of this stock were introduced into the Stock Center in 1992. These fish are characterized by a large dorsal fin and a thick caudal peduncle. The male possesses a relatively small sword when compared to *Xiphophorus. montezumae*. In addition, this stock carries sex-linked alleles for macromelanophore patterns. El Salto fish also show variation for an autosomal trait, caudal blotch (Cb and +), located at the base of the caudal fin. In addition, males show differences in the timing of sexual maturation, early differentiation (small size, S) or late differentiation (large size, L).

Sex determination / sexing:

The chromosomal sex determination mechanism is XX / XY. El Salto fish are sexed at two different times, depending on whether the pedigree is early maturing or late maturing. Early maturing males can be sexed at two months of age. Late maturing males are sexed at 3 to 4 months of age. All females show sexual differentiation at 2 to 3 months of age. The early males can reach sexual maturity at 3 months of age, while late maturing males are mature at 5 months of age or later. Also, early males may mature as small as 25 mm standard length (i.e., are small), while late males generally exceed 30 mm in length and may reach 40 mm in length (i.e., are large).

Scoring:

The macromelanophore pattern (M) is generally scored by placing the fish in an observation dish and observing them with the naked eye. Caudal blotch (Cb) is scored with the microscope. Scoring is more accurate on anesthetized animals. Maturation age and size are recorded for males.

Maintenance:

Three kinds of matings are set up to maintain all of the existing genotypes. These include two X-linked genotypes, [macromelanophore-pattern (X-M) and wild-type (X-+)] three Y-linked genotypes [wild-type & small (Y-+S), macromelanophore-pattern and small (Y-MS), and macromelanophore-pattern and large (Y-ML)] and the autosomal tail pattern, Cb. Matings should be designed so that all of the genotypes are discernable among the progeny. For example, one can not distinguish an X-M Y-MS male from an X-+ Y-MS male. Moreover, all matings should contain one parent that expresses Cb and one that does not. This ensures that Cb will be maintained in a heterozygous state (Cb/+).

A total of six matings are usually set up for each generation. A typical mating plan will look as follows:

X-+ X-+, Cb/+	(x)	X-+ Y-MS, +/+
X-+ X-+, Cb/+	(x)	X-+ Y-ML, +/+
X-M X-+, +/+	(x)	X-+ Y-+S, Cb/+

These three matings should be set up in duplicate, resulting in six matings. This helps ensure that all of the chromosome will be maintained in successive generations.

Stock source:

Prof. Klaus Kallman, the New York Aquarium, 9/10/92, 10/21/92, and 5/12/93.

Xiphophorus nezahualcoyotl, Ocampo

Female (+)

Male (ML)

Female (M)

Male (+OL)

Strain code: Ocampo

Phenotypes scored: Macromelanophore patterns and associations,
X-linked macro-pattern (X-M),
Y-linked macromelanophore-pattern associated with late maturation (Y-ML),
Y-linked wild type allele at the macromelanophore gene linked to orange sword color and late maturation (Y-+OL), and
X- linked wild type (X-+).
Both X-chromosomes are associated with early maturation (S).

Introduction:

The Ocampo stock originated from a tributary of the Rio Tamesi west of Ocampo, Tamaulipas, Mexico, and was brought to the Stock Center in 1984. There are genes in this stock that control maturation rates (P-factors) linked to the X and Y-chromosomes. An allele for early maturation and small size (S) is found on all X-chromosomes in this stock. All Y-chromosomes carry a P-factor for late maturation and large size (L). The males carrying the Y-linked wild-type allele also carry a single allele at a second Y-linked gene for orange sword color. The Y-

chromosome that carries the + allele at the macromelanophore locus also possess a factor that causes orange sword coloration. Y-chromosomes with the factor for early maturation occur in the natural population, but are not represented in this stock.

Sex determination / sexing:

Females are XX; males are XY. Ocampo fish can be sexed at 3 months of age. Males will reach sexual maturity at 4 to 6 months of age. Females are sexually mature once the gravidity spot is apparent, at about 4 months of age.

Scoring:

Several phenotypes are scored in the Ocampo stock. Some of the X and Y chromosomes carry a dominant allele for a macromelanophore pattern (M) as well. Both M and + alleles are maintained by mating heterozygous M (M+) fish with wild-type fish (++) resulting in a 1:1 ratio of wild-type to spotted offspring. The Y+OL chromosome is maintained by mating X-+ Y-+OL males to either ++ or M+ females. Females in this stock may be difficult to score for the M pattern and may not display spotting until after sexual maturation. It is, therefore, best to score the fish once they are fully mature. Sword color should be scored under the microscope with a black background as is done when scoring any sword color of any given strain. Males of this stock have been noted to express a pseudogravidity spot (pgs).

Stock Maintenance:

At least six matings are set up for each generation as reciprocal crosses. Special attention should be given to preserving the wild-type X-chromosome. At least two matings should be set up to produce wild-type males and females. The following mating schemes should ensure these objectives:

$X^{+}X^{+}$ (x) $X^{+}Y^{+OL}$ → 2 matings

$X^{+}X^{+}$ (x) $X^{+}Y^{M+L}$ → 2 matings

$X^{M}X^{+}$ (x) $X^{+}Y^{+OL}$ → 2 matings.

Matings with X^{M} males may also be used, as long as the Y chromosome with the M-allele is preserved.

Stock source:

Prof. Klaus Kallman, the New York Aquarium, on 9/10/92, 10/21/92, and 6/11/93.

Xiphophorus nigrensis

Female

Male

Strain code: nigrn

Phenotypes scored: Caudal blotch (Cb) and wild-type (+)

Introduction:

Specimens of *Xiphophorus nigrensis* were collected in a headwater cave of the Rio Choy, San Luis Potosi, Mexico, by Dominic Isla on February 17, 1999 and were received at the Stock Center on September 22, 1999. This stock represents the progeny of a single, wild-caught female. *X. nigrensis* resembles *X. pygmaeus* and was originally classified as a subspecies of *X. pygmaeus.* Not only is *X. nigrensis* similar in size and shape, but it shows similar coloration and pigment patterns. Both possess a single, dense mid-lateral stripe, which appears solid at birth, but separates into several lines over time. There are additional coalesced zigzag stripes along the lateral surface that are similar in appearance in both species. A unique character seen in both *X. nigrensis* and *X. multilineatus*, but not in any other swordtail species, is that the sword shows allometric growth. Consequently, the sword index is not constant in mature males, unlike other species, where this index is relatively constant. The sword is composed of unbranched rays and is often upturned and often lacks pigment in the distal dorsal surface of the sword (Kallman and Atz, 1967; Rauchenberger, et al., 1990).

Sexing:

Fish are sexed at two to three months of age.

Scoring:

Caudal blotch (Cb) is scored in this stock and is easier to observe in anesthetized animals using the dissecting microscope.

Maintenance:

Five to six matings should be established for each generation using reciprocal crosses. Cb should be maintained in a heterozygous fashion (Cb/+) if the fish are available. This is done by continually mating a Cb expressing fish to a fish lacking Cb. One may also want to set up 2 of

the matings using only fish not expressing Cb (+) to help ensure that the wild-type allele is not lost.

Stock source:

Dominic Isla, 9/29/99.

Xiphophorus pygmaeus

Female

Yellow Male

Tail Yellow Male

Wild-type Male

Strain code: pygIII

Phenotypes scored: In male, yellow (yel), tail-yellow (Ty) and wild-type (+)
In females, wild-type (+).

Introduction:

The *Xiphophorus pygmaeus* pygIII stock was collected in the town of Huichihuyan, Mexico, at 21° 28' 48.1" N, 98° 58' 0" W by Dr. Michael. Ryan of the University of Texas. This collection arrived at the Stock Center on May 18, 2002. The stock contains three male phenotypes, yellow (yel), tail-yellow (Ty) and wild-type (+). Females only express the + phenotype.

Sexing:

Fish are sexed at about 2 months of age. They mature at about 6 months of age.

Scoring:

Scoring the fish is done with the unaided eye. The fish can be caught and placed in an observation dish to discern if it is +, yel or Ty. Females, as well as males, should be scored for

these color variations. All mature fish should be measured upon discarding or fixing in order to collect data on maturation rates.

Maintenance:

Males inherit color patterns from their fathers. Because of this, 2 matings are set up to maintain each of the three male phenotypes. This results in 6 total matings for each generation. All females are wild-type and can be used in reciprocal crosses for the generation. A typical mating plan will look as follows:

2 + matings:

++ female (x) ++ male

2 yel matings:

++ female (x) yel male

2 Ty matings:

++ female (x) Ty male

Stock source:

Dr. Michael J. Ryan, University of Texas, 5/18/02.

Xiphophorus signum

Female

Male

Strain code: Signum

Phenotypes scored: Grave spot (Gr)

Introduction:

The original strain of *Xiphophorus signum* was collected in the Rio Chajmaic, Alta Verapaz, Guatemala, in 1963. The 'Ch' strain survived for more than 11 generations of inbreeding, but usually exhibited severely aberrant sex ratios (the first seven matings at the New York Aquarium produced 22 males and 205 females, Kallman and Atz, 1966) and was eventually lost. The current *X. signum* stock was received from Dr. J. H. Schroder, Munich, on September 28, 1993, but was derived from individuals of uncertain history. These fish were probably obtained from aquarist Manfred Meyer and were likely unrelated to the earlier strain.

This strain is a member of the southern swordtail clade and was described as a subspecies of *X. helleri* (Rosen and Kallman, 1969; Morizot and Siciliano, 1982). Dr. Rosen (1969) elevated *signum* to specific status with the most notable character being the presence of a grave spot, a micromelanophore pattern in the caudal fins of males and females. *X. signum* is not known to have ever successfully hybridized, i.e., produced offspring, with any other *X.* species (Kallman, pers. comm.).

Sexing:

X. signum fish are sexed at 4 to 6 months of age. It is important that sex ratios be determined as early as possible, in case of an extreme bias in sex ratios, and additional matings are needed to ensure adequate production of the next generation. Always retain at least one tank of males and one tank of females from the previous generation until it is positively established that males and females exist in the current generation.

Scoring:

The grave pattern (Gr) of micromelanophore spots typically involves the anterior portions of the first through fifth ventral bifurcated caudal fin rays and first appears in fish two to three weeks old (Kallman and Atz, 1966; Rauchenberger et al., 1990). This pattern is present in all *X*

signum fish and thus need not be scored individually, except to note obvious deviations from the norm in expression. The grave spot becomes part of the dorsal black margin of the sword in males, which makes the *X. signum* sword appear to have a very black anterior margin. The presence of the grave spot in females led Kallman and Atz (1966) to suggest that in *X. signum*, expression of the grave pattern has become independent of androgenic hormonal control.

Maintenance:

X. signum is maintained as an inbred strain. Four to six reciprocal crosses are set up between offspring from at least two different pedigrees when available to establish the subsequent generation.

Stock source:

Dr. J. H. Schroder, Munich, Germany, 9/28/93.

Xiphophorus variatus, Encino

Female

Male

Strain Code: Encino

Phenotypes scored: Black gonopodium (Gn), dorsal yellow and tail yellow (DyTy) or tail red (DyTr).

Introduction:

This strain of *Xiphophorus variatus* was collected by Dr. Kallman from the Arroyo Encino at Encino, Tamaulipas, Mexico. The arroyo flows into the Rio Sabinas of the Rio Tamesi system. The strain shows the black gonopodium (Gn) macromelanophore pattern, as well as the xanthophore/ erythrophore patterns dorsal yellow and tail yellow (DyTy) or tail red (DyTr). The black gonopodium trait is linked to the X-chromosome and is sex-limited, thus it is only exhibited in mature males. Three sex chromosomes are present:

X^+ (no macromelanophore pattern)
X^{Gn} (black gonopodium)
Y^+ (no macromelanophore pattern)

Although males of this strain develop dorsal yellow (Dy) and tail yellow (Ty) [sometimes called Tail Orange (To)], the inheritance is not well understood since these traits are sex limited to mature males. At least in the Rio Panuco basin (and perhaps elsewhere), all *Xiphophorus variatus* males may develop yellow dorsals and either red or yellow caudal fins. Caudal fin pigmentation in the Zarco and Encino populations is autosomally determined. The evidence for this was provided by the observation that, when strains with yellow or red caudal fin coloration and differently marked Y chromosomes were crossed with each other, in the F_2 generation the marker gene on the Y's segregated independently from caudal fin coloration (Kallman unpubl.). On the other hand, another *X. variatus* strain was reported to have these patterns on the sex chromosomes, but this evidence is not compelling (Anders et al., 1973).

Sex determination / sexing:

The male of this species is heterogametic (XY); the female is hemogametic (XX). Fish are sexed at about 2 months and become sexually mature at about 5 months of age. Sexual maturation in males can be inhibited by the presence of other males through a social and behavioral mechanism (see Borowsky 1973, 1978, 1987).

Stock maintenance:

This stock is maintained with 2 sets of crosses.

1st generation:

$X^{+}\ X^{+}$ (x) $X^{Gn}\ Y$ (2 of these matings)

All female offspring will be X-Gn X-+ and all male offspring will be X-+ Y.

$X^{+}\ X^{+}$ (x) $X^{+}\ Y$ (2 of these matings)

All female offspring will be X-+ X-+ and all male offspring will be X-+ Y.

All of the fish produced by the above matings are distinguishable and can be used to set up crosses for the next generation.

2nd generation:

$X^{Gn}\ X^{+}$ (x) $X^{+}Y$ (2 of these matings)

Female offspring will either be X-Gn X-+ or X-+ X-+, but these genotypes cannot be told apart. Do not use these females for next generation matings. The male offspring will either be X-Gn Y or X-+ Y. These male phenotypes are easily distinguishable and all of the males can be used for matings to produce the next generation.

$X^{+}\ X^{+}$ (x) $X^{+}\ Y$ (2 of these matings)

All female offspring will be X-+ X-+ and all male offspring will be X-+ Y. Any of these fish can be used to set up the next generation of crosses. The females, in particular, will be the only ones needed to set up the next generation.

After this generation has produced, the mating scheme will rotate back to the crosses designed for the 1st generation.

Stock source:

Prof. Klaus Kallman, the New York Aquarium, 8/20/93.

Xiphophorus variatus, Lineatus

Female

Male

Stock code: Huich

Phenotypes scored: Pigment pattern, Lineatus (Li) and wild type (+)

Introduction:

Progenitors of this strain of *Xiphophorus variatus* were collected from the Rio Huichihuayan, San Luis Potosi, Mexico. The Stock Center obtained these fish in 1996 from M. Schartl, who had maintained a stock in Germany. This stock has an X-linked lineatus pigment pattern gene, which is expressed as horizontal stripes on the flank. There is also a vertical barring pattern expressed in some dominant and aged males.

Sex determination / sexing:

Sex determination is XX / XY. Fish are sexed at 1.5 to 2 months of age and mature at about 5 months of age.

Scoring:

The phenotypes scored are the lineatus pattern (Li) or wild type (+). These traits must be scored under the dissecting microscope, particularly in younger fish and females.

Maintenance:

This stock is propagated by inter-pedigree crosses. The goal of the design of the matings is to maintain the lineatus pattern as well as wild type alleles. Three or four matings are set up crossing a lineatus expressing fish by a wild type fish. Another two matings are set up to maintain the wild type allele by crossing a wild type female with a wild type male. Ideally, at least two to three pedigrees are available at any time and the successive generation is produced via reciprocal crosses between pedigrees. If a brood is not produced after three months, the male is checked for sperm and a female is artificially inseminated.

Stock source:

Prof. Manfred Schartl, Wuerzburg, Germany, 1996.

Xiphophorus variatus, Zarco

Female (+, C)

Female (P-1, +)

Female (P-2, C)

Male (P-2Gn, +)

Strain code: Zarco

Phenotypes scored: Pigment patterns punctatus 1 (P-1) and punctatus 2 (P-2),
Black gonopodium (Gn),
Tail spot pattern crescent (C).

Introduction:

This strain of *Xiphophorus variatus* was collected from the Arroyo Zarco locality west of Encino, Tamaulipas, Mexico. The arroyo Zarco joins the arroyo Encino. The locale is described by Richard Borowsky (1984). The strain was kept at New York University until it was moved to University of Texas M. D. Anderson Cancer Center by Dr. Steven Kazianis. This strain is one of the few where individuals can develop melanoma in the absence of hybridization. The P-2 pigment pattern can eventually result in progressively larger black blotches as an animal ages. After about 1.5 years of age, these fish can develop external melanotic nodules (Borowsky, 1973 B; Schartl et al., 1995). Usually, this occurs in animals that are homozygous for P-2.

Sex Determination / sexing:

The male of this species is heterogametic (XY) and the female is homogametic (XX). Fish are sexed at 2 months and become sexually mature at approximately 4 months of age. In this species, early maturing males can inhibit maturation of other males by a "social mechanism" (see Borowsky 1973 B, 1978, 1987).

Scoring:

All phenotypes exhibited in this stock should be scored when the fish have reached full maturity. The strain shows 3 macromelanophore pigment patterns and a tail spot micromelanophore pattern, as well as a xanthophore / erythrophore pattern. The chromosomal linkage of these is as follows:

X-P-2 (Punctatus-2)
X-+ (wild type)
Y-P-1Gn (Punctatus-1 linked to Black Gonopodium)

This strain also possesses a micromelanophore tail spot pattern called crescent (C). This is autosomally inherited (LG XVII; Kazianis, Morizot, Nairn, Borowsky unpublished). The strain has a chromosome with C and the corresponding chromosome is wild type (+).

Although some males of this strain may develop Dorsal Yellow (Dy) and Tail Yellow (Ty) [sometimes Tail Orange (To)], the inheritance is not worked out since the trait is sex limited to mature males. Klaus Kallman has observed at least one *X. variatus* strain where such xanthophore/ erythrophore patterns are autosomally inherited. Some males may also develop a blue hue. These colorations may also be related to male dominance (see Borowsky, 1973 A.)

Crossing-over between the X and Y chromosomes is believed to have occurred several times and was manifested by females with P-1. In one case, such a crossover resulted in an X chromosome with P-1 and the Gn linked. A test cross was performed to verify this. Stock Center data also indicates a large frequency of XY females occurring in this stock. This evidence is supported by the occurrence of P-1 females and biased sex ratios. Proper care must be taken to ensure production of males and fish with the proper genetic make up. This is complicated by the fact that P-2 will mask P-1 and will be further discussed in the maintenance section.

Maintenance:

Two crosses should be set up for one generation. These are as follows:

X-+ X-+, C/+ (x) X-+ Y-P-1Gn, +/+

and

X-+ X-+, +/+ (x) X-P-2 Y-P-1Gn, C/+

Each type of cross needs three replicates. This makes a total of six matings set up for each generation. This mating scheme will compensate for biased sex ratios and masking of P-1 in P-2 females. The P-2/+ females are needed in the next generation. For each mating one fish is C and the other is + with respect to the tail pattern. This will ensure that C is maintained in a heterozygous state and that neither C or + is lost.

For the successive generation the following crosses must be established:

X-+ X-+, C/+ (x) X-+ Y-P-1Gn, +/+

and

X-P-2 X-+, C/+ (x) X-+ Y-P-1Gn, +/+

Also set up three replicates each for this generation. Greater numbers of fish are required to compensate for any skewed sex ratios and/or unexpected phenotypes. These two crosses will generate the proper fish needed for the original set of matings. Be careful in all generations to score fish accurately and rescore matings after they have been set up for a while and the fish have had a chance to age.

Stock source:

Dr. Steve Kazianis, New York, 9/6/96.

Xiphophorus xiphidium, Peduncular Spot

Female (+)

Male (Ps ct)

Stock code: Ps

Phenotypes scored: Tail pattern, peduncular spot (Ps), cut crescent (ct), and wild type (+).

Introduction:

Progenitors of this stock were collected from a small stream at Santa Engracia, Tamaulipas, Mexico, by Dr. Klaus Kallman in 1962. This site is geographically isolated from San Carlos, the locality of *Xiphophorus xiphidium* Sc. This locale is also a short distance from Rio Purificacion, a river inhabited by other populations of *X. xiphidium*. The *X. xiphidium* Ps stock is so named in reference to an allele present in this stock that encodes the tail spot pattern, peduncular spot (Ps).

Sexing:

X. xiphidium Ps fish are sexed at 2 months of age and rechecked a month later.

Scoring:

These fish can be scored for the three different tail patterns as soon as they reach sexual maturity (4 to 5 months old). As the fish age, the tail pattern may spread and Ps can mask 'ct' or '+' phenotypes. Three-dimensional growth can be associated with this pattern and should be noted.

Maintenance:

The mating scheme for this stock requires two generations to complete and requires fish from the *X. xiphidium* Sc strain. This scheme ensures that all of the tail spot patterns will be maintained. A heterozygous Ps fish (Ps +) is mated with a *xiphidium* Sc fish, known to be homozygous for cut crescent (ct ct). This mating produces both male and female offspring that are Ps ct and ct+. The first generation offspring are then inbred to produce the second generation, i.e., a Ps ct fish is crossed to a ct + fish. This mating produces both males and females that are Ps +, Ps ct, ct +, and ct ct. All fish, except the Ps+ fish, are discarded and the entire process is repeated. The mating scheme is listed below:

1st generation parentals:	Ps stock (Ps+)	(x)	Sc stock (ct ct)	(P_1)
F_1 phenotypes/genotypes:	Ps ct	,	ct +	(F_1)
2nd generation parentals:	Ps ct	(x)	ct +	(P_2)
F_2 phenotypes/genotypes:	Ps +, Ps ct, ct +, ct ct			(F_2).

Stock source:

Prof. Klaus Kallman, the New York Aquarium, 6/24/93.

Xiphophorus xiphidium, Rio Purificacion

Female (fl, ct)

Male (fl, +)

Strain code: RP

Phenotypes scored: Tail spot pattern, cut-crescent (ct), crescent (C), wild type (+)
Spotting pattern, flecked (fl) and wild type (+).

Introduction:

This stock arrived at the *Xiphophorus* Genetic Stock Center 11/9/98, from M. Schartl's laboratory in Wuerzburg, Germany. This stock was collected in the Rio Purificacion, near Barretal, Tamaulipas, Mexico. A pigment pattern, flecked (fl-1), described by Kallman and Atz (1966), is segregating in this stock. The inheritance of the flecked pattern is reported to be X-linked in the stock. Three alleles for tail spot patterns are segregating in the stock; crescent (C), wild type (+) and cut crescent (ct).

Sexing:

Fish of this stock are sexed at 1.5 to 2 months of age. These fish seem to mature earlier than the other stocks of *Xiphophours xiphidium.* The results of the sex designation should be confirmed after 2 weeks.

Scoring:

Fish are scored for the presence or absence of the flecked spotting pattern and the three possible tail spot alleles C, ct and wild-type (+).

Maintenance:

Six matings are set up as reciprocal crosses each generation for stock maintenance. Three of these matings are established to ensure production of flecked and wild type fish with crescent in a heterozygous state. To ensure heterozygosity of the tail pattern, one parent in a cross should be C and the other +. Another three matings are set up to produce flecked and wild-type animals as well. However, these other three matings are also designed to maintain cut-crescent in a heterozygous state by mating a ct fish to a + fish.

Stock source:

Prof. Manfred Schartl, Wuerzburg, Germany, received 11/9/98.

Xiphophorus xiphidium, San Carlos

Female

Male

Stock code: Sc

Phenotypes scored: Tail pattern, cut crescent (ct)

Introduction:

These fish were collected from a small spring in the Sierra San Carlos, Tamaulipas, Mexico. This stock is fixed for the allele encoding the cut crescent (ct) tail pattern. Two spots comprise this pattern: the more ventral spot appears fainter than a spot located on the dorsal edge of the tail fin. No other color patterns are scored. Occasionally, exophidic, 3-dimensional growths are associated with the tail pattern.

Sexing:

Individuals of this stock mature at a later age, about 4 to 6 months, than those of the RP strain. Fish are sexed at about 2 months of age and sex designation is then verified 2 to 4 weeks later.

Scoring:

This stock is fixed for the tail pattern ct, but presence for this pattern is scored and confirmed in fish at maturity.

Stock maintenance:

The Sc fish are maintained as a separate stock from the Ps stock. Three to four reciprocal crosses are set up to propagate each generation. The Sc fish are also used to maintain the Ps stock. Enough offspring must be produced (about 40 to 50 offspring) from two or more pedigrees to maintain both stocks. Sc is only used to maintain Ps every other generation and numbers can be adjusted accordingly.

Stock source:

Prof. Klaus Kallman, the New York Aquarium, 5/12/93.

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Appendix

"How the *Xiphophorus* Problem Arrived in San Marcos, Texas,"
K. D. Kallman, Marine Biotechnology, 3, S6-S16, 2001.

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MARINE BIOTECHNOLOGY

How the *Xiphophorus* Problem Arrived in San Marcos, Texas

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The Genetic Stock Center can trace its origin to Myron Gordon's doctoral research at Cornell University. He began his scientific work in 1924 while still an undergraduate student with 6 platyfish of a domesticated stock, and the following year he added some swordtails. Platyfish and swordtails were not new to him because already as a teenager he had kept many tropical fish. But a great enthusiasm for genetics was aroused by Professor A.C. Fraser in the Department of Plant Breeding. Other professors at Cornell who took a great interest in his work were H.D. Reed (Zoology), who was his official sponsor, and G.C. Embody (Limnology) and R.A. Emerson (Plant Breeding). Dr. Gordon concentrated first on platyfish, working out the genetics of various pigment patterns, and then on swordtails. He coined the terms micromelanophore and macromelanophore (Gordon, 1926).

It is an incredible coincidence that at the same time, Dr. Curt Kosswig in Germany also started research on swordtails. Neither Gordon nor Kosswig knew of the other's existence. But their initial contributions to the *Xiphophorus* melanoma problem were not the same (Atz, 1941). Kosswig (1928) pointed out that the melanoma was hereditary and in some way associated with melanophore patterns from the platyfish, while Gordon (1931) clearly identified the macromelanophore gene of *X. maculatus* as being responsible for the tumor.

The complementary relationship between laboratory and field research that has made the study of *Xiphophorus* so exciting and successful was initiated early in Gordon's career, and one hopes it will be continued in the future. Whereas the development of melanoma in certain platyfish-swordtail hybrids was predictable, the sporadic appearance of such growths in some platyfish presented a problem. While working on his thesis at Cornell, Gordon reasoned that platyfish were known only from the hobby and had been bred for many years under domestication and hybridized with swordtails. He could not determine whether platyfish of natural populations also developed pigment cell abnormalities or whether the occurrence of melanoma in the domesticated platyfish was due to the introgression of *helleri* genes. The sample of 83 platyfish from Mexico in museum collections was too small to provide the answer. He also thought it possible that platyfish and swordtails might hybridize in nature, because Meek (1904) had found them together in one location. He must go to Mexico himself!

None of the modern collecting paraphernalia were available to Gordon. There were no maps or rental cars, and only a few paved roads. He went in a model T Ford, loaded with camping equipment, shotguns, letters of introduction to every conceivable agency, and with milk and oil cans for shipping fish back to the United States.

In 1930 only 6 of the 22 presently described species of *Xiphophorus* were known, and virtually nothing was known about their habitat and distribution. Platyfish were assigned to *Platypoecilus* and swordtails were known as *Xiphophorus.* Gordon knew that 3 scientific collections of platyfish had been made. The first consisted of 2 fish collected by La Salle prior to 1866 in "Central America," a locality description utterly useless if you are trying to find the fish (Gordon,

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1947a). A second collection of 13 fish in 1867 by Dr. Francis Sumichrast, an ornithologist, came from Cosamaloapan on the Rio Papaloapan, Veracruz, Mexico, where the Smithsonian had maintained a field station for several decades. The third collection of 68 fish was made by Seth E. Meek in 1902 (Meek, 1904), near the railroad station of El Hule, Oaxaca, Mexico, the last stop of the Transisthmian Railroad before it passed over the Rio Papaloapan into the state of Veracruz. Meek, an ichthyologist, had traveled by railroad from Chiapas in the south to the state of Nuevo Leon in the north. He detrained every other stop to collect fishes and then continued his journey the next day. Myron, in his search for platyfish, made the most sensible decision: he headed for the Rio Papaloapan.

Gordon's 1930 Mexico Expedition was financially supported by the National Research Council and by the Museum of Zoology of the University of Michigan, which wanted to enlarge its fish collection. The expedition consisted of three men: Gordon, Edward Creaser, a malacologist from Cornell, and Ricardo Ostos, M.D. They drove to Laredo and had to ford the Rio Grande, then proceeded through Monterrey along the foot of the Sierra Madre to Ciudad Victoria. The section from Monterrey to Ciudad Victoria was unpaved and every stream had to be forded. They learned to wrap their socks around the sparkplugs to keep them dry. This entire distance of 267 km took 10 hours to drive. From Ciudad Victoria they continued to Ciudad Valles and then headed southeast to Tantoyuca, crossing the Rio Moctezuma at El Higo. Here they stayed for several days on Ricardo Ostos' ranch and explored the surrounding area on horseback (Figure 1). One day, 20 km south of Tantoyuca, they collected 4 immature swordtails near the Rio de los Hules and Rio Calaboza, which Gordon identified as *X. montezumae.* Actually, these fish represent the first specimens of *X. birchmanni,* a species not described until 1987. No further collections of *Xiphophorus* were made in this region until 1985. From Tantoyuca the expedition headed east to the Estero Cucharas, a small stream flowing into the Laguna Tamiahua, about 75 km south of Tampico. The road conditions were so horrendous that they had to rest for half an hour every 3 km. From there they turned south toward the Rio Cazones and then climbed up over terrible roads across the headwaters of the Rio Tecoluta to the Mexican Plateau. The Rio Tecoluta watershed is characterized by steep canyons and waterfalls several hundred meters high. In small streams on a narrow plateau above the falls at 1200-m altitude Dr. Gordon discovered a platyfish, somewhat similar to *X. variatus,* many years later described as *X. evelynae.* A series of hydroelectric power plants and dams had been constructed in this rather inaccessible region, and undoubtedly Gordon was aided by Claudio Martinez, an alumnus of Cornell and an engineer with the Mexican Power Company. The expedition headed for Mexico City, where they rested, and then drove to Veracruz.

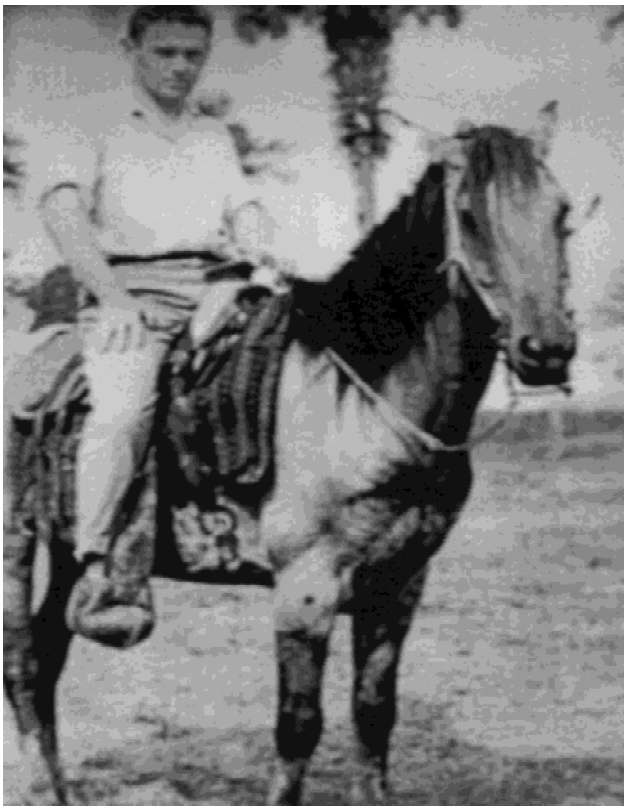

Figure 1. Dr. Myron Gordon at Tantoyuca, Veracruz, Mexico, 1930.

On the way they collected swordtails, *X. helleri,* at Jalapa and were puzzled by the many large males with incompletely formed gonopodia and undifferentiated gonads (Gordon, 1937a). The only record that I can find about their next move states that they hired a boat at Alvarado and headed up the Rio Papaloapan toward Cosamaloapan and El Hule, 45 and 85 km, respectively, to the south, but more than twice that distance by river. A railroad spur from Veracruz ends at Alvarado, but I do not think they took the train when they could have traveled on another one to El Hule. It is also unlikely that they took the Ford there, because the track to Alvarado on the narrow high ground between the high dunes to the left and the marshes to the right must have been blocked by the ever-present sand drifts. Gordon placed Alvarado only 20 miles from Veracruz; however, it is actually twice that far. I think he may have hired the launch at Boca del Rio, 20 km south of

Veracruz, and followed the coast to Alvarado, because he wrote many years later, "we sailed up the ocean-like mouth of the river" (Gordon, 1940). This is an apt description if you enter the river from the Gulf.

The quest for *X. maculatus* soon came to an anticlimactic end. The current of the river was swift and the motor of the little launch was too weak. The river meandered endlessly and appeared to follow the contour of a pretzel. For every mile they covered in a straight line, they had to travel three. The marshes on both sides of the river seemed to extend forever. Finally they reached Cosamaloapan, but then the rains came, and they had to turn around without having seen a single platyfish. They returned to Mexico City and made the slow trip north via the Mexican Plateau as far as San Luis Potosi. There they picked up a track through the Sierra Madre, passing through Tula (elev. 1400 m), still today a rather small, isolated desert town, to Ciudad Victoria (elev. 300 m). The section from Tula to Victoria was so horrible that Myron declared he would never make this trip again. On the way back the expedition collected *X. couchianus* near Monterrey, and introduced live specimens for the first time to the United States. Although no *X. maculatus* were caught, the trip was nevertheless a success. Over 100 species of fishes were collected, of which 10 were new to science ("Introducing Dr. Myron Gordon, *The Home Aquarium Bulletin,* March 5, 1935).

On March 6, 1932, the model T Ford was again heading south. Gordon was accompanied by John Ross and Joe Whetzel, sons of Cornell faculty members. According to Gordon (1940), the great business depression had been good for the tropical fish hobby, inasmuch as people stayed at home and became interested in tropical fish keeping. The Cornell University Mexico Expedition 1932 was supported by private funds from hobbyists, commercial tropical fish breeders, and the Heckscher Research Foundation at Cornell. The Shedd Aquarium in Chicago and the New York Aquarium also promised to buy exotic fish that the expedition hoped to bring back. The expedition arrived at the border in Laredo on March 12th, where it was unexpectedly delayed. Myron had shipped by Railroad Express Agency 120 one-gallon cans to the border, to be reshipped to various small Mexican railroad stations near areas where the expedition hoped to collect fish. The cans, painted on the inside with asphaltum varnish, were the shipping containers. A requested permit to bring the empty cans across the border free of customs duty, because they would eventually be returned to the United States, had not arrived. The customs officer demanded US $1.00 per can ($12.49 in current dollars), and this was far more than Gordon could afford. Frantic telegrams exchanged between Laredo and Mexico City elicited sympathetic advice but led nowhere. Why don't you buy goldfish, Myron was told, and place them into your jars? There is no duty on importing goldfish into Mexico, and your cans then become the shipping containers, which are not taxed. But Gordon eventually paid. He also complained about the price of gasoline, which was US $0.35 per gallon ($4.38 in current dollars), and remarked that nobody trusted Mexican gas pumps. Gasoline was first pumped into well-marked cans to measure accurately the amount purchased (Figure 2).

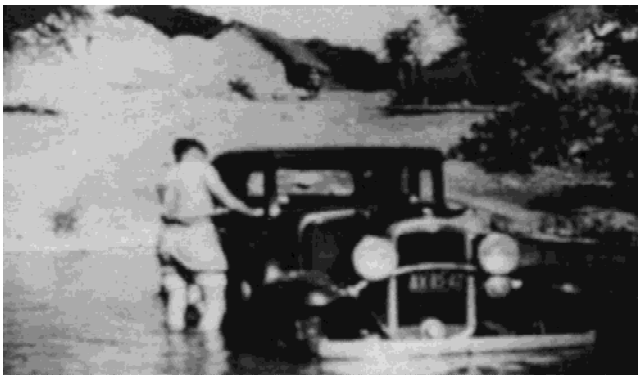

Figure 2. Dr. Gordon's model T Ford in Mexico, 1930.

Once beyond Monterrey the spirits of the expedition soared. The road was paved now with macadam or consisted of packed gravel and the many streams had all been bridged. Five hours later they arrived in Ciudad Victoria. The expedition was not eager to drive again the Victoria–Tula segment, and they had heard about a new road being built to Mexico City via Tamazunchale. The more they inquired from the locals about road conditions ahead, the more contradictory the information became. Finally they settled on going via Tula. They left early in the morning and arrived totally exhausted in Tula, long after nightfall, with an empty gas tank, having covered the 140 km in 15 hours. Afterwards Gordon (1940) wrote he could not understand what made him drive this route again. I presume he chose a known quantity, even if it was difficult, over something completely unknown. They experienced every imaginable road hazard: huge rocks strewn across the track, hidden steep ravines, axle-deep ruts, narrow mountain passes, and enormous climbs and descents. The road crossed seven ranges. The remainder of the drive across the Mexican Plateau to Mexico City, which took 8 days, was a "diabolical trial: parched, barren, dusty and death dealing". But they survived and rested up in comfort in Mexico City.

Today, Tula is not considered such a bad place for *Xiphophorus* researchers. Only 15 years ago we learned of a level path through the barrier range that leads to a valley at 1000-m elevation, only 20-km distant, with a small stream teaming with swordtails, *X. nezahualcoyotl.*

Early in April the expedition arrived in Veracruz, where they stored the model T. From there they proceeded in relative comfort by local train to El Hule, which had been renamed Papaloapan. For a belated impression of the joys of riding this train, the reader is referred to Theroux (1979). Papaloapan lies near the fall line. To the east of the railroad track stretches the low coastal plain with its enormous swamps, muddy lagoons, and oxbows, and to the west we find the beginning of the Piedmont of the Sierra Madre del Sur. Today, a narrow vehicle bridge crosses the river at Papaloapan with a toll station at its southern (Oaxaca) side. Truck traffic on the road is as busy as on the New Jersey Turnpike because it provides the least mountainous access to Puebla and Mexico City from the southern states. A new superhighway built through the marshes 30 km to the east is barely used because of its excessive toll rates.

But in 1932 the area was a sleepy backwater. The Standard Fruit Company had banana plantations in this area with wonderful facilities for its administrative staff. With letters of introduction to military and civil authorities, the second Cornell expedition was taken in by the Fruit Company and installed like royalty. After camping out for nearly a month, it felt wonderful. "The perfect service and comfort of our headquarters meant more to us than a paid-up, de luxe suite of rooms at Sun Valley Lodge" (Gordon, 1940). The company's officers must have looked on the three New Yorkers as strange little fish, indeed, to have traveled 3000 miles to catch 1-inch-long fish! Every facility of the company was placed at Dr. Gordon's disposal: vehicles, boats, horses, and guides. Three days' fishing effort yielded not a single platyfish. Gordon had not yet discovered its exact ecological niche. He thought platyfish, being rather broad and somewhat deep-bodied, could not be a stream fish, but must prefer quiet waters without current. So he concentrated on the nearby oxbows and swamps. From my own experience, I know how utterly depressing it can be to have come so far and fished for many days without catching a single specimen. Myron must have felt likewise, but he merely wrote they were "quite discouraged." Eventually, they headed upstream by boat from company headquarters and entered the Rio Tonto and one of its little side streams. And there Gordon caught his first *X. maculatus!*

Excitedly, and no doubt with satisfaction, he telegraphed his success to Cornell. On April 17, 1932, the *New York Times* carried a long article with the headlines: "Cancerous Fish Found in Mexico," "Oaxaca Jungle Pierced." A direct quotation from the telegram reads, "We found by actual count ninety-nine platyfish, some of which are suspiciously suffering with a small degree of melanosis. There is one in which the condition is well seen."

And of course the publicity hounds of Cornell sprang into action and added that the Cornell expedition attained its objective, "deep in the jungle recesses of the Mexican state of Oaxaca." In reality, the jungle had been long gone and the only *Xiphophorus* person, if we may call him that, to rhapsodize about the luxuriant vegetation was C.B. Heller, a 21-year-old German botanist who landed in Veracruz in 1845 (Siemens, 1990). One of his objectives had been to scout for areas suitable for German colonists, and while doing so he collected in the Rio Blanco drainage the first swordtail, which now carries his name. This river is the northernmost tributary to the Rio Papaloapan estuary, and the general area where Heller found the swordtail is approximately 100 km northwest of the village where Gordon caught his first platyfish.

The next day, the platyfish and also some swordtails were placed in the cans and successfully shipped by railroad to a commercial fish breeder in New Orleans. Later in the summer when it was warm up north, they were reshipped to Cornell. "Melanotic" platyfish, however, were not to be. Closer examination later showed that the darker fish merely had a well-expressed spot-sided pattern, and the single most heavily pigmented one exhibited a new pattern, called spotted-belly, Sb (Gordon and Smith, 1938a), or black-bottomed (Gordon, 1948), but there was no melanosis. It remains the only *Sb* fish that has ever been caught in the Rio Papaloapan drainage, although fish with somewhat similar patterns have been taken in some of the river systems to the east (Kallman, 1975). Next day the expedition members packed their bags and retraced their steps to Mexico City to collect goodeid fishes on the Mexican Plateau to satisfy their sponsors in the aquarium trade. Later, before the return trip north, Myron inquired at the Mexican Automobile Association about the new road being constructed beyond the old towns of Zimapan and Jacala to Tamazunchale at the foot of the Sierra Madre; they were not going to pass through Tula again. Assured that on average 12 cars per day traveled over this road, the expedition opted for the new highway (Gordon, 1933).

For 160 km beginning just beyond Zimapan the road passes from 2000-m elevation through lush forests of pine,

then cloud and deciduous forests reminiscent of the Smokies, to the tropical lowlands at Tamazunchale at about 300 m. The road crosses not a single stream along its entire length; it follows closely the top of the long divide between the Rio Amajaque to the east and the Rio Moctezuma to the west because this minimizes the ups and downs across mountain ranges. In several places the road straddles the very top of the narrow divide. There were long stretches where the road was still under construction. Not a single piece of mechanical equipment was used, only dynamite, crowbars, and pickaxes.

The expedition reached the Rio Moctezuma at Tamazunchale, but was unable to fish. The main river is too deep and swift as it comes out of a gorge, and Myron could not locate any side channels or small tributary streams. They pushed on another 30 km and were stopped at the Rio Axtia. Men used mules to pull the cars through this river, but it was late in the day and the men had stopped working. Seining in the river near their campsite yielded Montezuma swordtails, *X. cortezi,* and beautiful *X. variatus.* Many of these fish were preserved. The next morning they rushed to the railroad station in Ciudad Valles, 75 miles to the north, to claim their cans, which had been waiting for them for well over 6 weeks. The expedition returned to the Rio Axtla and filled 12 cans with 20 fish each. The next day the fish were successfully shipped by railroad to New Orleans. Farther north other successful collections of *X. xiphidium* were made. The expedition returned to Cornell by the end of May. Sorting through the preserved collections, Gordon was eventually left with a single fish that he could not identify; it turned out to be the first pygmy swordtail.

As early as 1931, Gordon had been wondering how many genes were involved in the exaggerated expression of the macromelanophore genes in hybrids with *helleri.* But he considered it an almost impossible task to determine: "While it is probable that many modifying factors are operating in *Xiphophorus* which influence the degree of melanosis, it is hopeless to establish the exact number without increased facilities" (Gordon, 1931). Like every investigator after him, Gordon needed more tanks! Between 1932 and 1938 Gordon made good use of the fishes he had brought back; he crossed *X. maculatus* from the Rio Papaloapan with 3 other species, *X. variatus, X. xiphidium,* and *X. couchianus.* In each of these crosses the expression of the macromelanophore gene of *X. maculatus* was enhanced, but to different degrees. The *maculatus* × *couchianus* hybrids were especially interesting because they exhibited the neoplastic disease at birth. Thus the modifier genes of these species could not be the same, but the question of whether they were different alleles at the same locus, or different genes altogether, was not addressed (Gordon and Smith, 1938a). These experiments also established that the occurrence of melanoma and pigment cell abnormalities was not restricted to *maculatus* × *helleri* hybrids, but was a general phenomenon in *Xiphophorus.*

Gordon (1937b) employed the concept of multiple factors to explain the formation of melanoma in the hybrids. Closely following Kosswig (1931) in Germany, he hypothesized that 2, but perhaps many more, modifier loci were contributed by *X. helleri.* But it is not clear from his data how he arrived at this conclusion, and a year later Gordon and Smith (1938a) stated the the 2-factor hypothesis was really a somewhat arbitrary construct. Later in the same year, Gordon and Smith (1938b) wrote that the swordtail contributes apparently more than one dominant modifier. Gordon thought that the two species, *maculatus* and *helleri,* possessed different alleles at the 2 loci, aa bb and AA BB, respectively. Those hybrids exhibiting the most advanced state of melanosis were thought to have inherited, in addition to the macromelanophore factor, all 4 dominant modifiers. But there was no independent confirmation that this interpretation was correct.

Detailed histological descriptions of the abnormal macromelanophore patterns were provided by Reed and Gordon (1931) and Gordon and Smith (1938b), who classified the melanotic overgrowths into 3 stages. The first stage is characterized by a macromelanophore hyperplasia in the corium, the second stage by an invasion of the muscular tissues by macromelanophores along the myoseptae with some tissue destruction of fin rays and the soft tissue between them, and the third stage by the appearance of invasive spindle-shaped cells, significant tissue destruction, and melanotic overgrowth. No metastases were observed. They emphasized that there is no sharp separation between the 3 stages and that the development of the pigmentation can stop at any stage. The histological description, of course, was important not only in its own right, but also because it served to make the *Xiphophorus* melanoma system highly pertinent to the medical community at large. "The spindle cells of the melanotic overgrowths in hybrid fishes histologically resemble the cells of mammalian melanosarcoma. They also are infiltrative and destructive to adjacent tissue" (Gordon and Smith, 1938b). This was followed 3 years later by a report of growing the *Xiphophorus* melanoma in tissue culture. "In its morphology and behavior, including its property of clasmatosis, it is identical with

the melanoblast of the mouse and human melanoma" (Grand et al., 1941). The fish melanoma had established its credentials.

In 1937 Professor Reed, who had been Gordon's sponsor for more than 12 years, died. Latent opposition to Gordon's project now manifested itself. Not only was Myron forced to relocate his laboratory, he also lost his financial support from the Heckscher Foundation at Cornell. Gordon, perhaps introduced by Professor G.M. Smith of Yale, then turned to Dr. Charles M. Breder, Jr., the director of the New York Aquarium. Breder had his own ideas of what a public aquarium should do. It should not be only a place to exhibit aquatic life and perhaps provide some education to the public, it should also carry on its own unique research. At the Aquarium scientists were already experimenting with electric eels, and the research on *Xiphophorus* melanoma seemed unique and important. Gordon was invited in.

In 1938 the New York Aquarium was located at the Battery in Castle Clinton, at the southern tip of Manhattan. The building had been constructed in 1807 as a fort in New York Harbor, known as the South-west Battery. Fourteen years later, after the fort became obsolete, it was ceded to New York City and given a new name: Castle Clinton. It was connected to Manhattan by a bridge and later by landfill and remodeled to serve as a place of entertainment until the mid century. Between 1855 and 1890 New York City served as the main landing place for immigrants, and a total of 7,690,606 passed through the building. After 6 years of extensive renovation, Castle Clinton reopened in 1896 as the New York Aquarium.

Gordon built his new laboratory with several hundred aquaria on the second floor above the larger exhibit tanks of the exhibition hall (Figure 3). He had also become a Fellow of the John Simon Guggenheim Foundation and secured a generous grant. The 101 platyfish he collected in 1932 were not sufficient. A new trip to Mexico was in the planning stage, one that he had been thinking about since 1932 (Gordon, 1940). He hoped to stay at Papaloapan for several weeks to study the fate of platyfish populations as the dry season progressed and the lagoons shrank in size and small arroyos dried out. In the meantime platyfish had been found at several places in the Rio Usumacinta system in Guatemala, indicating that *X. maculatus* might have a wide distribution. He had also heard about lakes in Chiapas near the border with Guatemala, and that was where he was going to search for platyfish. He still thought that platyfish preferred quiet waters, notwithstanding that he had caught none in the lagoons near Papaloapan. He must check out the lakes!

Figure 3. Dr. Gordon and his laboratory setup above the main exhibition tanks in the old New York Aquarium at the Battery, Manhattan, 1939.

In January 1939, Gordon drove south again, with Mr. J.W. Atz of the Aquarium. The car was again placed in storage in Veracruz, and they rode the train across the Isthmus of Tehuantepec to Arriaga near the Pacific coast. This was a daylong ride. From there they took a bus through the hot canyons and bone-dry mountains to Tuxtla Gutierrez, capital of the state of Chiapas, where they stayed overnight. The next day they continued by bus the slow agonizing crawl up the Sierra. The landscape certainly changed. They were now on the lush Atlantic versant of the mountains. The temperature dropped not only because the road kept climbing, but also because of the cloud cover and frequent fog. There were still magnificent forests covering the mountains. Upon their arrival at San Cristobal, well over 2000 m above sea level, they must have realized that their quest for platyfish had turned into a wild goose chase. They never made it to the lakes. The following morning, with the help of a local guide, they found a stream and seined it, but they collected only *Profundulus.* The area was too high for poeciliid fishes.

There are no records of how Gordon had learned about the lakes, and obviously he knew few details. The only lakes of any size in this region are the Lagunas de Montebello, now in a national park, a little bit more than 125 km beyond San Cristobal, and at much lower altitude. The lakes are within the range of *X. alvarezi.* Was it really such an outrageous idea to look for platies near San Cristobal? Taken out of context, it was a failure because none were brought back. But, in retrospect, I think Gordon was right to go there. He was interested in platyfish distribution. They had

been reported from Guatemala. He had found *X. helleri* and what is now known as *X. evelynae* at an altitude of 1200 m, and we now know that *X. cortezi, X. malinche,* and *X. xiphidium* may occur at similar elevations. "World Aeronautical Charts" of southern Mexico, which Gordon consulted, carried the following warning: "reported elevations could be off by several hundred meters." There was also a very important psychological factor involved. If it is really important to you to find out what *Xiphophorus* might occur in a certain region, you have to go there yourself and look; otherwise, the uncertainty is going to prey on your mind for years. It took also courage to go to Chiapas in 1939. It was one of the least developed areas of Mexico, and the natives were even more hostile to outsiders than they are today.

After picking up Evelyn Gordon, Myron's wife who had arrived in Veracruz by boat, they headed for Papaloapan, where they were again welcomed by the Standard Fruit Company. For an entire week they again searched for platyfish in the wrong location in the broad lagoons east of the railroad track, not realizing that their fish were close by. A small part of the swamp is isolated between the railroad and the higher ground to the west from which small springs were flowing. This part of the swamp drains through a few openings in the railroad embankment to a gradually widening lagoon (Zacatispan). Not much has changed since 1939 except that the swamp has been divided once more by Mexican Highway 145, about 100 to 200 m to the west of the railroad. It was in the springs and in the small shallow pools that formed in the swamp that Gordon caught his fish. On February 20, in a pool near railroad marker 149 km, they collected 954 platyfish. From another pool 500 m away, 1937 platyfish were collected between March 4 and 10. Over several weeks the expedition caught more than 3310 platyfish. Even though none of the fish exhibited *Sb,* they had hit the platyfish jackpot (Gordon, 1940).

More importantly, Dr. Gordon now recognized the precise habitat of *X. maculatus:* "Four species of platyfish, *P. couchianus, P. xiphidium, P. variatus* and *P. maculatus* have this point in common: all of them may be found in relative large numbers in regions of springs" (Gordon, 1947a). I may add this also holds true for 4 species of platyfish that have been discovered since then.

The most important platyfish turned out to be the strains Jp 163 A and 163 B. They have been distributed to many laboratories in the United States and Europe and form the baseline for many *Xiphophorus* studies. Their origin was a mere accident. In March 1939, after lengthy discussion at Papaloapan, it was decided that Atz would return to New York by steamer from Veracruz with a live subset of the Papaloapan platyfish. While he was waiting in Veracruz for passage to New York, disaster struck. The platyfish in the shipping cans developed "Ich" and died. Desperately, Atz hit upon a plan. If platies are so common in the Rio Papaloapan, perhaps they also occur in the Rio Jamapa drainage, which is the next, albeit small, river system to the north? He took the train out of Veracruz to Plaza de Agua (El Tejar), near today's Veracruz airport, where the railroad crosses a small tributary of the Rio Jamapa. This looked like a likely spot, and platyfish there were. Several hundred were collected and preserved, and several dozen were brought alive to New York. The experiment designated number 20, a mating of a spot-sided female, *Sp*/+, with a spotted-dorsal, stripe-sided male, *Sd/Sr,* gave rise to pedigree 163 (Gordon, 1947b). These fish and their descendants have been inbred by brother-to-sister matings ever since. After 9 generations the strain was split into 2, carrying *Sd* and *Sp* on their X chromosomes, respectively. Jp stands for Rio Jamapa. Strains Jp 163 A and B were born!

In 1971 I revisited the site. An abattoir, a mere roofed-over cement slab, stood next to the small brook, which received blood and other offal. There was nothing here but stinking mud. Seining was futile. But 3 km away in a small roadside stream I caught platyfish, some of which I brought back alive to the stock center in New York. One fish exhibited the spotted-dorsal pattern, Sd. For a number of reasons we maintained this gene and noticed minor, yet consistent differences in phenotype as compared with Sd in Jp 163 A. It turned out that two *Sd* factors exist in Jamapa. When *Sd* of Jp 163 A is introduced into *X. couchianus,* its expression is suppressed whereas that of *Sd* from the 1971 collection is enhanced. This illustrates that when patterns within a population appear identical, it does not necessarily mean they are caused by the same gene.

The Genetic Stock Center stayed at the New York Aquarium at the Battery for only a short time. Coney Island, the big amusement center of Brooklyn, which had its heyday in the early part of the 20th century, and the surrounding neighborhoods were steadily deteriorating. The New York City administration thought the area could be upgraded by relocating the New York Aquarium to Coney Island. Today only the outer shell of Castle Clinton remains. Inside, there is a ticket booth for the Statue of Liberty ferry, and at night rock concerts are held here.

The Genetics Laboratory found new quarters on the

6th (top) floor of the American Museum of Natural History, where three rooms of the bird department were not being used. The rooms had high ceilings and a roof that consisted of glass panels sloping somewhat towards the west. Each room had a western exposure that consisted almost entirely of glass. During the summer, the roof and windows were repeatedly whitewashed and a sprinkler system ran cold water over the roof. Nevertheless, during heat waves the water in the aquaria sometimes climbed to 32°C. At these temperatures the fish had brood intervals of 18 to 19 days. The fish did well.

In 1948, Gordon organized the first of a series of Pigment Cell Conferences. In his contribution to it, he analyzed "the effects of five primary genes on the site of melanoma" (Gordon, 1948). This paper is important, because all 5 macromelanophore genes of *X. maculatus* are listed here together, and it gives the impression, and certainly Gordon thought, that there are only 5 alleles at the macromelanophore locus: *Sd* (dorsal-spotted), *Sp* (spot-sided), *Sr* (stripe-sided), *N* (black-sided), and *Sb* (black-bottomed). In reality, the situation is much more complicated: *X. maculatus* exhibits 5 or 6 basic macromelanophore patterns corresponding largely to Gordon's 5 alleles. But there are consistent differences within each pattern between the 7 major river systems, and these differences are not caused only by genetic background, but rather reside within a particular macromelanophore factor (Kallman, 1970, 1975). Thus, instead of a single *Sd* factor, there are at least 7, perhaps even more because some populations may have more than one. The same holds true for the other patterns. Thus within this species at least 35 macromelanophore factors exist. There are additional macromelanophore factors in other species of *Xiphophorus.* One should also not forget that each collection in nature is only one tiny snapshot in place and time. I do not believe that Gordon and I have found every macromelanophore gene. Only a small fraction of these factors has ever been tested to determine how they are expressed in interspecific hybrids.

In 1948, Gordon still believed in his artificial scheme of at least 2 unlinked modifier loci involved in melanoma formation, but he seemed to lose confidence in it. He was puzzled why in crosses with *helleri* the *Sd, Sp,* and *Sb* factors readily gave rise to melanomas, whereas few tumors developed in *Sr* and *N* hybrids. In his opinion, different macromelanophore factors responded to different modifier systems, similar to gene interactions at the complex tail-spot locus (Gordon, 1956). For example, the modifier locus *E* interacts with one, and only one, *Co,* of the 9 tail spot alleles, and a second modifier locus, *Cg,* changes only the expression of *T.* In other words, 2 unlinked modifier loci may interact with *Sd, Sp,* or *Sb,* while 2 other loci may modify *N* or *Sr.* Dr. Gordon (1951a) expanded on this idea: "Apparently, the platyfish *Sr* gene requires a number of genic modifiers in homozygous state to induce the formation of the melanotic tumor." These ideas are somewhat similar to those expressed by Kazianis et al. (2001), who found that the *CDKN2X* gene seems to interact with *Sp* and *Sd* of Jp 163 in crosses with *helleri* but that other factors are responsible for melanoma formation in *variatus* × *helleri* and *maculatus* (Jp 163 A *Sd*) × *andersi* crosses.

Unfortunately, Gordon rarely presented a decisive cross that provided solid data as to the number of modifiers. His *helleri* × *maculatus* crosses in the 1930s were conducted with domesticated stocks of doubtful genotypes, and those listed in 1948 were also compromised by the introduction of a domesticated swordtail stock with the albino gene. Only one cross is free from all these defects (Berg and Gordon, 1953). It involves a cross of Jp 163 carrying *Sp* and *Sd* with *X. helleri* from the Arroyo Zacatispan. The first hybrid generation was then backcrossed to either parental strain. On face value, the range of macromelanophore expression of the *Sd* genotypes seems to point to a single modifier factor and that of the *Sp* genotypes to two. But one should note that of the 64 first-generation hybrids, 16 were listed as "normal." Unfortunately, the paper failed to define "normal," and I do not recall ever having seen such a hybrid with a macromelanophore pattern that was identical with that of the *maculatus* parent (i.e., "normal").

The observation that the expression of the macromelanophore gene of *maculatus* was also enhanced when introduced into other platyfish species (Gordon and Smith, 1938b) made Gordon wonder whether a similar reaction occurs in interpopulation crosses of *X. maculatus* (Atz, 1948). He again went to Mexico in 1948 with Atz and F.G. Wood, Jr., a graduate student from Yale University, and they headed from Veracruz by train for the Rio Coatzacoalcos. They got off at Almagres and Jesus Carranza, where in 1939, unbeknown to Gordon, Coronado had collected platyfish. They obtained 485 fish from these two sites and then headed toward the Rio Tonala, where more fish were collected. For the first time, live fish from Gordon's field trips were sent by air from Mexico City to New York. Subsequently, he discovered that *Sd* of the Rio Coatzacoalcos exhibited increased expression in a Jamapa genetic background.

In 1950 Gordon attended the International Cancer

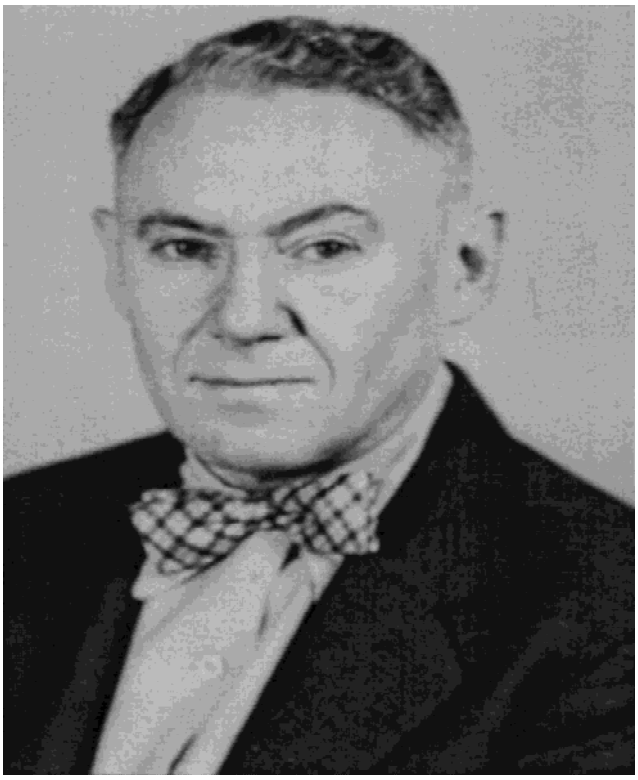

Figure 4. Dr. Myron Gordon, photograph taken in the late 1950s.

Congress in Paris, lectured on *Xiphophorus* melanoma before the Royal Society of Medicine in London on July 4, then flew to Turkey. Finally, 25 years after *Xiphophorus* research began in Germany and the United States, Gordon (Figure 4) and Kosswig met on July 5, 1950, in Istanbul (Gordon, 1952). Dr. Kosswig had invited him to speak at the university.

There are in the literature references to the "Gordon-Kosswig melanoma model" and to the "classical cross." These terms are poorly defined. I feel the term Gordon-Kosswig melanoma model should only be used to describe the rather general fact that an *X. maculatus* macromelanophore factor, when introduced into an *X. helleri* genotype, gives rise to melanoma. The term "classical" refers to something authoritative, or of long standing, or to a standard. The term classical cross is applied to matings involving Jp 163 A of *X. maculatus* with *Sd* and *X. helleri.* But because these crosses are not always made with the same *helleri* stock, the results cannot always be replicated. We are dealing with not one cross but many. The crosses with Jp 163 A and various *helleri* stocks only date back 25 to 35 years after this stock became widely distributed. Kosswig never made this cross, and Gordon only once (Berg and Gordon, 1953).

Strains Jp 163 A and B of *X. maculatus* have become the workhorses in *Xiphophorus* melanoma research. But how representative of *X. maculatus* are these stocks or the Jamapa population? The phenomenon of enhanced pigment gene expression in *maculatus* × *helleri* hybrids is also observed when the genes from other *maculatus* populations are introduced into the Jamapa stocks, except that the frequency of malignant melanoma is not as high (Gordon, 1951b; Kallman, 1970, 1975). Not enough crosses between *maculatus* populations that do *not* involve Jamapa have been made to determine whether changes in gene expression are of general occurrence in such hybrids. Preliminary evidence suggests that this may not be the case.

A gene sequence called *Xmark* (or *Xmark-2*) has been identified that maps closely to the macromelanophore locus (Schartl, 1990). It is present in all *Xiphophorus* fish with macromelanophore factors that may give rise to melanoma after hybridization, and it is absent from wild-type fish and fish with macromelanophore factors that do not change in expression after hybridization. *Xmark* has also been equated with the macromelanophore gene, and the macromelanophore gene has been called the oncogene or tumor gene, but this is misleading. There is only one *Xmark,* and it plays an important role in melanoma occurrence, but more than 50 macromelanophore factors are known from *X. maculatus* and other species. DNA sequences other than *Xmark* must control these patterns. Note that *X. variatus P-1* individuals lack *Xmark-2,* but they develop a macromelanophore pattern.

Atz (1962) applied to *Xiphophorus* the model of Fisher (1928) and Ford (1957) showing that a population with a potentially deleterious locus accumulates modifiers that will eventually mask its harmful effects. Whereas Kosswig (1931) and Gordon (1948) had always thought that the swordtail contributes specific genes that enhance the expression of the macromelanophore genes, Atz (1962), following Dr. D.E. Rosen, proposed that *helleri* and other species lack genes that control the expression of the macromelanophore factors of *maculatus.* This view was also more in line with Gordon's (1959) characterization of the melanoma cell as an incompletely differentiated pigment cell. Later, Anders (1967) in Germany expressed a similar view and presented a more detailed model. The expression of the macromelanophore gene is controlled by a regulatory gene, *Diff,* and both are species specific. Hybrids that inherit two *Diff* alleles of *helleri* develop malignant melanoma, whereas those that are heterozygous, one *Diff* factor from *helleri* and the other from *maculatus,* exhibit melanosis or benign melanoma (Anders et al., 1979). How could such an expla-

nation, so attractive in its simplicity, have escaped Gordon and Kosswig?

In a recent paper, Kazianis et al. (2001) found a highly significant correlation of the CDKN2X genotypes with pigmentation phenotypes and melanoma formation. They had tested the *Sp* gene of Jp 163 B in *couchianus* and *helleri* genetic backgrounds. In both crosses, there was a very high association between fishes with melanoma and the presence of two CDKN2X alleles inherited from either *couchianus* or *helleri.* Most fishes that did not develop melanomas were heterozygous at the *CDKN2X* locus. Similar results were obtained when the *Sd* gene of Jp 163 A was introduced into *X. helleri.* These results were interpreted as suggesting that *CDKN2X* might be the *Diff* tumor suppressor gene. Not mentioned in this paper was the well-known fact that the expression of *Sd* gene of Jp 163 A is suppressed in a *couchianus* background. Obviously, the structures of *CDKN2X* of *X. couchianus* and *X. helleri* cannot be identical. One should also recall Zander's (1969) report that the pigment gene of *maculatus* is kept constant, the degree of its expression is dependent upon the particular strain of *X. helleri.* If *CDKN2X* is one of the important players in melanoma formation in *Xiphophorus,* it may exist in different allelic states even within the same species.

Although *CDKN2X* is a likely candidate for *Diff,* the question of how many loci play a role in pigment cell proliferation after hybridization is still unanswered. The *Sd* phenotype of Jp 163 A exhibits little variation and is markedly different from that of F_1 hybrids, which show a 100-fold increase in pigmentation. A wide gulf in phenotype separated the most heavily pigmented *Sd* fish of Jp 163 A from the least pigmented F_1 hybrid. If only a single gene, e.g. *Diff,* were responsible for the change in expression, then a cross of F_1 to Jp 163 A should result in two nonoverlapping phenotypes: one identical with Jp 163 A and the other indistinguishable from that of F_1 hybrids. Why is such a simple cross not performed?

Myron Gordon died unexpectedly in 1959 at the age of 59 while Dr. Rosen and I were in San Salvador. Three weeks before, both of us, together with J.W. Atz, had received our doctorate degree from New York University under the sponsorship of Myron Gordon. Eventually I took over the laboratory, which stayed at the American Museum until 1968, when it moved to a newly built science facility, the Osborn Laboratory of Marine Sciences, at the New York Aquarium in Coney Island. I took the genetic research in a somewhat different direction focusing not only on pigment cells, but also on tissue transplantation, sex determination, and the hypothalamic-pituitary-gonadal (HPG) axis. The collaboration with the researchers in Texas dates back to 1975. The field work in Mexico, which continued at an ever-increasing rate, often with colleagues at Science Park, Smithville, and more recently, Southwest Texas State University, turned out to be extremely successful and led to many new discoveries. It was only natural that after my retirement in 1992, the laboratory would be moved to Texas.

There may be more to the segment of DNA that makes up the macromelanophore gene. The *P* locus controls the activity of the HPG axis, and its most obvious phenotypic effect is that it determines the age and size at which the fish become sexually mature (Kallman, 1989). Each species may have from 1 to 10 alleles and, as far as we know, these alleles are species specific. Depending upon its genotype, *X. maculatus* may mature as early as 8 weeks or as late as 2 years. Introduced into *helleri,* a *P* allele that causes maturity in *maculatus* at 12 weeks causes maturity in *helleri* at 3 years! Conversely, *P* alleles of *helleri* cause accelerated maturity in platyfish at 5 weeks. I find this change in *P* phenotype analogous to what happens to macromelanophore genes after hybridization. Moreover, is it a mere coincidence that the *P* locus is so closely linked to the macromelanophore gene that not a single crossover has yet been discovered? A third locus, again closely linked to the macromelanophore gene, controls pterinophore patterns. It, too, shows changes in phenotype after hybridization that range from no expression to erythrophoroma formation, albeit rarely (Gordon, 1950). I wonder whether there is not a common denominator responsible for all these changes. Perhaps, one should not focus exclusively on the macromelanophore gene, but on this entire interesting segment of DNA.

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