

GENETICS OF SPECIES DIFFER-
ENCES IN THE MORPHOLOGY
OF THE MALE GENITALIA
OF XIPHOPHORIN FISHES

MYRON GORDON AND
DONN ERIC ROSEN

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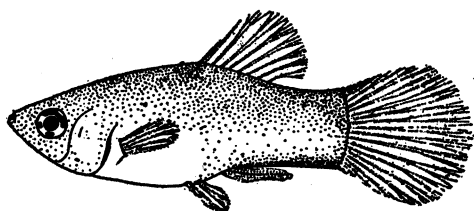
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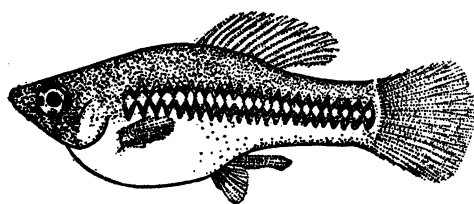
CONTENTS

INTRODUCTION	415
MORPHOLOGICAL AND TAXONOMIC ANALYSIS	417
Materials and Methods	417
The Gonopodium	418
Structure	418
Use in Courtship	421
Viviparity in Cyprinodont Fishes	421
Use in Taxonomy	422
The Gonopodium in Xiphophorin Taxonomy	424
Diagnostic Characters in Xiphophorins	424
Specific Differences in <i>Xiphophorus</i>	425
Specific Distinctions in <i>Platypoecilus</i>	427
Generalizations	427
GENETICS OF SPECIES DIFFERENCES IN THE MALE GENITALIA OF <i>Platypoecilus maculatus</i> AND <i>Xiphophorus hellerii</i>	434
Preliminary Analyses	434
Relationship between the Lengths of Fish and their Gonopodia	434
Lengths of Fish and Number of Distal Serrae	436
Lengths of Gonopodia and Heights of Distal Serrae	436
Number of Distal Serrae and their Heights	437
Inheritance of Shape of Spines in Ray 3	438
The P ₁ , F ₁ , and F ₂ Generations	438
The Backcross Generations	439
Analysis	440
Inheritance of Shape of Distal Serrae of Ray 4p	440
Index for Shape (<i>I_{DS}</i>)	440
The P ₁ , F ₁ , and F ₂ Generations	440
The Backcross Generations	443
Analysis	443
Relationship between Genetics of Widths and Heights of Distal Serrae	444
Preliminary Analysis	444
Inheritance of Width of Distal Serrae	445
Inheritance of Height of Distal Serrae	449
GENERAL DISCUSSION	450
Morphological Differentiation	450
Ecological Conditions	450
Behavioral Differentiation	450
Hybrid Sterility and Sex Chromosomes	450
Factors in Morphogenesis of Gonopodium	452
Genetics of Genitalic Characters in Insects	454
Species Differences in Genitalia as Isolating Mechanism	454
Mode of Inheritance and Evolution of Genitalic Elements in Xiphophorins	455
Taxonomic Implications	456
SUMMARY AND CONCLUSIONS	458
BIBLIOGRAPHY	460

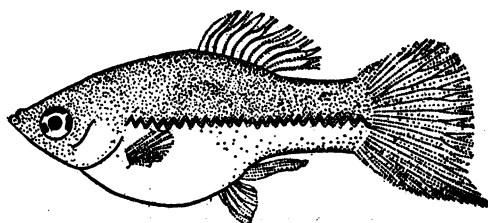
PLATYPOECILUS



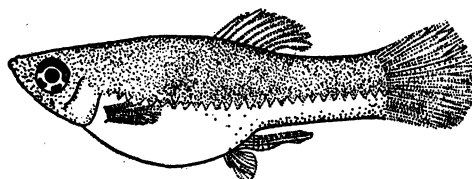
P. MACULATUS



P. VARIATUS



P. XIPHIDIUM

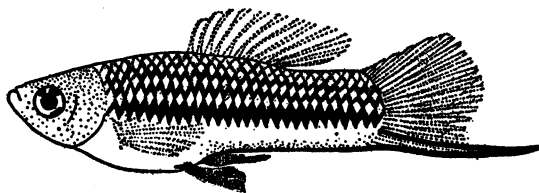


P. COUCHIANUS

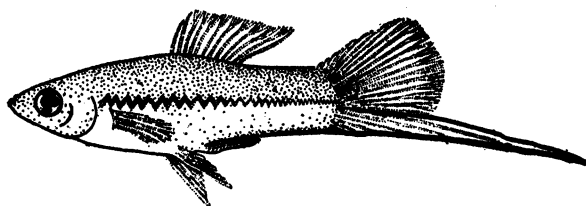
XIPHOPHORUS



X. PYGMAEUS



X. MONTEZUMAE



X. HELLERII

FIG. 1. Males of seven xiphophorin species.

INTRODUCTION

IN THE COURSE of genetic studies of normal and atypical pigment cell growth in the hybrids of the platyfish-swordtail group of fishes, relatively large numbers of mature hybrids were reared. These xiphophorin fishes provided the material for a genetic analysis of species differences as reflected in critical structures in the genitalia of the males.

In the xiphophorins, as in all members of the viviparous fish family Poeciliidae, fertilization is internal. The transfer of sperm in spermatophores from male to female is accomplished by the gonopodium, which is the elongated, stiffened, and generally highly modified anal fin. Ichthyologists have used the many fine complex and anatomic details in the gonopodia for the purpose of classifying these fishes. In a diagnosis of a newly discovered poeciliid fish today, the need for a detailed description of its gonopodium is taken for granted.

The xiphophorin fishes consist of four species of *Platypoecilus*, the platyfishes, and three species of *Xiphophorus*, the swordtails (fig. 1). In some streams and pools in their Mexican aquatic habitat two species may live together; in others, three species are sympatric. As yet not one hybrid between any of them has been found in over 12,000 xiphophorins collected in nature. Under laboratory conditions, however, not only do platyfish and swordtail species hybridize with members of their group, but platyfishes hybridize with swordtails.

Some zoologists have questioned the generic distinctness between *Platypoecilus* and *Xiphophorus*, but it is agreed that some of the xiphophorins are "good" species, particularly the sympatric ones. After considerable preliminary study of the suitability of various species, *Platypoecilus maculatus*, *Xiphophorus hellerii*, and their hybrids were chosen for analysis. These species are sympatric, living together in many localities in the Rio Papaloapan, Rio Coatzacoalcas, and Rio Tonala in Mexico and in the Rio Usumacinta in Guatemala.

Ordinarily, genetic studies of taxonomic characters that define closely allied animal species are difficult because distinct species

rarely hybridize. When related animal species do hybridize they often produce few or no viable and fertile male and female offspring. Further, fertile species hybrids when mated together seldom provide adequate numbers of second-generation individuals that attain sexual maturity. However, this paper will describe the results of analyzing 105 second-generation males derived from mating the platyfish and swordtail.

The relationship of this work, in the fields of ichthyology, comparative anatomy, genetics, and taxonomy, in short, evolution, to the subject matter of oncology may seem remote to some cancer research workers. In this connection, the senior author (1948a) has recently shown that the atypical growth of the macromelanophores leading to the progressive development of melanomas in platyfish-swordtail hybrids is the direct result of the artificial breakdown of the natural isolating mechanisms which exist between these species in their natural habitats. In nature, biological barriers prevent the two sympatric species from hybridizing, and in effect prevent an interchange of genes between the platyfish and swordtail. But under conditions of domestication the two species cross, and as a consequence of gene interchange between them new combinations of genes produce new cellular reactions in the hybrids. Specifically, the large black pigment cells, or macromelanophores, which are kept under normal control in the platyfish, grow atypically in the platyfish-swordtail hybrids.

Since the platyfish and the swordtail were derived from a common ancestral xiphophorin species, this study was undertaken in the hope of obtaining further information on the fundamental differences between the two species. This information, in turn, would be useful in tracing the biological changes that have taken place in the evolutionary history of these organisms. One of the answers being sought is the nature of the present-day incompatibility in the union of platyfish and swordtail genes.

This genetic and morphological analysis of the gonopodial elements in the xiphophorin fishes is divided into two parts. The first, the

morphological and taxonomic section, indicates the anatomic similarities and differences in the seven species. The second section presents the genetic analysis of the mode of inheritance of certain key gonopodial structures as revealed in *Platypoecilus maculatus*, *Xiphophorus hellerii*, and their F₁, F₂, and various backcross hybrids.

ACKNOWLEDGMENTS

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MORPHOLOGICAL AND TAXONOMIC ANALYSIS

MATERIALS AND METHODS

IN AN EARLIER, closely related study, Gordon and Benzer (1945) used 12 to 25 each of *Platypoecilus maculatus*, *P. variatus*, *P.*

fishes were carefully measured for standard length and depth of body and length and depth of caudal peduncle. The number of dorsal fin rays, a valuable taxonomic charac-

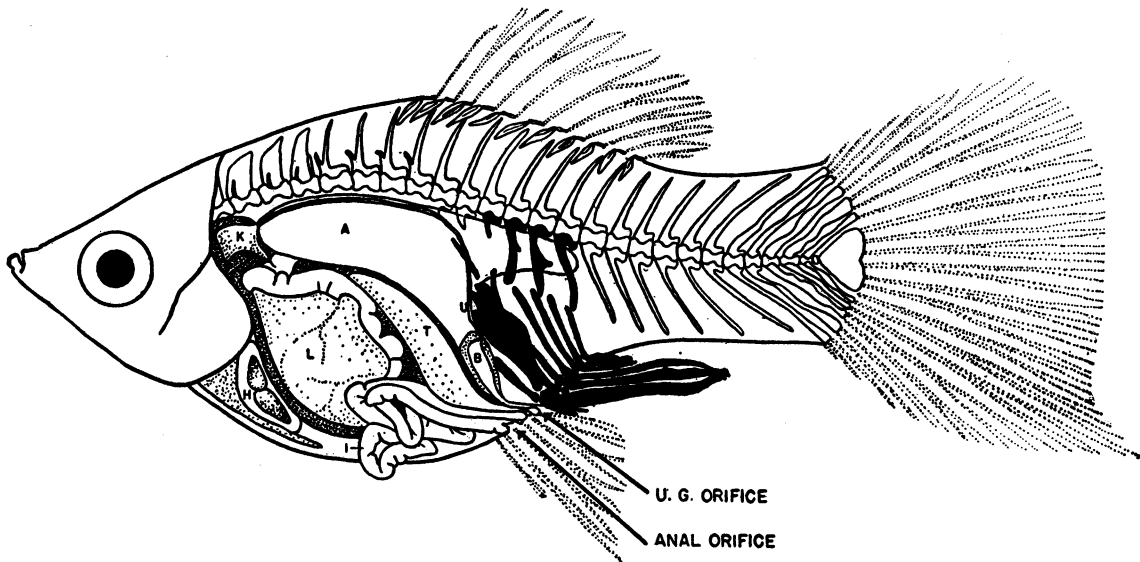


FIG. 2. The gonopodium in relation to the skeleton and organs in a male *Platypoecilus xiphidium*. The gonopodium and the gonopodial suspensorium including the gonactinosts and gonapophyses are indicated in solid black. Abbreviations: a, air bladder; b, urinary bladder; h, heart; i, intestine; k, kidney; l, liver; t, testis; u, urinary duct.

xiphidium, *P. couchianus*, *Xiphophorus pygmaeus*, *X. montezumae*, and *X. hellerii*, a total of 119 males, in an analysis of the gonopodial suspensorium system. Many of the animals used in that study were also used in this. Some additional specimens were examined, for it was found that while the gonopodial suspensoria developed normally in some males their gonopodia did not. In all, 208 gonopodia from "wild" stocks of platyfishes and swordtails were analyzed. In addition, the gonopodia of 237 aquarium-reared hybrid males were studied comparatively (fig. 2).

All the males were fixed either in 10 per cent formalin or in 70 per cent alcohol. In some instances the fishes were first killed in hot water (45° C.) before being fixed, a method worked out to cause the fins to expand and remain rigid. After fixation the

ter, was also determined. The genotypes of individuals, when known, and the phenotypes of all were recorded. The animals were stored in vials with numbered notes so that a particular gonopodium could always be referred to the individual from which it was taken. The gonopodium of each was cut close to the body. Some gonopodia were studied in fresh preparations. Then the fins were passed successively through increasing concentrations of alcohol and two changes of the absolute fluid. Later the gonopodia were transferred to xylol and finally to a drop of Canada balsam on a microscope slide. After proper orientation they were sealed under a cover slip. The gonopodia were studied under a compound microscope, and every detail of critical importance was recorded in a manner to be described.

THE GONOPODIUM

STRUCTURE

The anal fin of immature xiphophorin fishes, as well as that of the adult female is a single median appendage consisting of nine soft rays, each of which bifurcates a number of times with increasing growth. (According to general taxonomic practices in this group, the apparent tenth ray is not counted because it is merely the completely separated half of

low magnifications of a dissecting microscope, complete outlines and contours of the gonopodium can be appreciated. By a combination of these details of the external features of fresh fins with the details of the inner structures of the gonopodium in terms of its segments, such as hooks, claws, rami, serrae, and other types, the stereograms shown in figure 4 were constructed.

The broad surface along the shaft of the third ray of the gonopodium is made possible

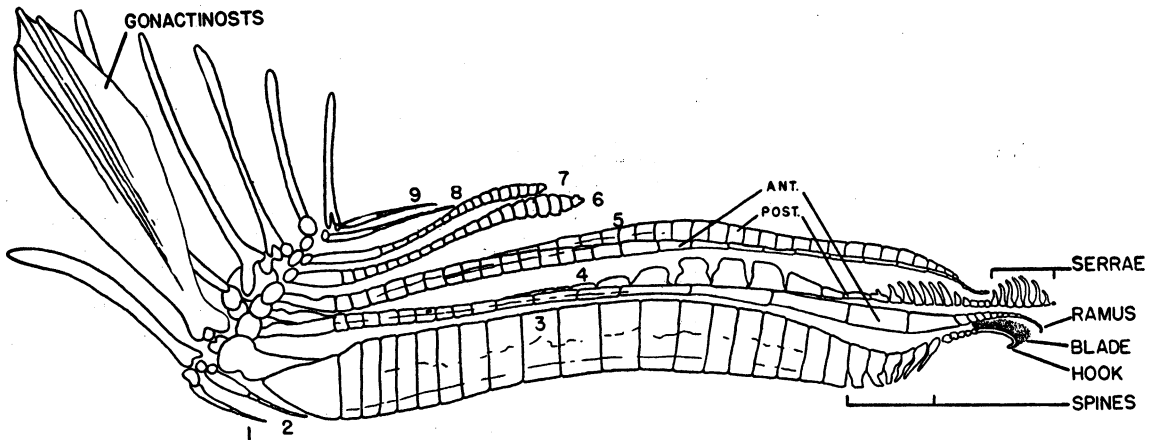


FIG. 3. Anal fin rays in the gonopodium of *Platypoecilus variatus*, including pterygial elements and gonactinosts. The most important elements in the gonopodium are contained in the third, fourth, and fifth rays which are much elongated and strengthened. The anterior (ant.) and posterior (post.) halves of rays 4 and 5 are indicated; in ray 3 they are fused.

the ninth.) In the adult female the rays are scarcely modified except for some slight strengthening of the third ray. The fourth ray is the longest, and the others are progressively shorter (pls. 17, 18).

The modified, masculinized anal fin of the adult male is a highly complicated organ. The third, fourth, and fifth fin rays, called by Langer (1913) "the 3-4-5 complex," are the longest and strongest, while the others are relatively unmodified (fig. 3). The manner in which the gonopodium articulates with its suspensorium and the way the latter is attached to the skeleton can be seen in figure 2. Details on the xiphophorins' suspensoria are given by Gordon and Benzer (1945).

When the anal fin of the male is removed and studied in a fresh state under the fairly

by the arrangement of the various segments within that ray. From observations made from cleared fins under a compound microscope, it has been determined, particularly by varying the focus, that the third ray (and every fin ray) is in reality a bilaterally symmetrical structure consisting of two almost mirror-image components, one on each side of the median plane. The distal tip of the third ray ends in a strong, enlarged, medianly fused segment which is generally called the terminal hook. When observed from above, this region is relatively thin in outline, and the distal tip is slightly notched (fig. 5). The hook characterizes the xiphophorin fishes and separates them taxonomically from other poeciliids. Adjacent to the terminal hook there is a series of small, paired, irregu-

larly ovoid, disc-like subterminal segments. The dorsal margins of the terminal hook and most of the subterminal segments adjoin the so-called "membranous hook," a tough, slightly opaque tissue. In a number of

covered by an epidermal membrane which later recedes in mature males, exposing some terminal elements.

Adjacent to the rather flat, disc-like subterminal segments in the third gonopodial

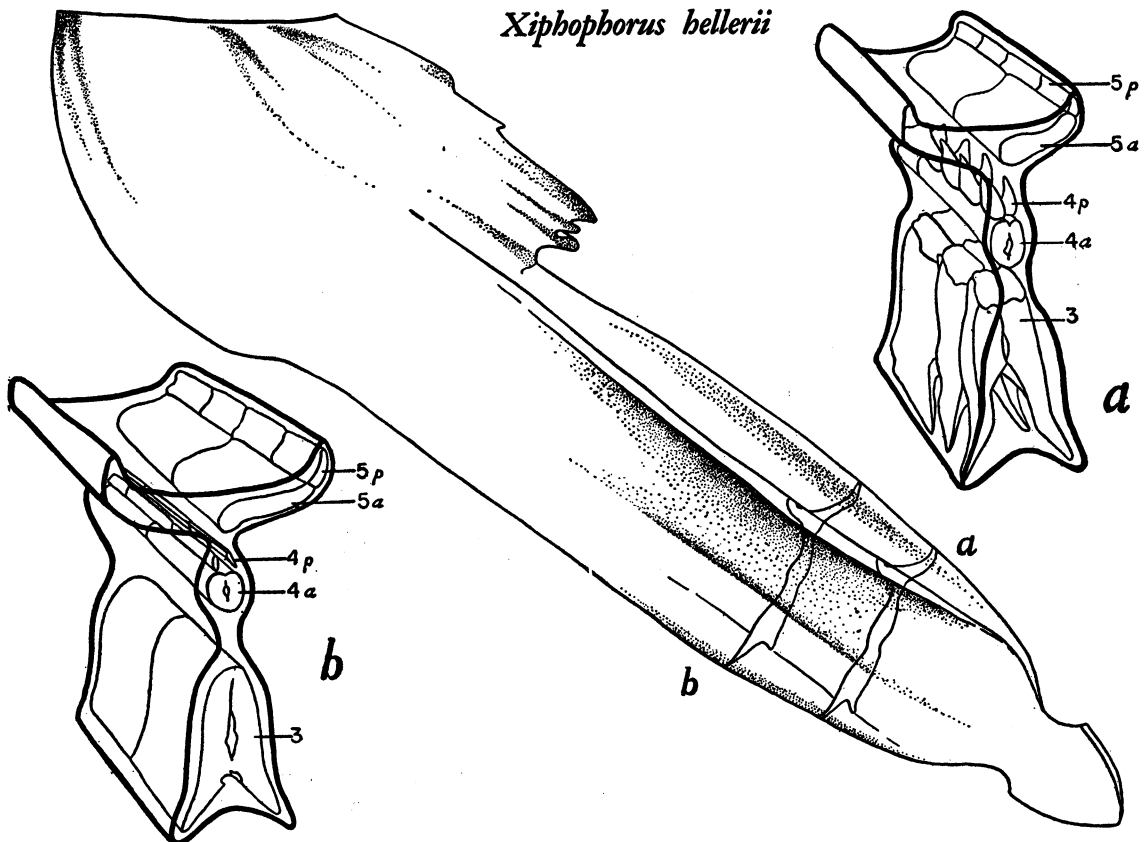


FIG. 4. Stereogram representing a xiphophorin gonopodium. The middle figure represents the contours of the entire fin. A represents an interior section of the fin taken from the distal region; B, a section taken from a more proximal area. For clarity, only one-half of the fifth ray is drawn. For detailed analysis, see text.

non-xiphophorin poeciliids this thick membrane is terminal and may function as a "hook" (Hubbs, 1924, 1926), but in xiphophorins it is always surrounded by other tissues. Grobstein (1940) called this tough tissue the blade and, incidentally, he said that it is the last structure formed in the morphogenesis of the platyfish gonopodium. The blade, the terminal hook, the subterminal segments of the third ray, and the terminal structures of the other fin rays are first

ray of the typical xiphophorin there is a series of about seven stout spines. These are all broad at their base (at their dorsal margins) but taper to narrow terminal points (ventrally). In general they are like irregularly shaped commas. Some are as crooked as a dog's hind leg. Viewed in three dimensions, each spine appears shaped like a gourd, with a fairly round base and a tapering end piece. Each spine is in reality a dual structure, so the spinous series of segments are

usually composed of seven paired elements. The tips of these spines flare away from each other to such an extent that the distance between the pointed tips of a pair of spines is greater than the width from base to base. This dual construction of the spines, when

thesis marks, (), forming in length a long, relatively narrow, rod-shaped structure. At its terminus a series of segments form a "ramus." Along the dorsal rim of the long row of rod-shaped segments of ray 4a lie those of ray 4p. The distal tip of ray 4p is

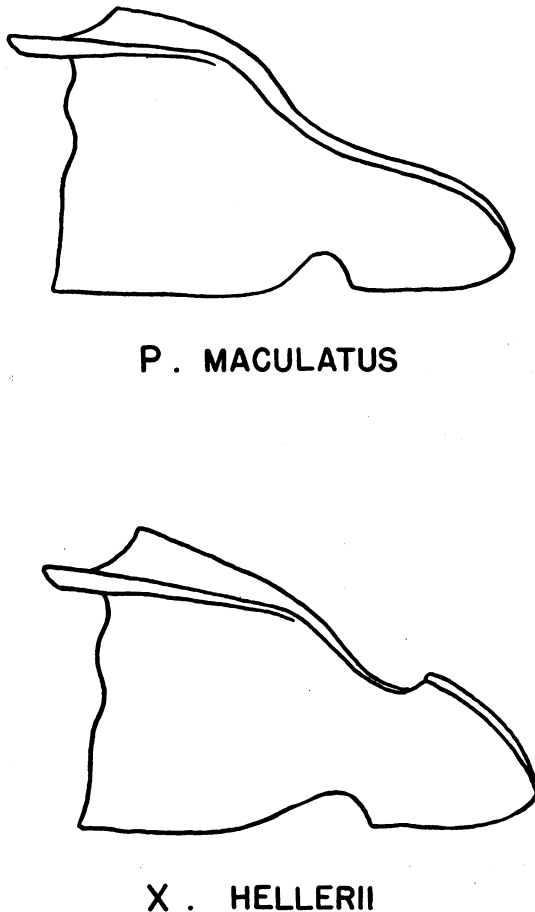


FIG. 5. External contours of the distal tips of gonopodia in the platyfish and swordtail.

arranged in series, resembles a double row of tent poles over which a canopy is spread and supported. Thus for some length along the third ray a fairly broad surface is created.

Ray 4 is bifurcate for its entire length, and each branch of the anterior portion (4a) and posterior portion (4p) is composed of paired segments quite different in structure and arrangement. The segments of 4a appear in cross section to be like opposing paren-

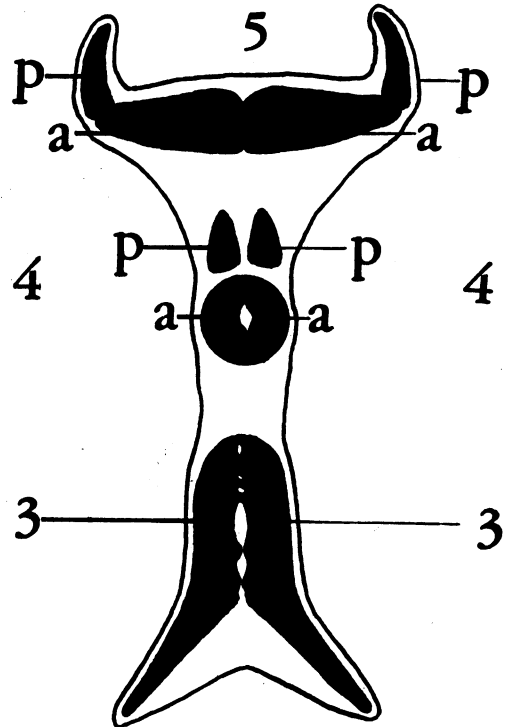


FIG. 6. Cross section at the level of the spines in the gonopodium.

composed of a small terminal segment, which is preceded by a series of pointed elements known as "distal serrae." Next comes a series of flat, disc-shaped segments, after which there lies another series of proximal serrae. From there on the segments of 4p are not radically modified.

The paired structures which 4a and 4p each create when viewed in three dimensions are as follows. The segments of 4a form a continuous narrow rod. Over them the dual distal and proximal gourd-like serrae (4p-4p) rest, with their tips pointed directly upward. They form a narrow parallel series of supporting points all along the distal portion of the gonopodium. The paired elements of ray 4 (4p-4p) are immediately ventral to the

segments of ray 5, particularly the paired elements 5a-5a.

Ray 5 is bifurcate, and each of its two branches, the anterior (5a) and the posterior (5p), is different in shape and in position. In the gonopodium the pair of 5a segments are flanked by the paired segments of ray 5p and are arranged as follows:

5p, 5a, 5a, 5p

In cross section the outer halves of segments 5p flare upward. Thus the four parts of ray 5 form an extremely broad, U-shaped framework. The integumentary tissue covers this framework which in length produces a shallow, trough-like area. This was called the "spoon" by Langer (1913).

Viewed in cross section in the region of the spines, the gonopodium resembles (fig. 6) an I-beam with slightly fluted dorsal and ventral surfaces. The broad dorsal surface of the gonopodium, when held in a position of rest, is formed by 5p-5a-5a-5p segments. Immediately below the 5a-5a sections there is an upright strip formed by the 4p and 4a segments, and below the latter are the bases of segments of ray 3. The broad ventral surface is formed by the paired spinous processes of ray 3 which flare widely. The dorsal and ventral surfaces of the gonopodium converge distally to form a narrow blade, with a blunt tip at the apex.

USE IN COURTSHIP

When a live adult male xiphophorin is observed at rest, its anal fin appears to be spear-shaped and is pointed caudally. It is carried close to the body, with its long axis virtually parallel with the long axis of the fish (pls. 17, 18).

During courtship and mating the position of the gonopodium changes. When the male is at rest, the gonopodium is directed posteriorly. In mating it is pivoted in an arc of about 145° so that its distal tip is pointed in an anterior direction. The transfer of spermatophores from male to female is accomplished, according to Clark, Aronson, and Gordon (1949), when the tip of the gonopodium makes close contact with the genital pore of the female. Instantaneous contacts of male and female genitalia are insufficient. The gono-

podial tip acts as a holdfast, and a contact lasting a second or two is required for the definitive copulation that produces an effective insemination. The structures in the tips are different in the platyfish and swordtail (fig. 5), but apparently this in itself is no mechanical barrier to effective hybridization. Sengün (1949) analyzed the function of the tip of the gonopodia in *Lebistes*

VIVIPARITY IN CYPRINODONT FISHES

In cyprinodont fishes the discovery of viviparity and sexual dimorphism of the anal fin dates back over a century. According to Gill (1882), the earliest notice of viviparity was contained in a letter written in 1769 and addressed to the Royal Academy of Sciences at Paris by Don Joseph Anthony de Alzate y Ramirez (published in 1772, and republished by Cuvier and Valenciennes in 1846). Gill's translation is in part as follows: "If you press the belly of the Mexican viviparous scaley fishes with your fingers, you force out the fry before their time, and upon inspecting them through the microscope, you may discern the circulation of the blood, such as it is to be when the fish is grown up. If you throw the little fishes into the water, they will swim as well as if they had been long accustomed to live in that element. The fins and tails of the males are larger and blacker than those of the females, so that the sex is easily distinguished at first sight."

The early manuscript of Peter Artedi, dated 1735, recently revealed by Merriman (1941) contains figures of a male *Anableps* with its prominent gonopodium. This early contribution antedates that of Bloch (1795) who in his ichthyological atlas showed the female of the viviparous four-eyed fish with an embryo and the male fish with its penis-like genitalium. These observations on sexual dimorphism and viviparity in *Anableps* were confirmed by Cuvier and Valenciennes (1846) and by Wyman (1857). Bloch and Schneider (1801) described scientifically the first poeciliid, *Poecilia vivipara*, apparently fully aware, as evidenced in the specific name they gave it, of its viviparous method of reproduction. A few years later Le Sueur (1821), in his description of *Mollienisia latipinna*, illustrated a fully developed male mollie with its

definitely modified, rod-shaped, anal fin. Duvernoy (1844), in a detailed study of the anatomy of *Poecilia surinamensis*, showed how its tissues were adjusted for viviparous reproduction.

Dowler (1855) claimed to have discovered the phenomenon of viviparity in a fish from Louisiana, but Agassiz (1856) denied this assertion, saying he previously (1853) had described the sexual dimorphism and viviparity in *Mollienisia latipinna* and had watched their copulatory behavior. Previously the male was regarded as *Mollienisia latipinna* of Le Sueur, but the female had been known as *Poecilia multilineata* of Cuvier and Valenciennes.

Fitz Gerald (1872) observed the courtship behavior of a viviparous *Poecilia* species from the island of New Providence, Bahamas, saying: "The anal fin of the male consists principally of a long spine. In its normal position this spine lies close under the body and reached backward nearly to the commencement of the caudal fin, and is evidently the intromittent organ. It is furnished with a hinge joint and is capable of being thrown forward at an acute angle, being also susceptible of a slight lateral movement. The act of copulation takes place by the male rising perpendicularly under and a little to one side of the female and making a dart at her with the spine, which is brought laterally forward at the instant of connection. The act is almost momentary and is hardly noticeable without the closest attention." A somewhat similar behavior was reported in *Girardinus* by Zolotinsky (1901) and others. Fraser-Brunner (1947) explained how the pelvic fin was coordinated with the gonopodium at the time of insemination in the guppy (*Lebistes*).

The fine structural details of the masculinized anal fin and adaptations for viviparity in *Girardinus* were described by von Ihering (1883) and in *Gambusia* by Ryder (1882, 1885), Kuntz (1913), and others. Dulzetto (1931) illustrated the cellular elements in the gonopodium of *Gambusia*. Adaptations for viviparity and internal fertilization by means of gonopodia in poeciliid and non-poeciliid fishes were outlined and discussed by Turner (1940, 1947b), Scrim-

shaw (1945), and Kosswig (1948). Kulkarni (1940) claims that the Indian cyprinodont *Horaichthys seinai* has a gonopodium more elaborate than any poeciliid; although oviparous, it has internal fertilization.

The developmental stages in the transformation of anal fins into gonopodia in *Platy-poecilus* were indicated by Tasker (MS); this study was continued by Grumbach (1935) who supplied additional histological details of the gonopodial elements during the regeneration process. These studies were extended by the critical experiments of Grobstein (1940, 1948). Later, Turner (1941) described the morphogenesis of the gonopodium in *Gambusia*, and Cummings (1943) studied the development of these structures in *Mollienisia*.

USE IN TAXONOMY

The use of the mature male's anal fin for classificatory purposes was inaugurated by J. J. Heckel in 1848 in his description of *Xiphophorus hellerii* and two other species. The generic name was given to the swordtail for its pointed anal fin and not, as many aquarists believe, for the sword-like extension of its caudal fin. Heckel's figures of *X. hellerii* and drawings of its gonopodium were done with great care, yet the full taxonomic significance of the finer details of the gonopodia escaped him, for Heckel included in the genus *Xiphophorus* two additional, quite dissimilar fishes now known as *Pseudoxiphophorus bimaculatus* (Heckel) and *Gambusia gracilis* (Heckel).

In presenting a faunal list of fishes of Cuba, Poey (1854) was the first to use gonopodial characters in a diagnostic description of the poeciliids. He demonstrated and illustrated the differences between the gonopodia of *Gambusia*, *Girardinus*, and *Limia*, all of which he named.

Girard (1859) evaluated the differences between the genera *Limia* and *Poecilia*, in part, in terms of the structure of the males' anal fins and decided that the genera were distinct. The importance of gonopodial criteria was illustrated anew when Girard was obliged to describe a new poeciliid from northern Mexico on the basis of a female specimen only. He called it *Limia couchiana*,

but it turned out to be a platyfish, *Platy-poecilus couchianus*.

There is no indication that Gunther (1866), in his "Catalogue of the fishes in the British Museum," paid any special attention to gonopodial characters. For example, he shifted the northern platyfish *couchianus* from the genus *Limia* to *Poecilia* rather than to his own newly erected genus *Platypoecilus*. Later Charles Darwin (1871), in "The descent of man" illustrated, on Gunther's advice, the differences in the structures of the caudal fin of male and female "*Xiphophorus hellerii*" as an example of secondary sexual characters. Garman (1859a) in his monograph on the cyprinodonts was one of the first to figure the gonopodial suspensoria of many poeciliid species, and he mentioned the structure of their gonopodia too, but he did not then see their systematic importance. He relied rather upon such structures as teeth and alimentary equipment. In a supplementary paper (1895b) he speculated on the probable action of the gonopodia of *Anableps*, some of which twist to the right and some to the left, but this was more entertaining than instructive. Jordan and Evermann (1896-1900) in their great summary of the fishes of North and Middle America mentioned but did not use gonopodia taxonomically.

In Germany, where tropical fish-keeping spread rapidly, Bade (1904) described by photomicrographs the differences between the gonopodia of two species of *Girardinus* and two species of *Mollienisia*. Eigenmann (1903, 1907) not only illustrated the gonopodia of *Gambusia* and *Poecilia*, but he indicated their taxonomic value in his description of the poeciliids of the Brazilian Rio Grande do Sul and of the La Plata basin.

The term gonopodium was coined by Philippi (1906, 1908) who, in great detail, described the male poeciliid anal fin and its suspensorium. The developmental aspects of these studies were emphasized by Langer (1913) whose work, especially on *Xiphophorus hellerii* and *Platypoecilus maculatus*, was outstanding.

The same year Regan (1913) published an exhaustive taxonomic review and revision of the poeciliids which has been the basis of

modern classification of this group. Henn (1916), in a well-rounded presentation of the fishes of central South America, followed Regan's lead and utilized gonopodial characters in his critical diagnoses. Henn remarked that no new poeciliid fish should be described without a thorough analysis of its gonopodium. At the same time he feared that this new taxonomic practice, if used loosely, would lead to needless multiplication of genera. This opinion was shared by Meek and Hildebrand (1916) in their survey of the fresh-water fishes of Panama. Geiser (1923) used characters of gonopodia to differentiate between three difficult geographical groups of *Gambusia*.

Hubbs (1924), in the second radical revision of the viviparous cyprinodonts, went further than Regan in utilizing genital characters. He elevated the poeciliid fishes to the rank of family (the Poeciliidae), on a par with six additional families which together constituted the Order Cyprinodontes. In Poeciliidae, Hubbs established nine new tribes and an equal number of new genera. In 1926, he designated seven additional genera and five new species, saying he had discovered a remarkable connection between their geographical distribution and their gonopodial characters. Later Howell Rivero and Hubbs (1936) showed, by a study of the gonopodia, a closer affinity of two alfarinid cyprinodonts.

The increase in descriptions of new poeciliid fishes is partly the result of increasing attention to their gonopodial structures. Von Ihering (1931) described 13 genera from Brazil, including descriptions of their gonopodia; Breder (1934) indicated a new species from the Bahamas; Myers (1935) described several from Hispaniola; Hubbs (1936) found a new genus in Yucatan; and Rivas (1944) diagnosed those from Cuba. In this connection, Howell Rivero and Rivas (1944) said in their descriptions of *Dactylophallus* and *Allodontium* from Cuba that these fish had gonopodial features that are essentially similar but that their gonopodial suspensoria are different. A somewhat similar association is found in two species of *Platy-poecilus*, *P. variatus* and *P. xiphidium*.

THE GONOPODIUM IN XIPHOPHORIN TAXONOMY

It was Regan (1913) who first utilized the characters of the masculinized anal fin of poeciliids as the chief criteria in a broad and basic revision of the group, although several systematists had previously used these characters in a limited way. As one result of his study, Regan pointed out that the species of *Platypoecilus* and of *Xiphophorus*, which previously had been widely separated in most of the earlier classifications, were in reality closely related. He showed that *Xiphophorus hellerii* Heckel (1848) and *Xiphophorus montezumae* Jordan and Snyder (1899) were correctly associated. He also indicated that *Platypoecilus maculatus* Gunther (1866) and *Platypoecilus couchianus* (Girard) were related species, although Girard (1857) described it originally as a *Limia* and Gunther (1866) placed it in *Poecilia*.

In the same year of Regan's revision, Langer (1913) from his analysis of the anatomy of the gonopodia and their suspensoria of *Xiphophorus (strigatus) hellerii* and *Platypoecilus maculatus* suggested that the two genera be joined, but he did not formalize this suggestion taxonomically.

Gordon and Benzer (1945), in their historical survey of studies of the gonopodial suspensoria of poeciliids, indicated that some of the credit for the discovery, in 1913, of the close phylogenetic alliance of the platyfishes and swordtails must go to the amateur tropical fish fanciers. German aquarists obtained several pairs of live platyfish (*P. maculatus*) in 1907 directly from Central America; two years later they also secured a number of live swordtails (*X. hellerii*). In 1910 they reported in their aquarium journals (the *Wochenschrift für Aquarien- und Terrarienkunde* and the *Blätter für Aquarien- und Terrarienkunde*) that they had succeeded in hybridizing these fishes. From these journals Regan and Langer probably learned that platyfish and swordtails hybridized. This must have aided them in establishing the kinship of these two species on anatomical criteria.

Hubbs (1924) established the Tribe Xiphophorini for *Xiphophorus* and *Platypoecilus*,

pointing out the distinctness of their gonopodia from those of other poeciliids. In 1930, after many additional specimens of various xiphophorins were obtained from Mexico, Hubbs and Gordon, in a manuscript as yet unpublished, showed that *Platypoecilus variatus* Meek (1904) was a valid species, although Regan (1913) did not recognize it. Hubbs and Gordon also described *Platypoecilus xiphidium* from the Rio Sota la Marina. This name was first used in a minor publication by Gordon (1932). Later Hubbs and Gordon (1943) formally described the pygmy swordtail, *Xiphophorus pygmaeus*, indicating parallel structures in its gonopodium with other xiphophorins, particularly *X. montezumae*, a photomicrograph of which was presented by Gordon and Benzer (1945). Breider's (1939) illustrations of the gonopodia of all xiphophorins, except *X. pygmaeus* and *P. couchianus*, were drawn sketchily and were not suitable for critical comparison.

DIAGNOSTIC CHARACTERS IN XIPHOPHORINS

The diagnostic characters of the gonopodium used in taxonomy are generally found in the 3-4-5 ray complex. The various segments of the rays are classified according to their shape and number, but the number of segments indicated refers to the number of paired elements. Counts of the various segments are usually made from the distal to the proximal points of reference (figs. 3, 7).

Ray 3 has a terminal hook, four subterminal segments, and seven spines; the remaining relatively undifferentiated segments are not counted.

Ray 4a (anterior half) has a series of terminal segments which make up what has been termed a ramus. In the generalized gonopodium the ramus is made up of four terminal segments. This is preceded by 10 subterminal segments; the rest are relatively undifferentiated.

Ray 4p (posterior half) is a complex consisting of a small terminal segment preceded by the distal serrae which are made up of five tooth-like processes. Proximally they are joined by a series of four subterminal segments and then by another series of six tooth-like processes, called proximal serrae.

These in turn are preceded by relatively undifferentiated segments.

Ray 5a (anterior half) has a terminal segment called a claw, preceded by a long series of subterminal segments. (The claw as here defined is synonymous with the term "hook of ray 5" of other authors. A distinctive name is desirable to distinguish this element from the hook of ray 3.)

tively weak in *X. pygmaeus* and *X. montezumae* (figs. 8, 9).

The hook of the third ray in *X. hellerii* is consistently short and crescent shaped. However, in *X. pygmaeus* and *X. montezumae* the hooks are consistently long and scythe shaped.

The spines of the third ray in *X. hellerii* are S-shaped, and the pointed processes of

RAYS	SEGMENTS IN XIPHOPHORIN GONOPODIUM																					
	21	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	
5 P	SEG																					
5 A	SEG																					
4 P	PRO SEG			PRO SER					SUB SEG				DIS SER				C					
4 A	PRO SEG					SUB SEG										CR						T
3	PRO SEG						SP								SUB SEG				H			

FIG. 7. Method for recording frequencies of gonopodial elements. The arrow points to the distal tip of the gonopodium. 5p and 4p represent the posterior halves of these rays; 5a and 4a, the anterior halves. Abbreviations: c, claw; cr, cephalic ramus; dist ser, distal serrae; h, hook; pro seg, proximal segments; pro ser, proximal serrae; seg, segments; sp, spines; sub seg, subterminal segments; t, terminal segment.

Ray 5p (posterior half) consists of a series of tapering segments which are sharpest at the distal point and broadest at the proximal end.

Taxonomically some segments are more important than others. For example, the presence of a claw on ray 5a indicates that the specimen is a swordtail. The relative size of the claw distinguishes each of the three swordtail species (see table 3). In the platyfishes, the arrangement of the segments in the distal serrae, as well as the shapes of the spines, is important. *P. variatus* and *P. xiphidium* have quite similar gonopodia, but their shapes at their distal ends beyond the "notch" vary, the former being longer than high, the other being just as high as long.

SPECIFIC DIFFERENCES IN *Xiphophorus*

The terminal segment of the fifth ray, the claw, is quite strong in *X. hellerii* but rela-

tively weak in *X. pygmaeus* and *X. montezumae* are not S-shaped, and the pointed processes of the proximal spines are directed towards the distal tip of the gonopodium.

The blade in *X. hellerii* is pointed at both its proximal and distal tips, while in *X. pygmaeus* and *X. montezumae* the blades are rounded and blunt.

The ramus in *X. hellerii* is quite strong; the terminal segments of the fourth anterior ray are rectilinear and do not taper. The distal segments are directed anteriorly and form an acute angle. In the ramus of *X. pygmaeus* the terminal segments taper in a relatively straight line. The terminal segments in *X. montezumae* taper and produce a curve.

The distal serrae of the fourth posterior ray in *X. hellerii* are three in number and are short and stout. In both *X. pygmaeus* and *X. montezumae* the distal serrae are five in num-

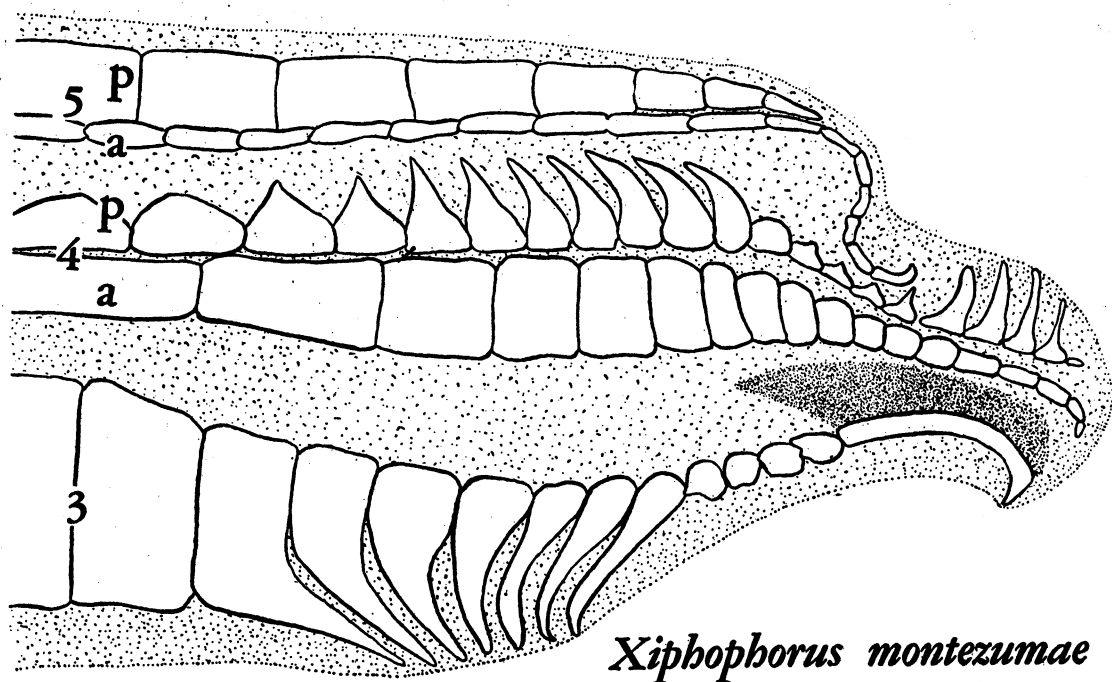
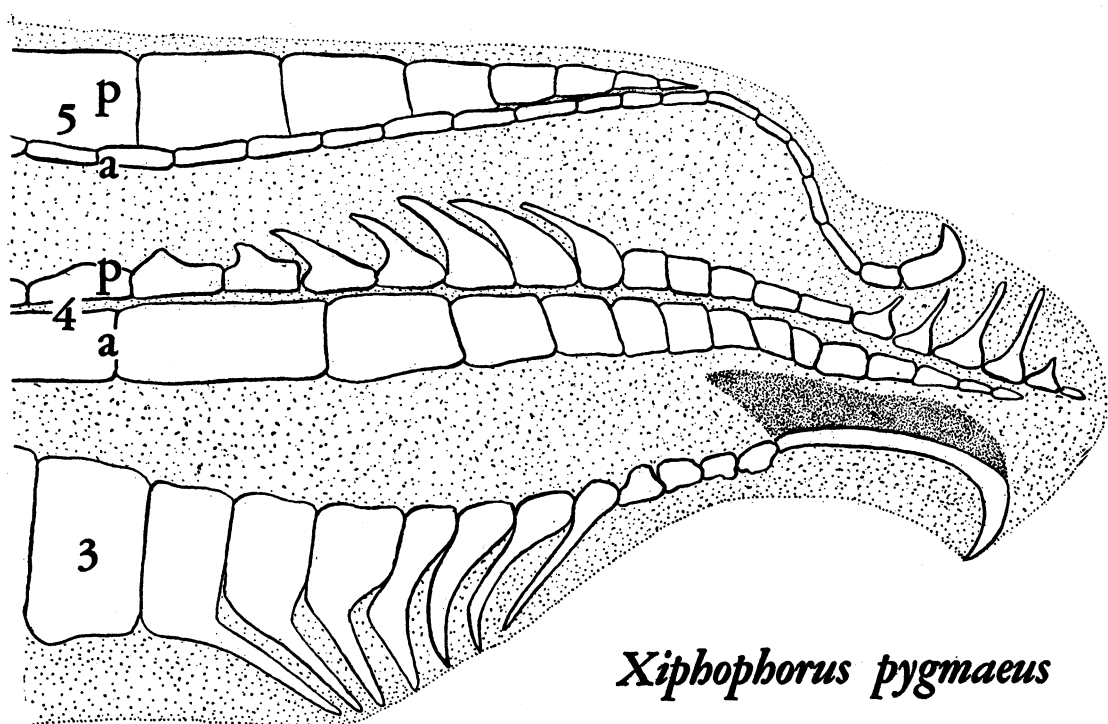
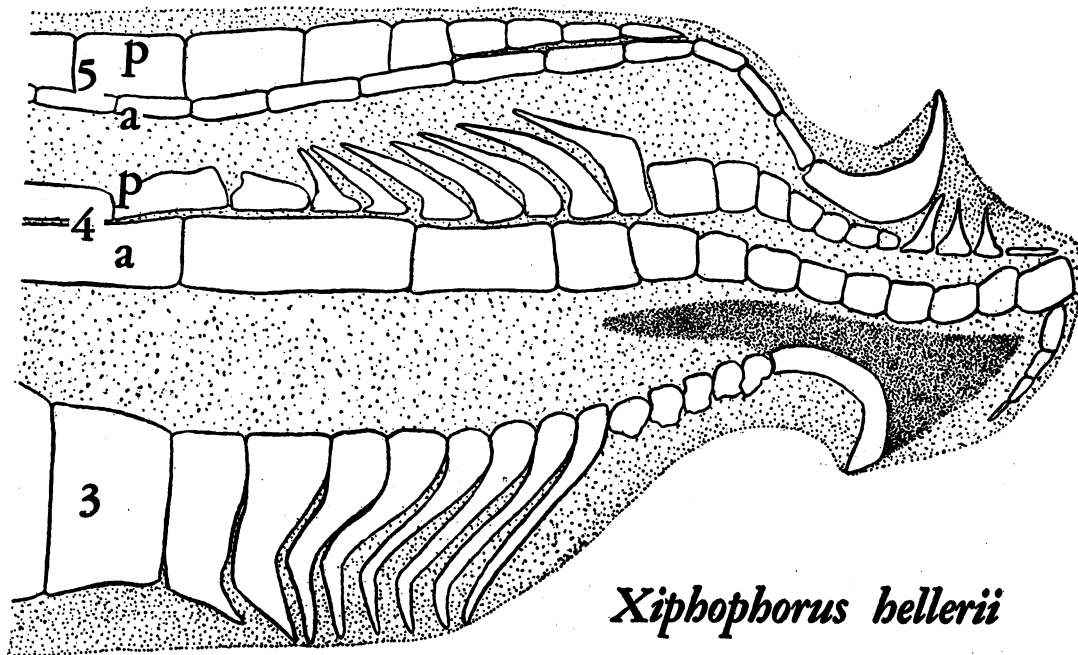


FIG. 8. Gonopodia of two species of swordtails, *Xiphophorus pygmaeus* and *X. montezumae*.

FIG. 9. Gonopodium of *Xiphophorus hellerii*.

ber and are relatively tall and slender. In the description of the holotype of *X. pygmaeus*, Hubbs and Gordon (1943) indicated that the distal serrae in ray 4p are short. In a study of a larger series we have found that the distal serrae are relatively tall and slender.

The proximal serrae of the fourth posterior ray in *X. hellerii* are slender, while similar segments in the other two species are stout.

SPECIFIC DISTINCTIONS IN *Platypoecilus*

The hooks of the third rays appear to be quite similar in the four species (figs. 10, 11).

The processes of the proximal spine of the third ray of *P. maculatus* are long, overlapping, and directed strongly towards the distal tips of the gonopodium. Their tips are blunt. Similar processes in *P. variatus* and *P. xiphidium* are relatively short, and their tips are more broadly blunt. In *P. couchianus* these processes are relatively short and are sharply pointed. The proximal borders of the proximal spines of the gonopodium of *P. couchianus* are scalloped.

The tips of the distal serrae of the fourth posterior ray in *P. couchianus* tend to con-

verge, while in the three other species the distal serrae are, for the most part, parallel to one another.

GENERALIZATIONS

The claw is present in all species of *Xiphophorus* but is absent in most of *Platypoecilus*. But the fact that the claw, although present, is weak in two of the *Xiphophorus* species, *montezumae* and *pygmaeus*, and always strong in *hellerii* may indicate, if this were an important criterion, a closer phylogenetic alliance of *montezumae* and *pygmaeus* to each other than to *hellerii*.

The number of segments in the spines of the third ray and proximal serrae of the fourth posterior ray does not differ significantly in the xiphophorin species (see table 1). The low number of segments in the distal serrae of the fourth posterior ray is significantly different in *X. hellerii*. At the same time, its coefficient of variability is the highest, 20.950. Comparable coefficients in the other xiphophorins are consistently lower, 9.350 to 16.090. (The coefficient of variability as devised by Pearson was used here to measure the dispersion of recorded values around the

TABLE 1

STATISTICAL ANALYSIS OF THE FREQUENCIES OF THE SPINES AND THE DISTAL AND PROXIMAL SERRAE IN THE GONOPODIA OF XIPHOPHORIN FISHES

Ray Elements	Segment Types		
	3 Spines	4p Distal Serrae	4p Proximal Serrae
<i>Platypoecilus maculatus</i>			
\bar{m}^a	7.650 ± 0.188	5.750 ± 0.207	7.200 ± 0.207
V^b	10.990 ± 1.740	16.090 ± 2.540	12.830 ± 2.030
Range	6-9	4-7	6-9
<i>Platypoecilus variatus</i>			
\bar{m}	7.833 ± 0.218	6.055 ± 0.206	6.945 ± 0.150
V	11.800 ± 1.970	14.430 ± 2.400	9.180 ± 1.530
Range	6-9	5-8	6-8
<i>Platypoecilus xiphidium</i>			
\bar{m}	6.833 ± 0.271	6.170 ± 0.167	7.055 ± 0.274
V	13.720 ± 2.800	9.350 ± 1.910	13.460 ± 2.750
Range	6-8	5-7	6-9
<i>Platypoecilus couchianus</i>			
\bar{m}	6.600 ± 0.183	4.850 ± 0.131	6.650 ± 0.233
V	12.440 ± 1.960	12.100 ± 1.910	15.640 ± 2.470
Range	5-8	4-6	5-9
<i>Xiphophorus pygmaeus</i>			
\bar{m}	7.470 ± 0.140	4.500 ± 0.130	6.390 ± 0.230
V	8.670 ± 1.280	13.710 ± 2.020	16.710 ± 2.460
Range	6-9	3-6	4-8
<i>Xiphophorus montezumae</i>			
\bar{m}	7.550 ± 0.220	4.700 ± 0.110	7.850 ± 0.280
V	12.740 ± 2.010	10.640 ± 1.680	16.220 ± 2.560
Range	6-10	4-6	6-10
<i>Xiphophorus hellerii</i>			
\bar{m}	7.730 ± 0.260	3.060 ± 0.170	7.330 ± 0.160
V	12.820 ± 2.340	20.950 ± 3.970	8.430 ± 1.540
Range	6-10	2-4	6-8

* \bar{m} , arithmetic mean, plus and minus its standard error.

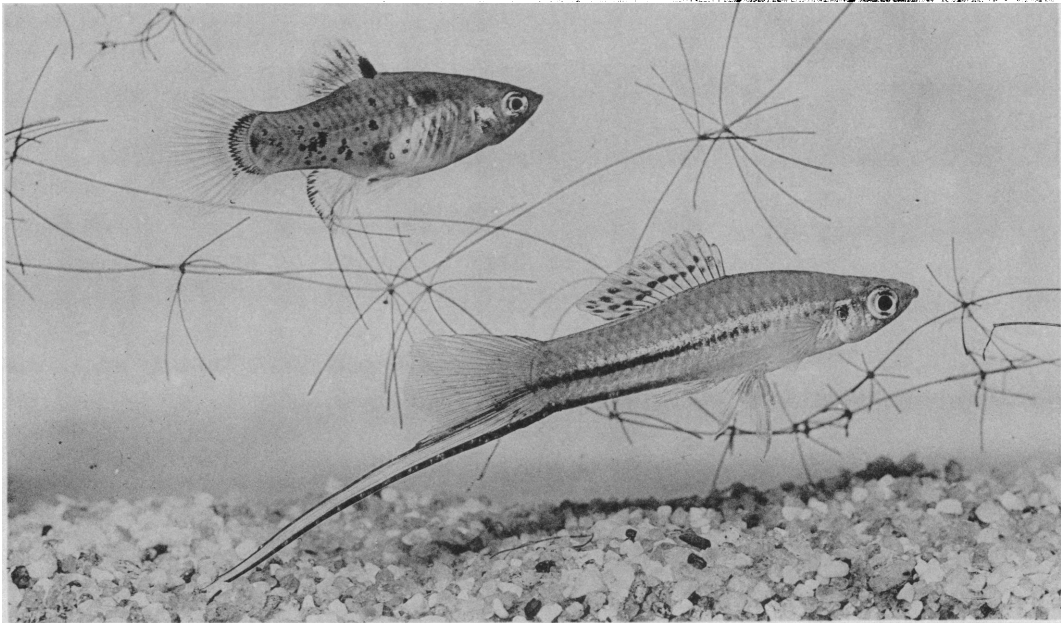
^b V , coefficient of relative dispersion and variability, plus and minus its standard error. For explanation of V , see text, page 427.

mean for a sample. The coefficients were derived so that samples could be made comparable with reference to their degree of variability.)

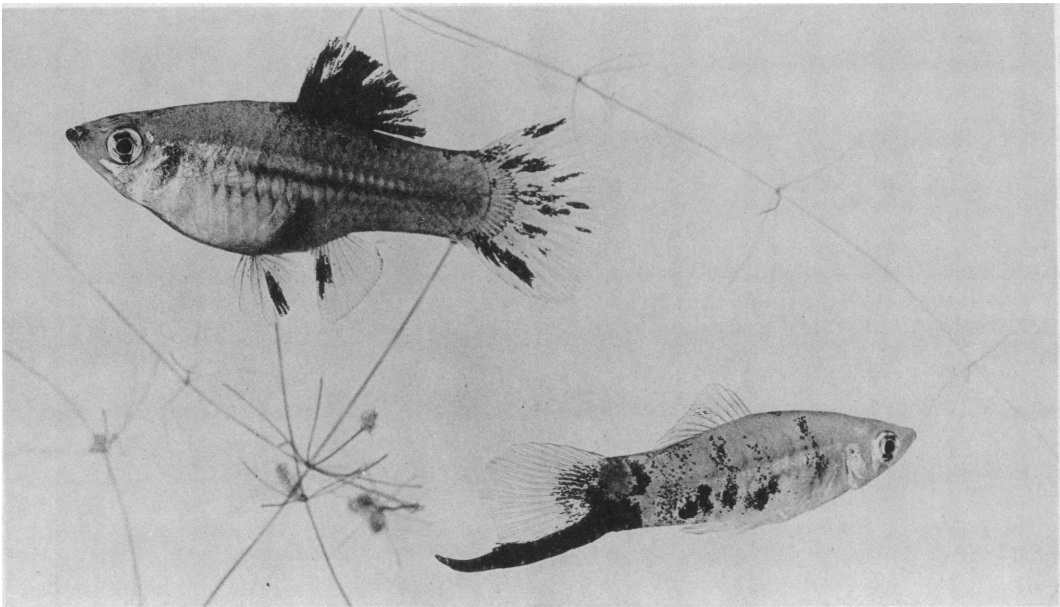
The spines of *P. couchianus*, *P. xiphidium*, and *P. variatus* have broad bases; their broad shafts point anteriorly. The spines of the three species of *Xiphophorus* and of *P. maculatus* have broad bases but rather slender shafts. The shafts, particularly those of the

most proximal spines, overlap and are for the most part directed towards the distal tip of the gonopodium.

Some of the outstanding points of differences between the seven xiphophorin fishes, summarized in table 2 and figure 12, were utilized to construct a taxonomic key (table 3) which may serve as an aid in the identification of the species.



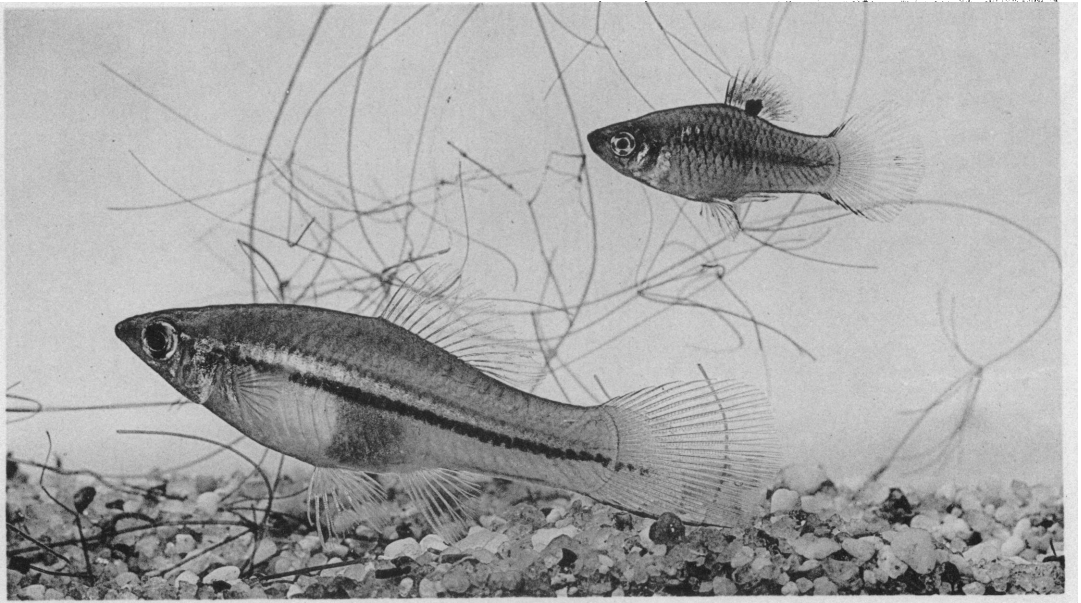
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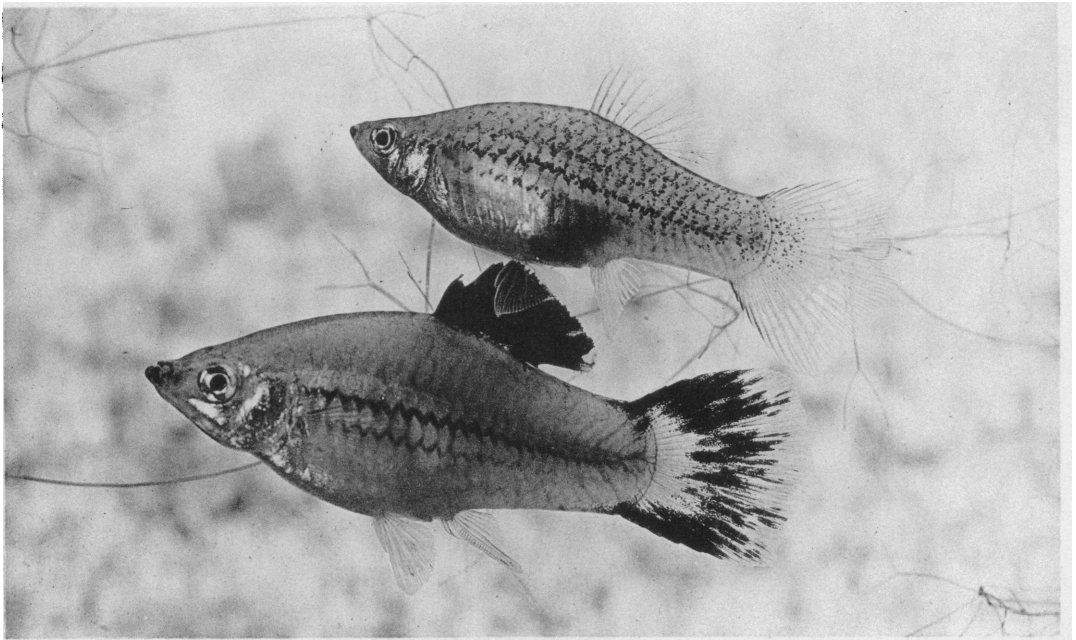
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1. *Platypoecilus maculatus* (platyfish) female, above, with the spot-sided gene (*Sp*) on one X chromosome and the spotted-dorsal gene (*Sd*) on the other X: (X)*Sp*/(X)*Sd*. *Xiphophorus hellerii* (swordtail) male, below, is (X') + / (X') +. Both $\times 1$

2. The first-generation platyfish-swordtail hybrids from the above parents. The female hybrid, above, is (X)*Sp*/(X') +. The male hybrid, below, is (X)*Sd*/(X') +. Note the modified anal fin, the gonopodium, in the male in comparison with the undifferentiated anal fin in the female hybrid, also that the spotted-dorsal gene (*Sd*) is found in one hybrid and the spot-sided gene (*Sp*) in the other. Both $\times 1$



1



2

1. *Platypoecilus maculatus* (platyfish) male, above, has the spotted-dorsal gene on the Y chromosome and the stripe-sided gene on the X: $(X)Sr/(Y)Sd$. Below, the *Xiphophorus hellerii* (swordtail) female is $(X')+/ (X')+$. Both $\times 1$

2. The first-generation platyfish-swordtail hybrids from the above parents. Above, a female hybrid has the stripe-sided gene (Sr): $(X)Sr/(X')+$. Below, a sterile spotted-dorsal hybrid male has the Sd gene on the Y: $(Y)Sd/(X')+$. Both $\times 1$

TABLE 2
FORM AND FREQUENCY OF THE ELEMENTS IN THE 3-4-5 RAY COMPLEX IN THE GONOPODIA OF XIPHOPODIN FISHES^a

Nos.	Species	Ray 3				Ray 4a			Ray 4p		Ray 5a
		Hook	Sub-terminal Segments	Spines of Shafts	Blade	Ramus	Sub-terminal Segments	Distal Serrae Shafts	Sub-terminal Segments	Proximal Serrae Shafts	
20	<i>Pm</i> ^b	Scythe shaped	4 ± 1 Irreg. ^c	8 ± 1 Slender	Rounded	3 ± 1 Curved	10 ± 1	6 ± 1 Tall, pointed, upright	3 ± 1 Irreg., rectang.	7 ± 1 Incl.	Wanting
18	<i>Pv</i>	Scythe shaped	4 ± 1 Irreg.	8 ± 1 Heavy	Rounded	3 ± 1 Slight. curved	10 ± 1	6 ± 1 Tall, pointed, upright	3 ± 1 Irreg., rectang.	7 ± 1 Incl.	Wanting
12	<i>Px</i>	Scythe shaped	4 ± 1 Irreg.	7 ± 1 Heavy	Rounded	2 ± 1 Slight.	13 ± 1	6 ± 1 Tall, pointed, upright	3 ± 1 Irreg., rectang.	8 ± 1 Incl.	Wanting
20	<i>Pc</i>	Scythe shaped	4 ± 1 Irreg.	7 ± 1 Heavy	Rounded	2 ± 1 Slight.	10 ± 1	5 ± 1 Tall, pointed, upright	3 ± 1 Irreg., rectang.	7 ± 1 Incl.	Wanting
23	<i>Xp</i>	Scythe shaped	4 ± 1 Irreg.	7 ± 1 Slender	Rounded	2 ± 1 Slight. curved	10 ± 1	4 ± 1 Tall, pointed, upright	5 ± 1 Reg., rectang.	6 ± 1 Incl.	Short, broad
20	<i>Xm</i>	Scythe shaped	4 ± 1 Irreg.	7 ± 1 Slender	Rounded	2 ± 1 Slight. curved	12 ± 1	5 ± 1 Tall, pointed, upright	5 ± 1 Reg., pointed	8 ± 1 Incl. slight.	Short, slender
15	<i>Xh</i>	Crescent shaped	4 ± 1 Irreg.	8 ± 1 Slender	Pointed	5 ± 1 Ang.	9 ± 1	3 ± 1 Short, pointed, upright	6 ± 1 Reg., rectang.	7 ± 1 Incl.	Long, broad
128 (Total)											

^a The number of segments in each category of morphological types is indicated by a figure which represents a frequency with its average variation.

^b Species: *Pm*, *Platyopocilus maculatus*; *Pv*, *P. variatus*; *Px*, *P. xiphidius*; *Pc*, *P. couchianus*; *Xp*, *Xiphophorus pygmaeus*; *Xm*, *X. montezumae*; *Xh*, *X. hellerii*.

^c Abbreviations: ang., angular; blade, shape of blade; incl., inclined; irreg., irregular; pyramid., pyramiding; rectang., rectangular; reg., regular; slight., slightly.

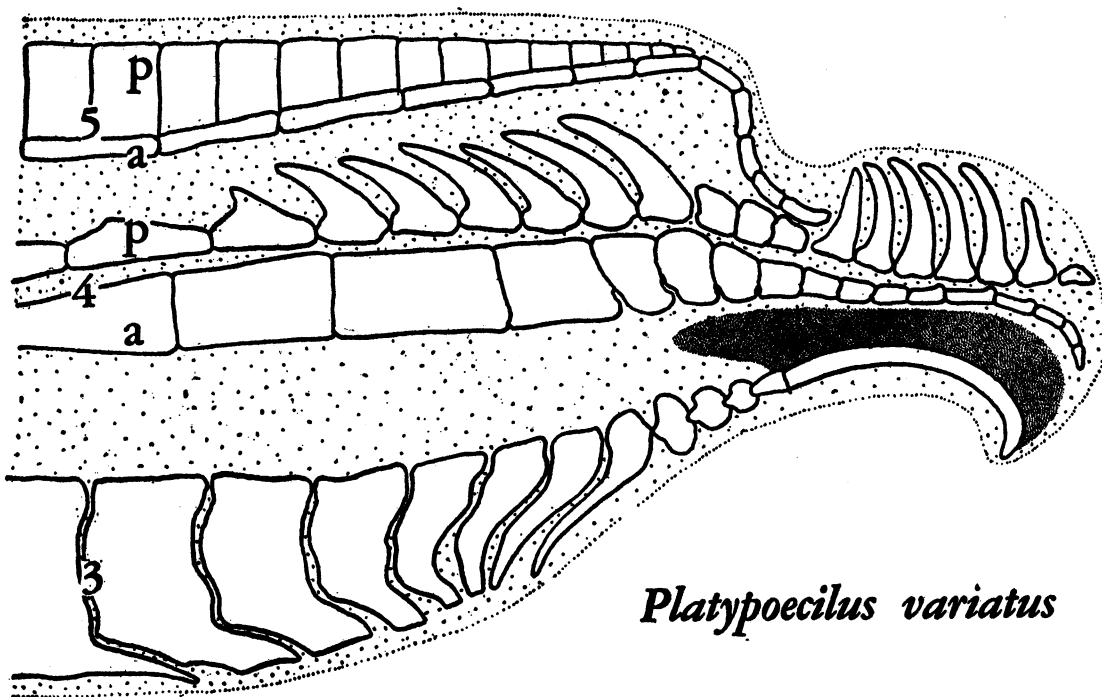
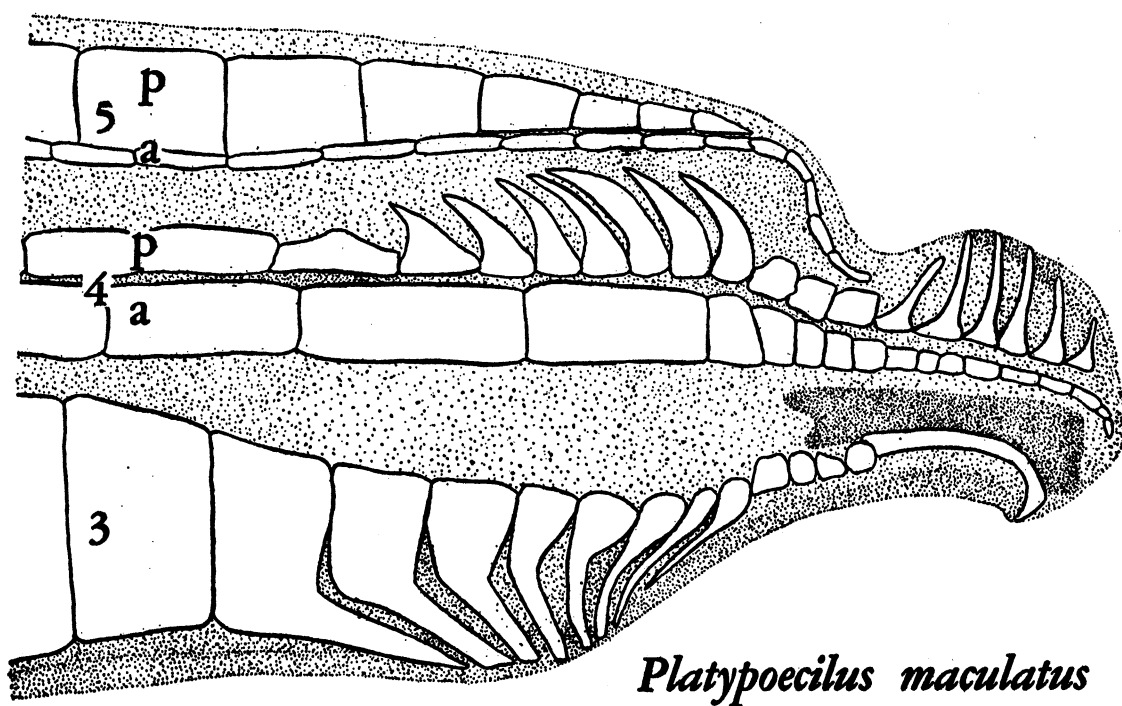


FIG. 10. Gonopodia of two species of platyfishes, *Platypoecilus maculatus* and *P. variatus*.

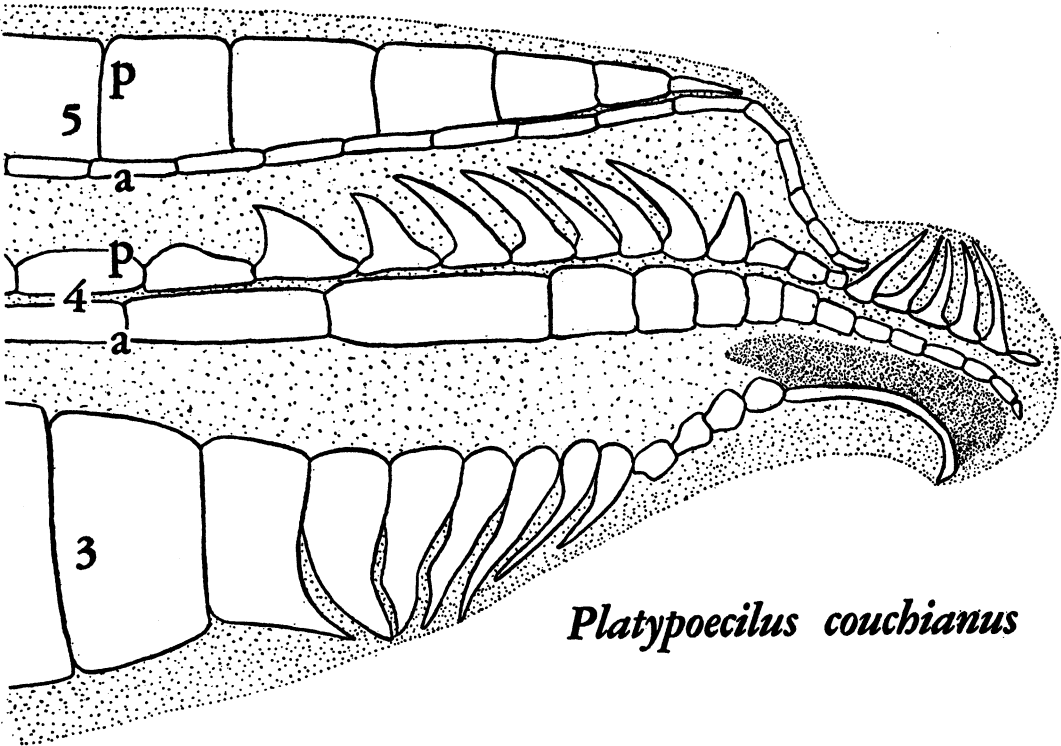
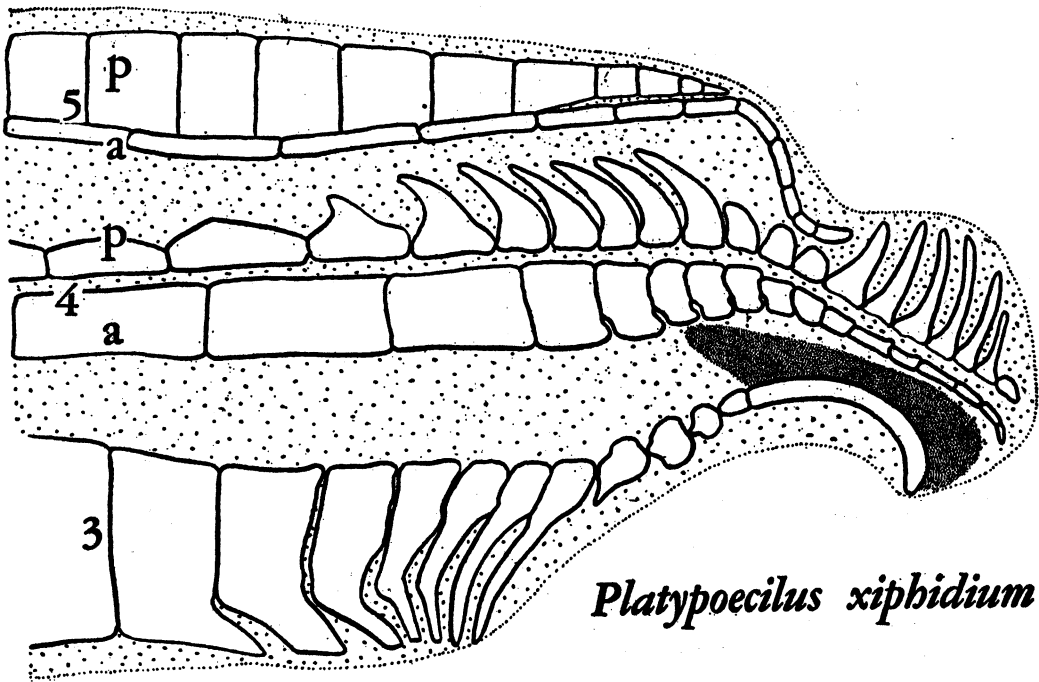


FIG 11. Gonopodia of two species of platyfishes, *Platypoecilus xiphidium* and *P. couchianus*.

TABLE 3

KEY TO THE XIPHOPHORIN FISHES BASED ON GONOPODIAL CHARACTERS

- A. Claw present at terminus of ray 5a XIPHOPHORUS
- B. Claw large, as large as hook on ray 3; distal serrae on ray 4p usually three in number; ramus of ray 4a strongly angular, extending beyond ray 4p *X. hellerii*
- BB. Claw small, one-fifth as large as hook on ray 3; distal serrae on ray 4p usually four or five in number; ramus of ray 4a not angular, not extending beyond ray 4p.
- C. Claw thin, one-half as thick as hook on ray 3; ramus of ray 4a curved, extending to end of ray 4p *X. montezumae*
- CC. Claw thick, two times as thick as hook on ray 3; ramus of ray 4a not curved, not extending to end of ray 4p *X. pygmaeus*
- AA. Claw wanting at terminus of ray 5a PLATYPOECILUS
- B. Tips of distal serrae of ray 4p close to each other, the spread of the two outermost ones from base to base four times the distance from tip to tip; the proximal borders of the proximal spines of ray 3 scalloped in part; all spines of ray 3 pointed at tips *P. couchianus*
- BB. Tip of distal serrae of ray 4p widely spaced, the spread of the two outermost ones from base to base less than two times the distance from tip to tip; the proximal borders of the proximal spines of ray 3 not scalloped; most spines of ray 3 blunt at tips.
- C. The long anterior processes of proximal spines of ray 3 overlapping; the anterior border of the most proximal spine two or more times as long as its posterior border . . . *P. maculatus*
- CC. The anterior processes of proximal spines of ray 3 short, hardly overlapping; the anterior border of the most proximal spine only 1.5 times, or less, as long as its posterior border.
- D. Distance between distal tip of terminal segment of ray 5a to proximal tip of hook (ray 3) equal to distance between terminal segment of ray 5a and distal tip of hook (ray 3) *P. xiphidium*
- DD. Distance between distal tip of terminal segment of ray 5a to proximal tip of hook (ray 3) two-thirds the distance between terminal segment of ray 5a and distal tip of hook (ray 3) *P. variatus*

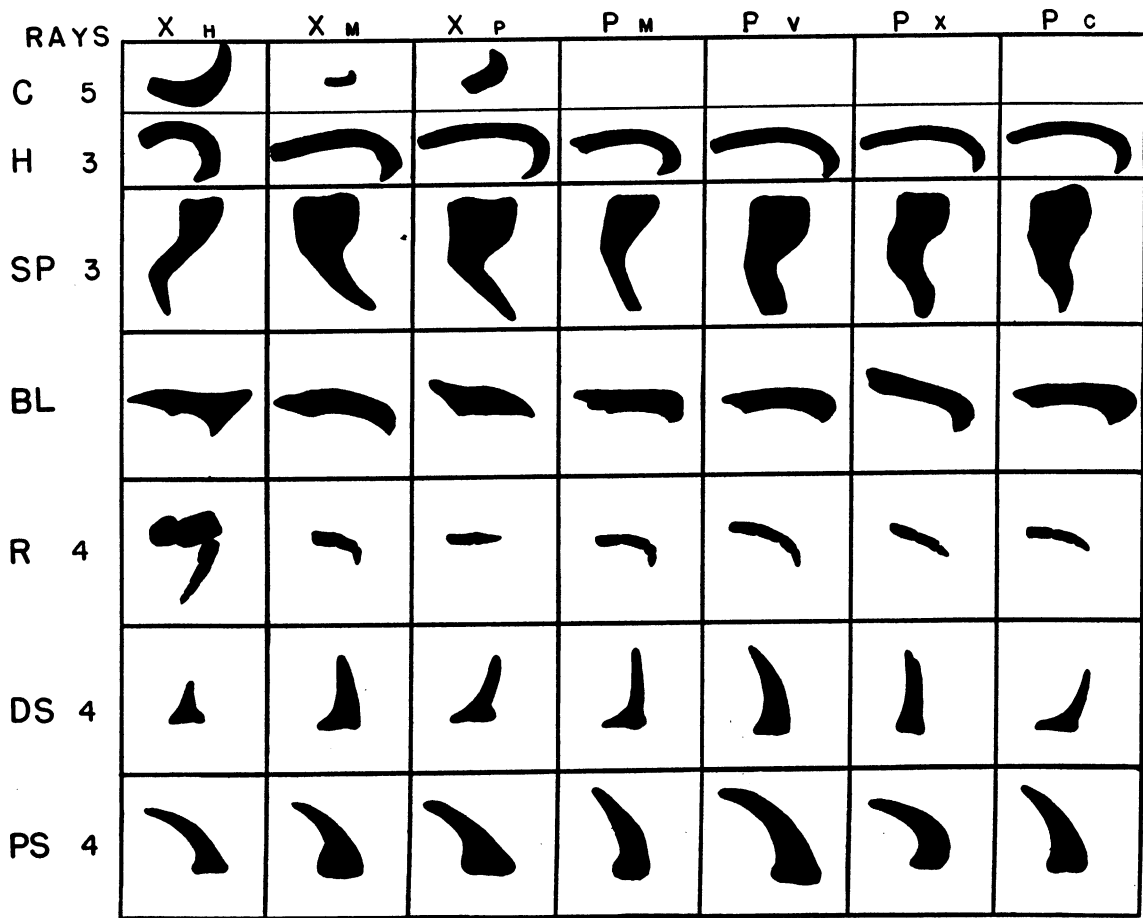


FIG. 12. Diagnostic elements in the xiphophorin gonopodia. The spines and distal serrae of *Xiphophorus hellerii*, *Platypoecilus maculatus*, and their various hybrids were studied genetically. Species: *Xh*, *X. hellerii*; *Xm*, *X. montezumae*; *Xp*, *X. pygmaeus*; *Pm*, *P. maculatus*; *Pv*, *P. variatus*; *Px*, *P. xiphidium*; *Pc*, *P. couchianus*. Abbreviations: bl, blade; c, claw; ds, distal serrae; h, hook; ps, proximal serrae; r, ramus; sp, spines.

GENETICS OF SPECIES DIFFERENCES IN THE MALE GENITALIA OF *PLATYPOECILUS MACULATUS* AND *XIPHOPHORUS HELLERII*

THE CHARACTERS in the gonopodia of the platyfish, the swordtail, and their hybrids that are best suited to a genetic analysis are, first, the sharp differences in the shape of the spines of the third ray and, second, the differences in the shape of the distal serrae of the fourth ray. Measurements and counts were made of these structures in the males of the platyfish, the swordtail, the first- and second-generation hybrids, and three types of back-cross generation hybrids.

The most obvious differential feature in the xiphophorin gonopodia, the claw, which is present in the swordtails but wanting in most platyfishes, was considered but not used. Great difficulties were encountered in measuring accurately the shape of the claws, particularly those in the second-generation hybrids, the most critical group of all. Somewhat similar difficulties were encountered with other apparently good key characters. For example, the variation in the number of distal serrae in both platyfish and swordtails was so great and the number of specimens so relatively few that statistically significant results from their analysis were not obtained. Unsatisfactory results were found also in the analysis of the number of subterminal segments in the third rays and in the fourth posterior rays. The difficulties in evaluating the hooks and blades were great. The analysis of the claws proved unfruitful. All these structures are extremely variable in form, particularly the blades, and no satisfactory method was found to describe them adequately in quantitative terms. The ramus, an apparently useful character for this study, could not be used because it is extremely variable both in shape and in the number of its segments.

The characters in the gonopodia that proved to be workable were the distinctive shapes of spines of the third ray and the shapes of the distal serrae of the fourth posterior ray. The analyses of these structures follow.

PRELIMINARY ANALYSES

Gonopodia in their development and differentiation are sensitive to non-genetic influences such as temperature variables. To evaluate the constancy or variability of certain structures of the gonopodium and their relationship to the gonopodium and to the fish as a whole, several preliminary statistical analyses were made.

If the form and frequency of the distal serrae remain constant in the gonopodium of a given species, regardless of the standard lengths of the specimens and the lengths of their gonopodia, then standards of the forms and frequencies of these segments of the gonopodia can be used with some assurance for comparative analyses of the genitalia differences in various species. If the form and frequency of the segment vary with the standard lengths of the specimens and the lengths of their gonopodia, an adjustment for this relationship must be made for purposes of a more accurate comparison of the differences.

RELATIONSHIP BETWEEN THE LENGTHS OF FISH AND THEIR GONOPODIA

The first correlation was made to determine the relationship of the total length of gonopodium to the standard length of the specimen. The length of the gonopodium was measured from its distal tip to the base of its first ray. The standard length of the fish was measured from the tip of its snout to the base of the hyphural bone (end of caudal peduncle).

Figure 13A shows the correlation between the standard length of the fish and the length of the gonopodium. In this correlation 28 fish (*P. maculatus*) of the seventh inbred generation, 30⁷, of the Rio Jamapa stock are represented. The standard length of the specimens ranged from 17 mm. to 24 mm. Two squares on graph paper were allowed

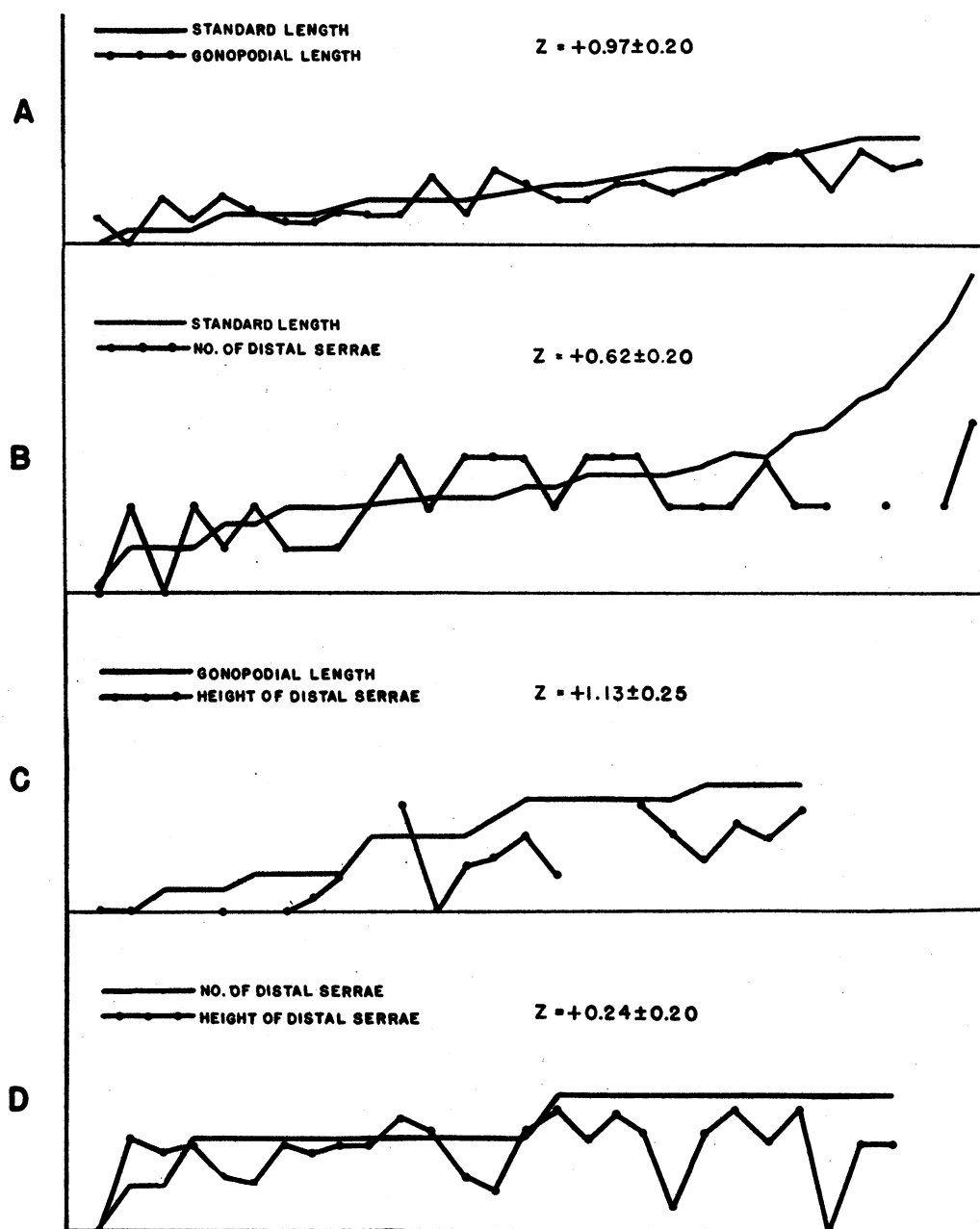


FIG. 13. Studies in variations of the distal serrae of the gonopodia of *Platypoecilus maculatus*. Correlations based on (A) 28 paired counts, (B) 29 paired counts, (C) 24 paired counts, (D) 27 paired counts. The breaks in the lines in B and C are explained in the text. z represents the corrected correlation coefficient, r .

for every millimeter unit of difference. For example, a specimen of 17 mm. was given a value of one unit; of 18 mm., a value of two

units, and so on. Then the units that represented the various standard lengths of the 28 specimens were arranged in an arithmetic

series progressing from the lowest (17 mm. = 1 unit) to the highest (24 mm. = 7 units). These standard lengths in units are represented by the smooth line on the graph.

Relative values were then obtained for the lengths of the gonopodia which ranged from 4 mm. to 6 mm. in the same 28 specimens. The 4-mm. gonopodia were given the value of one unit; 5-mm., two units; and 6-mm., three units. Each unit for gonopodium size was represented by three squares on the graph for purposes of clarifying the relationships of the two curves.

Beginning at the extreme left of figure 13A, the first standard length value lies at 1 (this starts the smooth line), while the value of the gonopodium length lies at 2 (this starts the dotted line). A comparison of these two lines indicates a closeness of relationship which is generally maintained throughout. This positive relationship is borne out by statistical analysis of the 28 paired measurements that relate the standard length of the fish to the length of its gonopodium. The coefficient of correlation (r) is $+75 \pm 0.08$. With the use of Fisher's suggestion, as given in Simpson and Roe (1939), the corrected correlation coefficient, z , was found to be $+0.97 \pm 0.20$. These determinations reflect a significantly high correlation and indicate that the lengths of gonopodia increase with increasing size of the fish.

LENGTHS OF FISH AND NUMBER OF DISTAL SERRAE

Figure 13B shows the correlation of the standard length of the fish and the number of distal serrae in its gonopodium. For this correlation 28 fish (*P. maculatus*) were used; these were collected from the Rio Papaloapan in 1939 (Gordon, 1947b). This strain was used because the range of standard lengths was greater than in the previous 307 group stock. The total range in the number of segments in distal serrae was found to be generally small, and in order to compare their frequencies adequately a greater range of standard lengths was required.

The number of segments in the gonopodia of the fish varied from four to eight, while their standard lengths varied from 17.5 mm. to 38.0 mm. In figure 13B the smooth

line represents the standard lengths of 28 fishes, while the dotted line represents the frequency of the segments in distal serrae of their gonopodia. This graph shows the position of two standard lengths of fishes for which no comparable distal serrae counts were available. These unpaired values were included in this graph in order to obtain a more complete standard length curve. A statistical treatment of the available paired values indicates that the coefficient of correlation, r , is $+0.55 \pm 0.13$ and the corrected r value, or z , is $+0.62 \pm 0.20$.

These determinations indicate a significant positive relationship between the standard lengths of the fish and the number of distal serrae in their gonopodia.

LENGTHS OF GONOPODIA AND HEIGHTS OF DISTAL SERRAE

Figure 13C shows the correlation between the lengths of the gonopodia and the heights of their distal serrae. For the study of this correlation 24 wild-caught fish from the Rio Jamapa were used (Gordon, 1947b). The lengths of their gonopodia measured from 5.50 mm. to 7.25 mm. Some gonopodia had five distal serrae, most had six, and some had seven. The heights of the distal serrae segments in each series were measured under a microscope by use of an eyepiece micrometer, by comparing the number of squares in the Whipple disc occupied by the heights of the segments. Then the average height of the segments was obtained for each gonopodium. The height averages varied from 3.50 to 5.18 micrometer units, and these were checked against the lengths of the gonopodia. Since the value of 3.50 was the lowest, it was placed at the lowermost point on the graph along the dotted line, and the other height values were plotted accordingly, with the uppermost value standing at 5.18. The breaks in the dotted line representing the heights of the distal serrae are the result of using gonopodia having imperfectly formed distal serrae.

A statistical analysis of the data revealed a definitely positive correlation; r is $+0.81 \pm 0.08$ and z is $+1.13 \pm 0.25$. This indicates a significant positive relationship between the

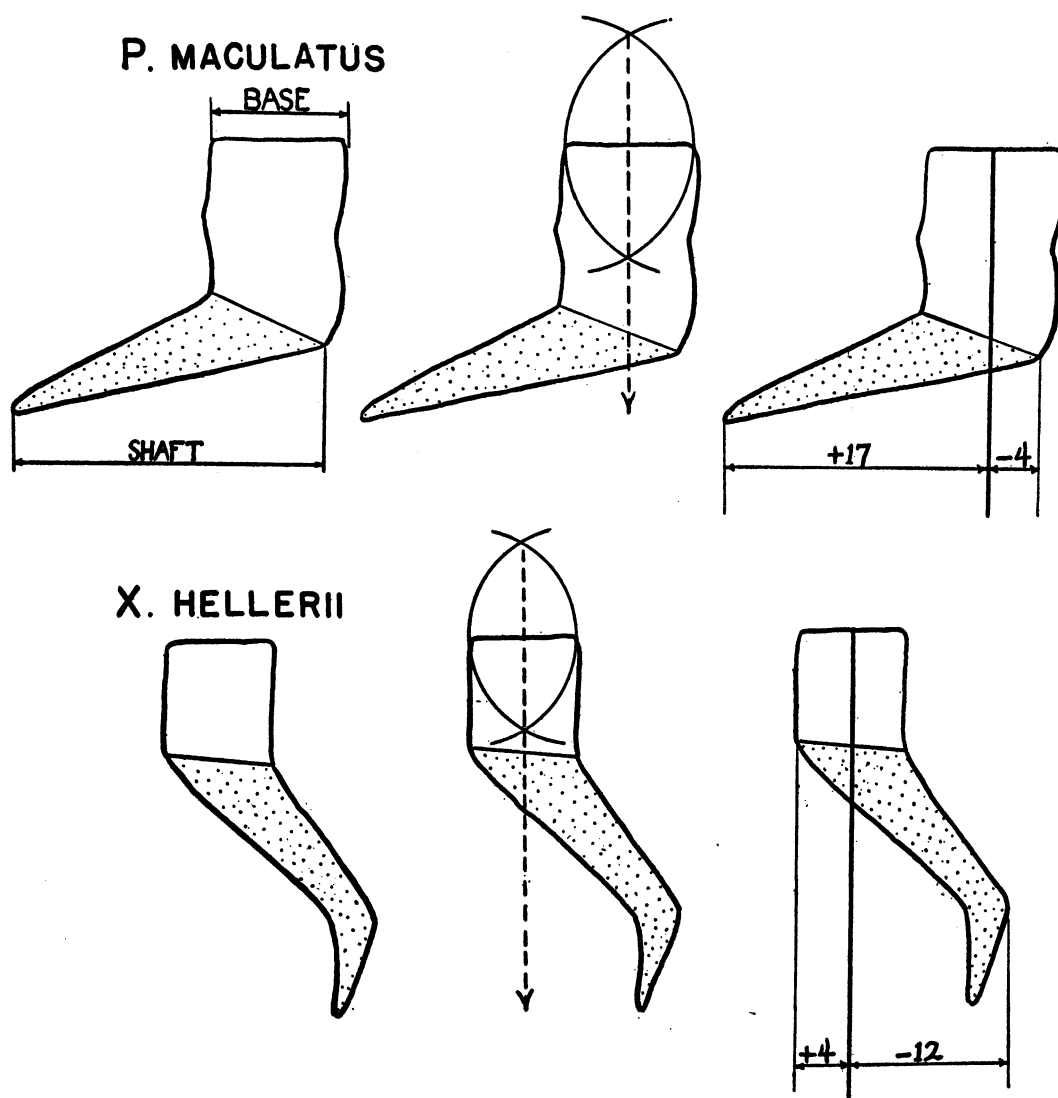


FIG. 14. Method of measuring gonopodial spines. The overlap of the shapes is represented in units. In this figure the platyfish spine has 17 plus and 4 minus units, giving it an index shape of 4.25.

lengths of the gonopodia and the heights of their distal serrae.

NUMBER OF DISTAL SERRAE AND THEIR HEIGHTS

Figure 13D shows the correlation between the number of distal serrae and their average heights. The fishes used for this study were the same as those used in the first correlation, stock 30⁷. The number of segments in the distal serrae varied from four to seven, while

their average heights ranged from 3.06 to 4.39 micrometer units. The smooth line represents the number of segments and the dotted line the average height of the segments in micrometer units. The statistical analysis indicated that the coefficient, r , had the low, non-significant value of $+0.24 \pm 0.18$ and z had the value of $+0.24 \pm 0.20$. Thus it appears that the number of distal serrae and their average heights vary independently of each other.

INHERITANCE OF SHAPE OF SPINES IN RAY 3

THE P₁, F₁, AND F₂ GENERATIONS

The differences in the shape of the spines in the gonopodia of the common platyfish, *P. maculatus*, and swordtail, *X. hellerii*, are reflected in part in the positional relationship of its shaft to its base (table 2 and fig. 12). The entire spine of the platyfish in outline resembles the shape of the foot of a digitigrade mammal, while that of the swordtail appears like the foot of an unguligrade. The shaft of the spine (the toe-like piece) of the platyfish extends far beyond its base in a distal direction with reference to the attachment of the gonopodium to the body. The shaft of the spine of the swordtail extends in the opposite direction. The degree of overlapping of the spines in each species was measured and evaluated, to obtain a numerical index (I_s) representing the shape of the spines for purposes of comparing these elements of the platyfish with those of the swordtail. Camera lucida drawings of all the spines in a given gonopodium were made on one-tenth-inch, cross-section graph paper, working at 80- \times magnification. In each drawing of a spine its base line was bisected, then a perpendicular line was drawn through the spine.

A line so drawn divided the spines of the platyfish and of the swordtail into two sharply contrasting types. The shaft of the platyfish

\bar{m}	<i>P. maculatus</i> (P)	<i>X. hellerii</i> (X)
	1.864	0.364
Range	1.315-2.328	0.254-0.637

spine extends far to the left, while that of the swordtail spine extends to the right (fig. 14). The degree of extension, or amount of overlap, of one spine over the adjacent one was measured by counting the number of unit squares indicated on the graph paper. Unit squares to the left of the perpendicular line were designated as plus values, while those to the right of the line were regarded as minus units, and each square was given the value of one unit. In figure 14 the spine of the platyfish has 17 plus and four minus units. After all the spines (usually eight) of

TABLE 4

AVERAGE VALUES AND COEFFICIENTS OF VARIABILITY OF THE INDICES OF OVERLAP OF THE SPINES FROM THE GONOPODIA OF *P. maculatus*, *X. hellerii*, AND THEIR HYBRIDS

N ^a	Group	\bar{m}	V
27	<i>P. maculatus</i>	1.864 \pm 0.057	15.933 \pm 2.168
16	<i>X. hellerii</i>	0.364 \pm 0.022	24.450 \pm 4.327
8	F ₁ (Px)	0.912 \pm 0.105	32.675 \pm 8.169
28	F ₂ (Group I)	0.569 \pm 0.021	18.453 \pm 2.659
77	F ₂ (Group II)	1.016 \pm 0.033	28.543 \pm 2.317
39	PXP (h57)	1.546 \pm 0.072	28.978 \pm 3.278
39	PXPP (h73)	1.767 \pm 0.094	33.446 \pm 3.783
46	PXX (hR)	0.917 \pm 0.049	32.933 \pm 3.777

^a N, number of individuals; \bar{m} , average value; V, coefficient of variability.

the gonopodium are measured in the manner just indicated, a total of the plus values (p) and the total of the minus values (m) are obtained. Then, the total plus values, $\Sigma(p)$, are divided by the total minus values, $\Sigma(m)$, and the result determines the index of overlap, I_s . See table 4 and the following formula:

$$p_1 + p_2 + p_3 \cdots + p_n = \Sigma(p) \quad \frac{\Sigma(p)}{\Sigma(m)} = I_s$$

$$m_1 + m_2 + m_3 \cdots + m_n = \Sigma(m)$$

By the use of these methods it was found that the spine indices for the two species and their first- and second-generation hybrids are as indicated in table 4, and summarized as follows:

	<i>P. maculatus</i> (P)	<i>X. hellerii</i> (X)	F ₁ (PX)	F ₂ (PX) ²
\bar{m}	1.864	0.364	0.912	I = 0.569 II = 1.016
Range	1.315-2.328	0.254-0.637	1.279-0.530	I = 0.735-0.358 II = 1.750-0.442

The above determination indicates a significant difference between the I_s of platyfish and of swordtail. The I_s is intermediate in the F₁ (fig. 16).

The F₂ population was studied in two groups. Group I represents 28 F₂ individuals derived from a single F₁ female, whereas group II represents 77 F₂ individuals obtained from several other F₁ females. The ranges of the two F₂ samples were found to be significantly different as calculated by the χ^2 test of association, the upper limits of the two distributions being about 1.000 index units

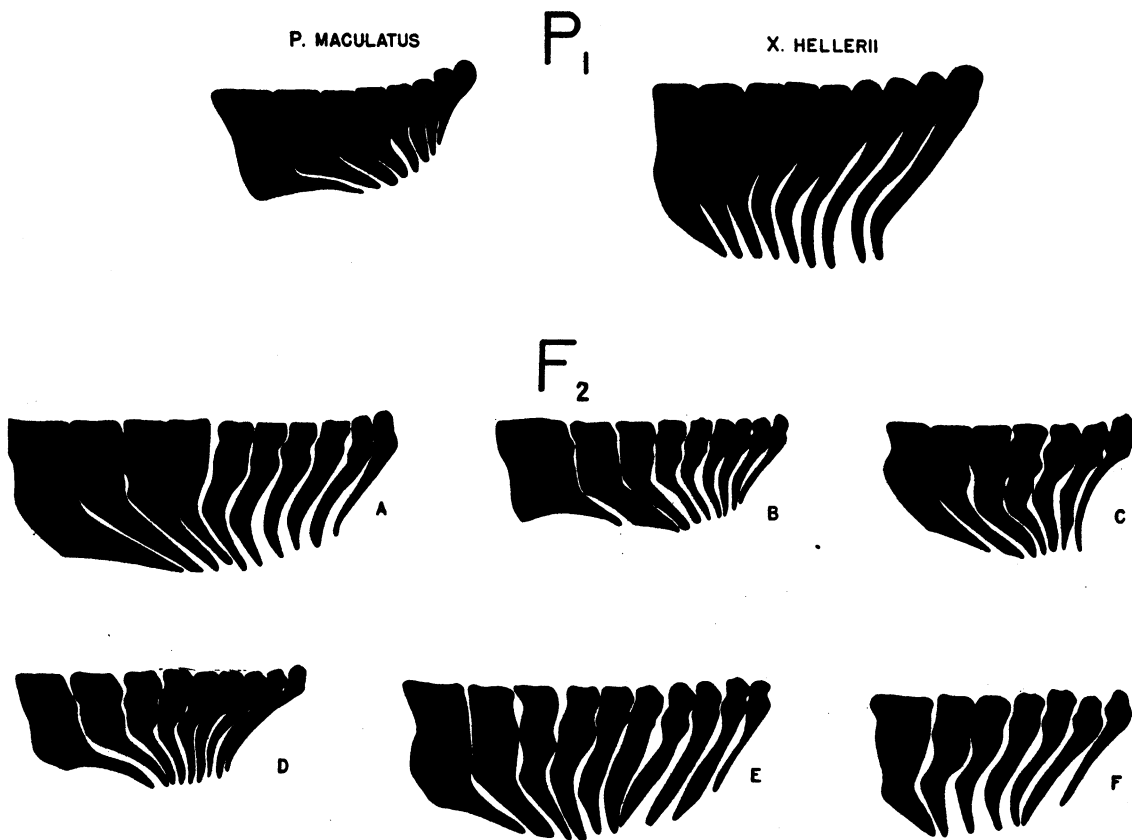


FIG. 15. Diagrams representing the shape of the spines of the platyfish, the swordtail, and their second-generation hybrids. The parental types among the F_2 individuals are indicated in A and F. The intermediates B, C, D, and E were selected from the F_2 group to show a graded series.

apart. Their coefficients of variability were noticeably different (group I: $V=18.453$; group II: $V=28.543$). Group I had an I_s distribution that was very similar to that of *X. helleri*. Group II, however, had a nearly normal I_s distribution. Among the F_2 in group II some parental types were recovered (figs. 15, 16).

Just why these two F_2 groups were different cannot be explained completely, but it is likely that the parent or parents of group I were genetically different from other F_1 for the particular trait being analyzed.

THE BACKCROSS GENERATIONS

When an F_1 *P. maculatus*-*X. hellerii* female hybrid (PX) was backcrossed to a *X. hellerii* male (X) they produced, among others, 46 male PXX backcross hybrids having perfectly developed gonopodia. The spine index,

I_s , for the PXX hybrids was 0.917, with a range of 1.875 to 0.415.

When an F_1 *P. maculatus*-*X. hellerii* female hybrid (PX) was backcrossed to a *P. maculatus* male (P) they produced, among others, 39 male PXP backcross hybrids having gonopodia that were analyzed. Their I_s was 1.546, with a range of 0.761 to 2.848. The difference between the I_s of the PXP hybrids and the I_s of the P_1 *P. maculatus* is not significant when calculated by the χ^2 method.

When the PXP backcross hybrid male was crossed back again to a female *P. maculatus* (P) of the Rio Jamapa population, they produced 47 PXPP double backcross hybrids, all of which were male. The gonopodia of 39 of them were studied, and the I_s was 1.767, with a range of distribution of 0.935 to 3.319. This was not significantly different from the I_s of PXP hybrids or P_1 *P. maculatus*.

ANALYSIS

A survey of the data indicates the following essential points on the genetics of the shape of the spines, I_s (fig. 16):

1. The F_1 are intermediate between the two P_1 .

2. The F_2 have approximately the same mean value as the F_1 .

3. The F_2 hybrids have I_s values that are more widely dispersed than those of F_1 .

4. Some of the F_2 hybrids reach the high values of the P_1 *P. maculatus* and the low values of the P_1 *X. hellerii*.

Because the number of F_1 hybrids is small and their variability relatively great, and because the two groups of the F_2 hybrids were dissimilar, no definite conclusions could be reached concerning the possible method of the inheritance of the spines. If, however, the F_2 hybrids of group II are considered alone and compared with their F_1 and the two P_1 , a more definite pattern of inheritance appears, and the data can best be explained on the basis of a small number of multiple factors.

This interpretation is supported by the results obtained from a series of backcross experiments. From these experimental data the following critical points were obtained:

1. The F_1 hybrids backcrossed to *P. maculatus* produced PXP fish whose I_s values approach those of *P. maculatus*.

2. The PXP hybrids crossed back to *P. maculatus* produced PXPP fish whose I_s values approach those of *P. maculatus* still more.

3. F_1 hybrids backcrossed to *X. hellerii* produced PXX fish whose I_s values approach those of the F_1 hybrids, although the trend is definitely towards those of *X. hellerii*.

It seems that if the multiple factor concept is to be used as an aid in interpreting the results, the number of factors must be small.

INHERITANCE OF SHAPE OF DISTAL SERRAE OF RAY 4P

INDEX FOR SHAPE (I_{DS})

The index representing the shape of the distal serrae, I_{DS} , was obtained from meas-

urements made as follows: Each segment of the series was drawn with the aid of a camera lucida on one-tenth-inch, cross-section graph paper, the magnification being 80 \times . The width, w , of each segment was determined by counting the number of unit squares occupied by the base. By a similar method the height, h , of this segment was obtained. By actual measurement the width was about 0.05 mm. and the height about 0.10 mm.

TABLE 5

AVERAGE VALUES AND COEFFICIENTS OF VARIABILITY OF THE SHAPE INDICES OF THE DISTAL SERRAE FROM THE GONOPODIA OF *P. maculatus*, *X. hellerii*, AND THEIR HYBRIDS

N ^a	Group	\bar{m}	V
27	<i>P. maculatus</i>	0.471 \pm 0.014	13.376 \pm 2.113
16	<i>X. hellerii</i>	0.739 \pm 0.038	20.568 \pm 3.634
8	F_1 (PX)	0.536 \pm 0.032	15.672 \pm 4.190
105	F_2 (PX) [*]	0.672 \pm 0.022	31.845 \pm 2.299
39	PXP (h57)	0.426 \pm 0.014	20.892 \pm 2.363
39	PXPP (h73)	0.412 \pm 0.017	23.058 \pm 2.975
46	PXX (hR)	0.668 \pm 0.023	22.156 \pm 2.475

^a N, number of individuals; \bar{m} , average value; V, coefficient of variability.

The index was first obtained for each segment by dividing the value of the width by that of the height, w/h , that of the first segment being w_1/h_1 . The index, I_{DS} , for the entire series of n segments is as follows:

$$\frac{w_1}{h_1} + \frac{w_2}{h_2} + \frac{w_3}{h_3} \cdots + \frac{w_n}{h_n}$$

$$= I_1 + I_2 + I_3 \cdots + I_n = \frac{\Sigma(I)}{n} = I_{DS}$$

THE P_1 , F_1 , F_2 GENERATIONS

The average mean values of distal serrae indices, I_{DS} , and their ranges for *P. maculatus*, *X. hellerii*, and their F_1 and F_2 hybrids were found to be as in table 5, summarized as follows:

	<i>P. maculatus</i>	<i>X. hellerii</i>	F_1 (PX)	F_2 (PX) [*]
\bar{m}	0.471	0.739	0.536	0.672
Range	0.352-0.551	0.557-1.185	0.414-0.639	0.353-1.750

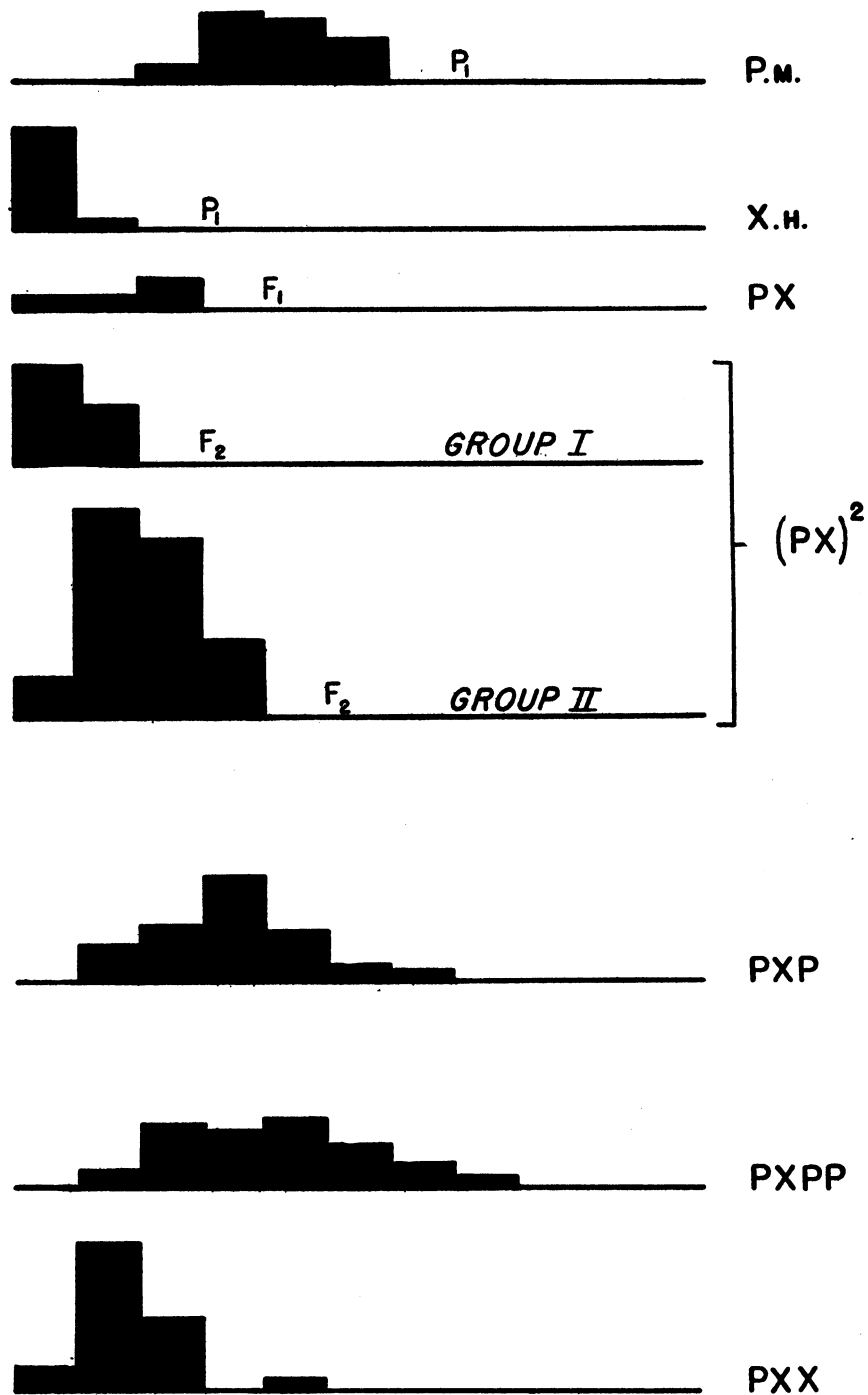


FIG. 16. Histograms representing the shape of spines in the gonopodia of *Platypoecilus maculatus*, *Xiphophorus hellerii*, and their hybrids. *Pm*, *P. maculatus*; *Xh*, *X. hellerii*; PX, F₁ hybrids between *Pm* and *Xh*; (PX)², F₂ hybrids of *Pm* and *Xh*; PXP, backcross hybrids of PX and *Pm*; PXPP, backcross hybrids of PXP and *Pm*; PXX, backcross hybrids of PX and *Xh*.

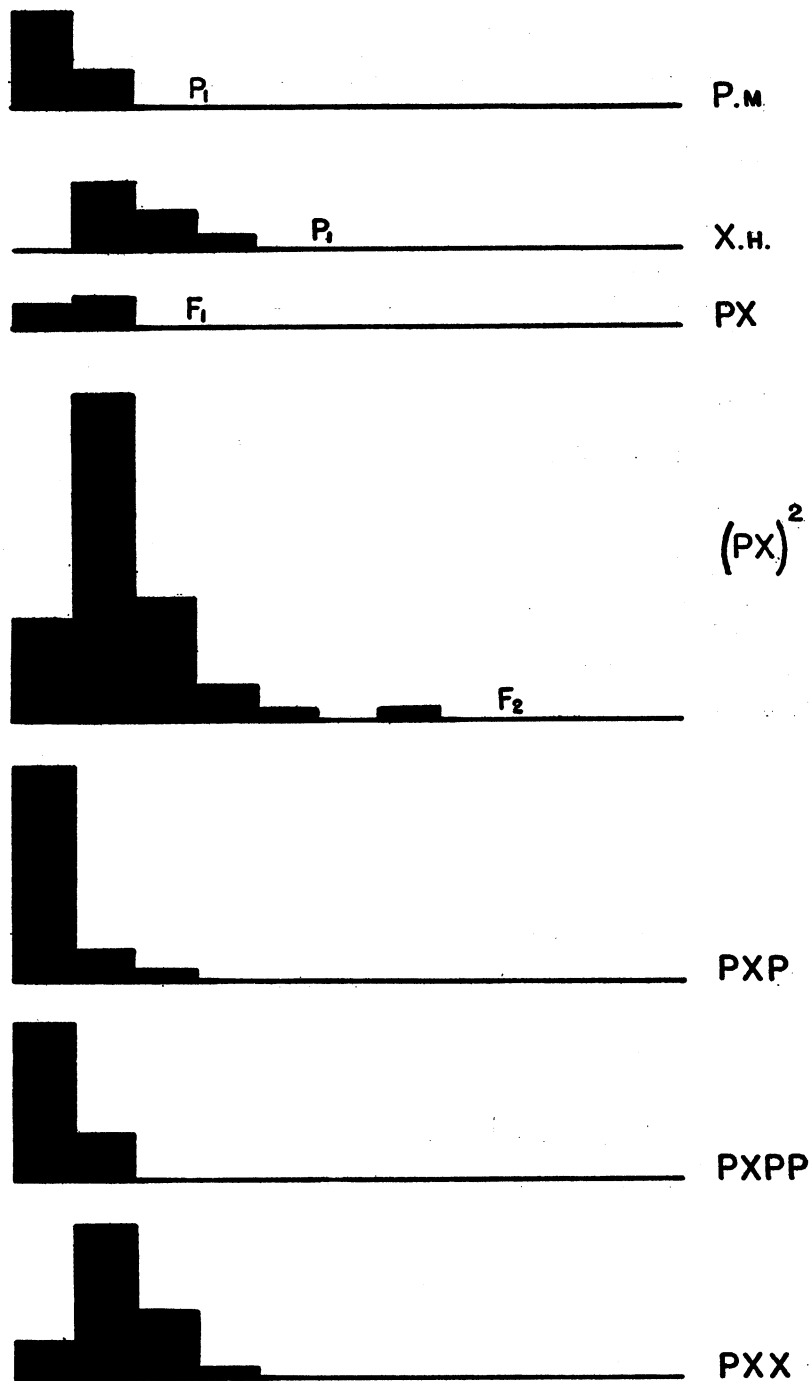


FIG. 17. Histograms representing the shape of distal serrae in the gonopodia of *Platypoecilus maculatus*, *Xiphophorus hellerii*, and their hybrids. *Pm*, *P. maculatus*; *Xh*, *X. hellerii*; *PX*, F_1 hybrids between *Pm* and *Xh*; $(PX)^2$, F_2 hybrids of *Pm* and *Xh*; *PXP*, backcross hybrids of *PX* and *Pm*; *PXPP*, backcross hybrids of *PXP* and *Pm*; *PXX*, backcross hybrids of *PX* and *Xh*.

The I_{DS} of *P. maculatus* differs slightly but significantly from that of *X. hellerii*, according to the χ^2 method of analysis. The smallness of χ^2 is, in part, due to considerable overlapping in the two distributions (fig. 17).

The I_{DS} of the F_1 hybrids is intermediate, but closer to that of *P. maculatus*.

THE BACKCROSS GENERATIONS

The F_1 hybrid (PX) backcrossed to *P. maculatus* (P), produced PXP hybrids that have an I_{DS} of 0.426, which is below that of the F_1 . This I_{DS} approaches closely that of *P. maculatus* and indeed goes beyond that of P_1 .

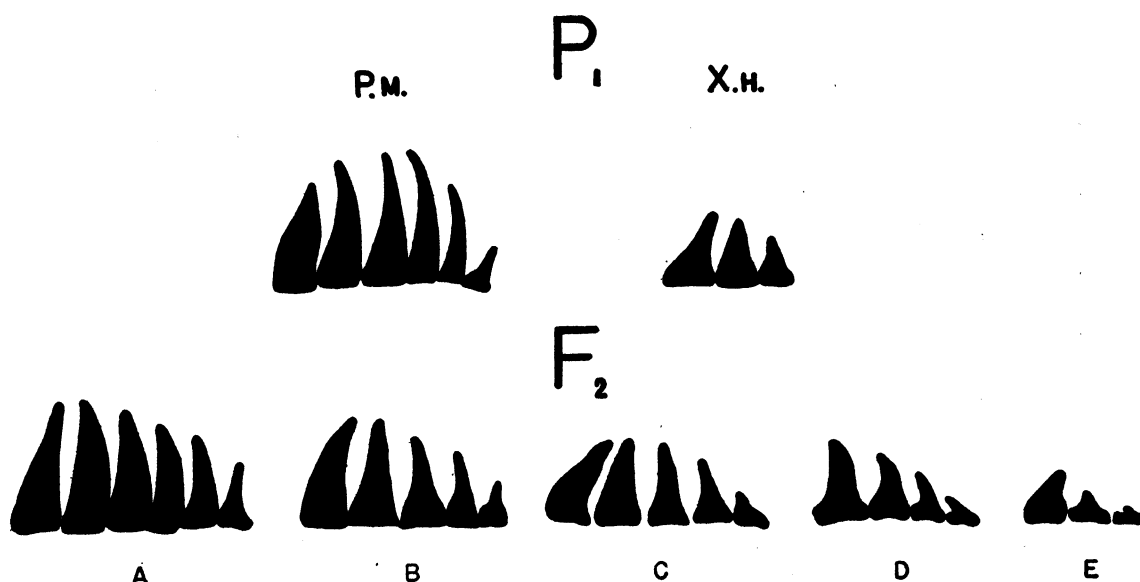


FIG. 18. Diagrams representing the shapes of the distal serrae of the platyfish, swordtail, and their second-generation hybrids. The parental types were found among the F_2 : A and E. The other F_2 , B, C, and D, represent intermediates.

In the previous analysis, the F_2 population was made up of two groups which differed significantly in the shape of the gonopodial spines, but the F_2 individuals of group I and group II showed no difference whatever in the shape of their distal serrae. The two groups were therefore combined and treated as a single F_2 population. The I_{DS} of the F_2 hybrids is approximately intermediate and quite close to that of the F_1 . The variability and dispersion of the individual indices are much greater than those of the F_1 or P_1 . Some of these values fall slightly below those of *P. maculatus*, while others go considerably beyond those of *X. hellerii*.

In the F_2 , some index values were found similar to those representing the parents, P_1 . However, these similar values do not necessarily represent morphologically identical structures (fig. 18).

The backcross hybrid PXP crossed back again to *P. maculatus* (P) produced PXPP hybrids that have an I_{DS} of 0.412, which again is below the comparable value of *P. maculatus*. This is even below the I_{DS} of the PXP hybrids.

The F_1 hybrids (PX) backcrossed to *X. hellerii* (X) produced PXX hybrids that have an I_{DS} of 0.668, which is greater than that of the F_1 . The PXX hybrids have an I_{DS} within the limits of *X. hellerii*, although it must be recalled that the PXP hybrids have an I_{DS} beyond that of *P. maculatus*. The deviation of the PXX hybrids from *X. hellerii* is small.

ANALYSIS

These data do not create a pattern that is necessary for a definitive interpretation of the results. For example, the I_{DS} for the platy-

fish approached that of the swordtail so closely that the F_2 distribution pattern of I_{DS} was indistinct.

Owing to the limitations established by the P_1 , the data on the backcross hybrids were inconclusive also. However, there is trace of significance in the fact that PXP hybrids have I_{DS} approaching those of the platyfish

varied with their widths or varied independently. In the gonopodia of *P. maculatus* the heights of the distal serrae far exceed their widths. In *X. hellerii*, however, the heights are only slightly greater than their widths. The distribution of the paired measurements is indicated in figure 19. In the platyfish-swordtail F_1 hybrids the heights

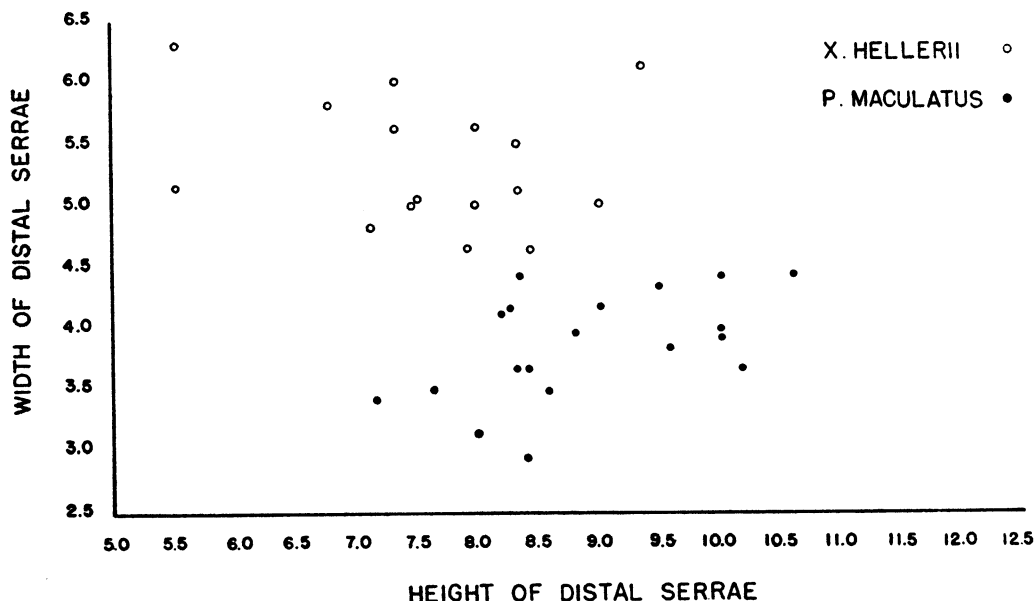


FIG. 19. Scatter diagram showing distribution patterns of the relationships of the width and height of the distal serrae in the platyfish and the swordtail. Note distinctness of the two distributions. The numbers along the coordinates represent units of measurements which are explained in the text.

parent, and the PXX hybrids have indices approaching those of the swordtail.

Consideration of these data seemed to indicate that shape of the distal serrae cannot be evaluated as a single unit. A preliminary analysis was therefore made to determine the relationship of the heights of the distal serrae to their widths.

RELATIONSHIP BETWEEN GENETICS OF WIDTHS AND HEIGHTS OF DISTAL SERRAE

PRELIMINARY ANALYSIS

Calculations were made to determine whether the heights of the distal serrae

and widths were intermediate. In the F_2 the parental types were found, as well as all possible intermediates.

The distribution of the paired measurements of heights to widths, measured from the distal serrae of 95 F_2 hybrids, is indicated in figure 20. The regression lines are approximately at right angles, and this signifies that the two variables are distributed independently. The actual correlation between the paired measurements as expressed by r is $+0.05 \pm 0.10$. This value indicates that the two variables react independently. This is just what might be expected if it is assumed that the heights and widths are genetically independent.

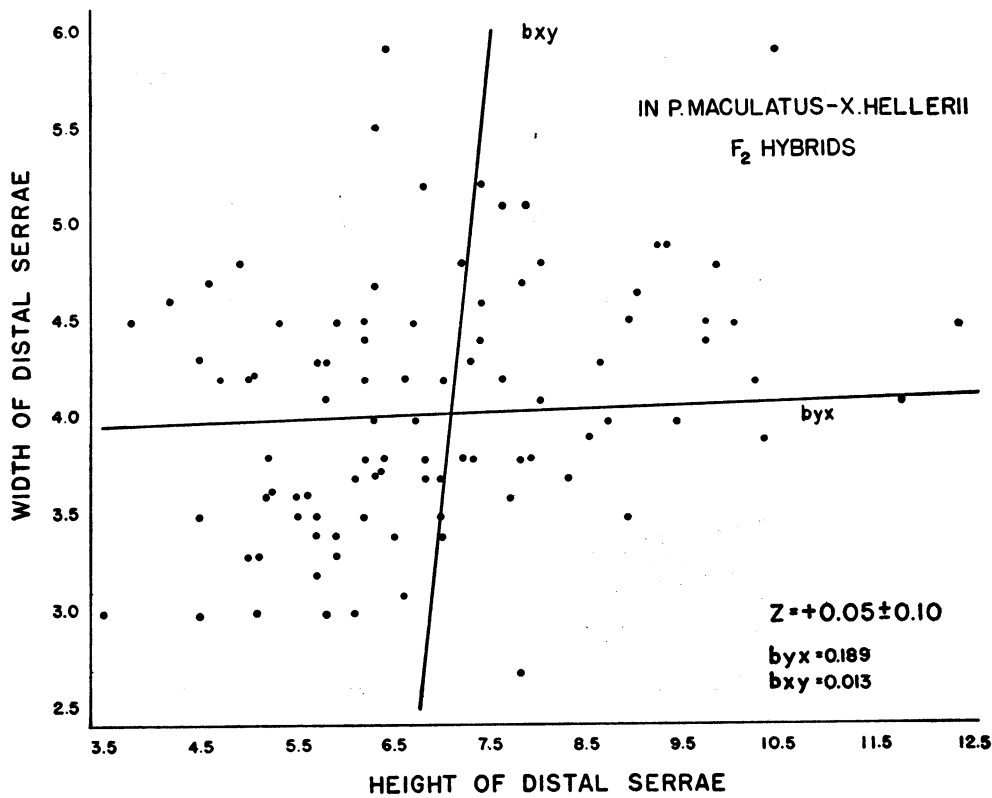


FIG. 20. Scatter diagram showing distribution patterns of the relationship of width and height of the distal serrae of the second-generation hybrids of the platyfish and swordtail. The slopes of the lines of regression are indicated by byx and bxy . The calculated correlation coefficient for the width and height is represented by z . The numbers along the coordinates represent units of measurement which are explained in the text.

The width and height of the serrae in each gonopodium were measured on one-tenth-inch, cross-section graph paper where each one-tenth-inch square represented one unit. The width of the base and height of the shaft of each segment were recorded, and their values were placed in separate columns, each of which contained as many values as there were segments in a group of serrae. A total for all the widths (w) and heights (h) was taken, and each total was divided by the number of segments to determine the average dimension. The averages of the actual measurements expressed as \bar{m}_w and \bar{m}_h are given in tables 6 and 7.

INHERITANCE OF WIDTH OF DISTAL SERRAE

The average values of the width of the distal serrae and their range for *P. maculatus*,

X. hellerii, and the F_1 and F_2 hybrids are given in table 6 and are summarized as follows:

	<i>P.</i> <i>maculatus</i>	<i>X.</i> <i>hellerii</i>	F_1	F_2
\bar{m}_w	3.9	5.4	4.4	4.1
Range	2.9-4.4	4.7-6.3	3.6-5.2	2.7-5.9

The histogram shown in figure 21 indicates a sharp difference between the widths of the distal serrae of the platyfish and those of the swordtail; the values are intermediate in the F_1 generation hybrids, and they have an approximately normal distribution in hybrid populations of the F_2 generation.

The range of distribution of the F_2 values falls one unit short of the highest limit set by *X. hellerii*, while it extends down to the lower limit set by *P. maculatus*. When calculated by Pearson's coefficient, V , the vari-

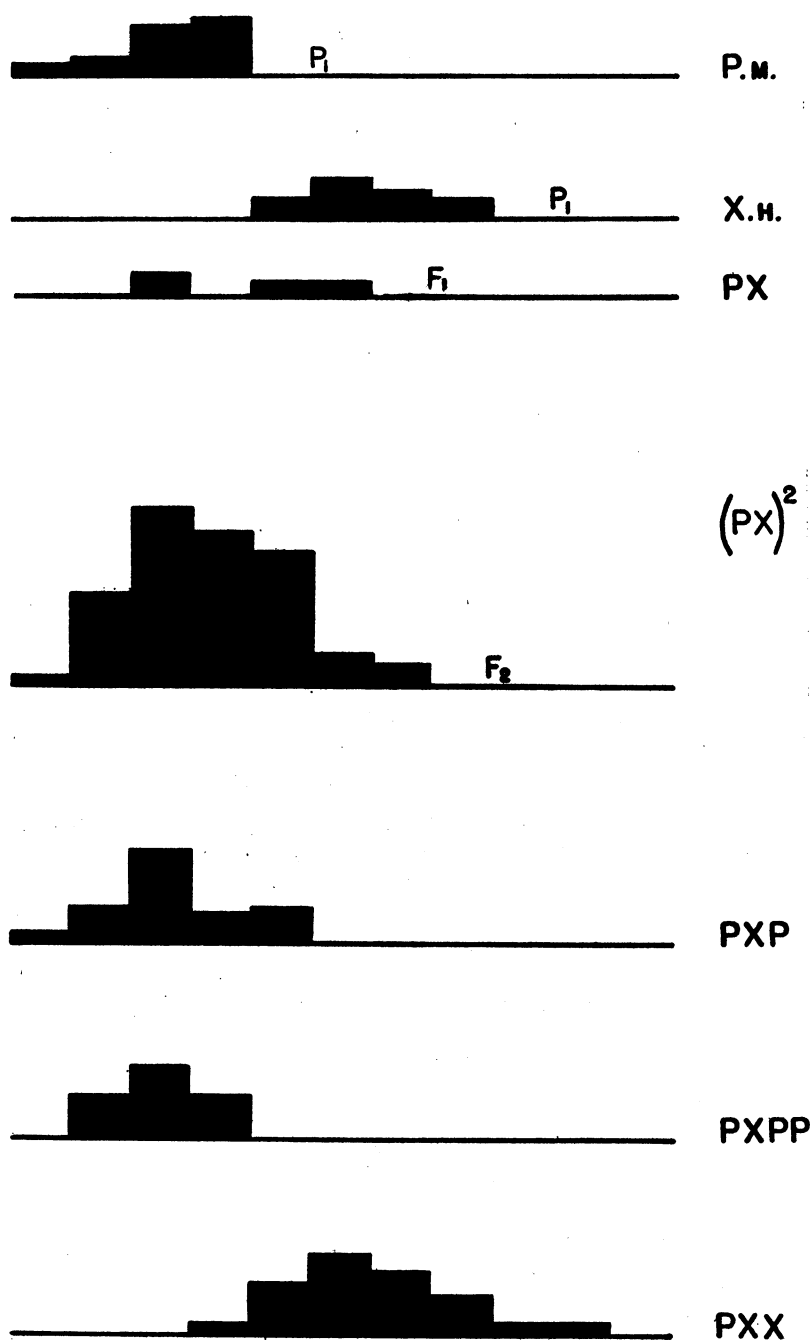


FIG. 21. Histograms representing the width of the distal serrae in the gonopodium of *Platypoecilus maculatus*, *Xiphophorus hellerii*, and their hybrids. *Pm*, *P. maculatus*; *Xh*, *X. hellerii*; PX, F₁ hybrids between *Pm* and *Xh*; (PX)², F₂ hybrids of *Pm* and *Xh*; PXP, backcross hybrids of PX and *Pm*; PXPP, backcross hybrids of PXP and *Pm*; backcross hybrids of PX and *Xh*.

TABLE 6

AVERAGE VALUES AND COEFFICIENTS OF VARIABILITY OF THE WIDTHS OF THE BASES OF THE DISTAL SERRAE FROM THE GONOPODIA OF *P. maculatus*, *X. hellerii*, AND THEIR HYBRIDS

N ^a	Group	\bar{m}_w	V
27	<i>P. maculatus</i>	3.9 ± 0.1	10.7 ± 1.7
16	<i>X. hellerii</i>	5.4 ± 0.1	9.8 ± 1.7
8	F ₁ (PX)	4.4 ± 0.3	10.1 ± 2.7
105	F ₂ (PX) ²	4.1 ± 0.1	15.9 ± 1.1
39	h57 (PXP)	3.8 ± 0.1	13.7 ± 1.8
39	h73 (PXPP)	3.5 ± 0.1	9.9 ± 1.4
46	hR (PXX)	5.3 ± 0.1	11.2 ± 1.3

^a N, number of individuals; \bar{m}_w , average value of widths of distal serrae; V, coefficient of variability.

TABLE 7

AVERAGE VALUES AND COEFFICIENTS OF VARIABILITY OF THE HEIGHTS OF THE DISTAL SERRAE FROM THE GONOPODIA OF *P. maculatus*, *X. hellerii*, AND THEIR HYBRIDS

N ^a	Group	\bar{m}_h	V
27	<i>P. maculatus</i>	9.1 ± 0.3	13.2 ± 2.1
16	<i>X. hellerii</i>	7.6 ± 0.2	13.6 ± 2.4
8	F ₁ (PX)	9.1 ± 0.8	22.2 ± 5.9
105	F ₂ (PX) ²	7.1 ± 0.2	34.4 ± 2.5
39	h57 (PXP)	10.7 ± 0.3	14.4 ± 1.9
39	h73 (PXPP)	10.4 ± 0.3	14.5 ± 2.1
46	hR (PXX)	8.4 ± 0.2	29.1 ± 3.3

^a N, number of individuals; \bar{m}_h , average value of height of distal serrae; V, coefficient of variability.

ability and dispersion of the values for the widths in the F₂ population were found to be noticeably higher than the variability and dispersion in the F₁ and P₁, as follows:

<i>P.</i> <i>maculatus</i>	<i>X.</i> <i>hellerii</i>	F ₁	F ₂
V 10.7 ± 1.7	9.8 ± 1.7	10.1 ± 2.7	15.9 ± 1.1

The means and ranges for the backcross are as follows:

	PXP	PXPP	PXX
\bar{m}_w	3.8	3.5	5.3
Range	2.8–4.8	3.1–4.2	4.2–7.0

The histogram and tests of association according to the χ^2 method of analysis indicate that the values of width of the PXP backcross hybrids and the double PXPP backcross hybrids are not significantly different from the corresponding values of the platyfish. The values for the PXX hybrids are not significantly different from those of the swordtail.

These data and figure 21 indicate that the dispersion and variability of the PXP hybrids, representing the first backcrosses to *P. maculatus*, and the PXX hybrids, representing a backcross to *X. hellerii*, are slightly greater than those of the parental types. Compared with *P. maculatus*, the PXPP hybrids have a smaller range of dispersion and a lower, though not significantly different, coefficient of variability. The V values are given in table 6.

The data collected on the widths of the distal serrae indicate these primary patterns of inheritance:

1. The F₁ are intermediate.
2. The F₂ have approximately the same mean value as the F₁.
3. The width values of the F₂ are more widely dispersed and more variable than those of the F₁ or P₁.
4. Some of the F₂ hybrids reach the high limit of *X. hellerii* and the low limit of *P. maculatus*.

These details can best be interpreted genetically if it is assumed that a series of multiple factors determines the width. Because 10 out of 105 F₂ hybrids had width patterns in the distal serrae comparable to those found in the parental types, it may be assumed the number of factors is probably small. This supposition is supported by study of the coefficients of variability of the F₂ population which are only slightly higher than the coefficients of the F₁ and the two P₁ generations.

In addition, the results obtained from the various backcross generations indicate the presence of relatively few multiple factors. For example, the PXP hybrids are essentially the same as the platyfish for the trait under discussion, and this is also true of the PXPP hybrids. However, the PXPP group is much more uniform, being less variable than the platyfish parent. The PXX hybrids are es-

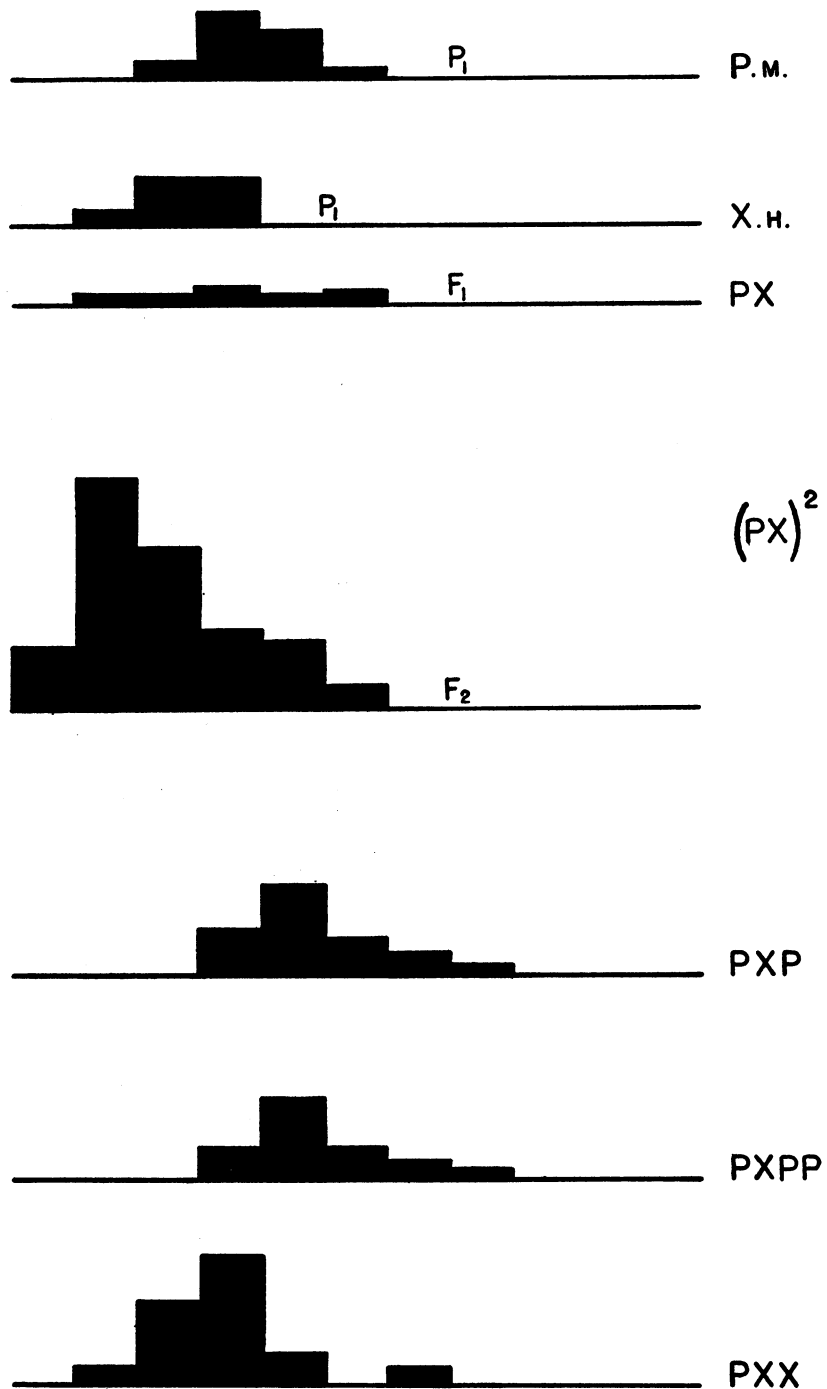


FIG. 22. Histograms representing the height of the distal serrae in the gonopodium of *Platypoecilus maculatus*, *Xiphophorus hellerii*, and their hybrids. *Pm*, *P. maculatus*, *Xh*, *X. hellerii*; PX, F₁ hybrids between *Pm* and *Xh*; $(PX)^2$, F₂ hybrids of *Pm* and *Xh*; PXP, backcross hybrids of PX and *Pm*; PXPP, backcross hybrids of PXP and *Pm*; PXX, backcross hybrids of PX and *Xh*.

sentially similar to the swordtail. In all backcross categories the coefficients of variability are always less than those of the F_2 populations.

INHERITANCE OF HEIGHT OF DISTAL SERRAE

The average values for the heights of the distal serrae and their ranges for *P. maculatus*, *X. hellerii*, and the F_1 and F_2 populations were:

	<i>P.</i> <i>maculatus</i>	<i>X.</i> <i>hellerii</i>	F_1	F_2
\bar{m}_h	9.1	7.6	9.1	7.1
Range	7.2-12.3	5.5-9.3	6.2-11.9	3.5-12.3

The platyfish differs from the swordtail for heights of the distal serrae slightly but significantly according to χ^2 calculations. The histogram (fig. 22) indicates considerable overlapping, with *P. maculatus* having the higher and *X. hellerii* the lower values.

While the heights in the F_1 populations happen to be of the same mean value as those of the platyfish, the coefficient of variability of the F_1 is considerably greater, almost twice that of P_1 . This suggests that the true mean value might have been intermediate had a larger sample been available.

The F_2 hybrids are extremely variable, as indicated by the high coefficient, V . The mean of the F_2 population is essentially like that of the swordtail. This is reflected in figure 22 by the skewness in the distribution of the height value, which is definitely in the direction of the swordtail. This is in contrast to the results obtained in evaluating the widths of these same segments, where the F_1 and F_2 distributions had mean values that were intermediate.

The comparable values for the various backcross generations are as follows:

	PXP	PXPP	PXX
\bar{m}_h	10.7	10.4	8.4
Range	8.3-14.9	8.0-14.1	5.0-12.5

The histogram (fig. 22) and the χ^2 calculations indicate no significant differences between the various backcross hybrids and the parental types. The first, PXP, and the second, PXPP, backcross platyfish hybrids have closely similar average values and distributions.

The PXX backcross swordtail hybrids

have a higher, but not significantly higher, average value than has the swordtail alone.

The coefficients of dispersion and variability are alike in the PXP and PXPP hybrids but slightly larger than the coefficient for the platyfish. The coefficient for the PXX hybrids is quite high, 29.1, this being slightly over twice as great as the indicated variability for *X. hellerii*. The V values are given in table 7.

The data on the values representing the heights of the distal serrae can be summarized as follows:

1. The F_1 are like the platyfish parent (not intermediate).

2. The F_2 have a mean value like that of the swordtail (not intermediate or like the F_1).

3. The F_2 are more variable and their range is greater than those of F_1 or P_1 .

These details are so inconsistent that they cannot be easily fitted into any genetic pattern, nor are the details concerning the backcross hybrids helpful.

A parallelism was observed between the results obtained from studies of the heights of the distal serrae and the shape index of the distal serrae. This can be appreciated by a comparison of figures 17 and 22. In a sense, figure 17 is a reciprocal of figure 22. This can be explained by the fact that the values for height were used as denominators of the fraction from which the shape index was derived, as w/h .

One of the reasons why these two evaluations appear to be reciprocal in the P_1 and the backcross generation is that the values of w vary inversely with the values of h . For example, in *P. maculatus* the low values for w and the high values for h are always associated to produce the characteristic platyfish pattern. This same principle is true in some measure for a backcross to *P. maculatus*, where there are more low values of w recombining with high values of h to produce a definite platyfish shape.

The reason why the F_2 distributions do not reflect the reciprocal pattern completely is that the height and width factors are independent and recombining at random to produce all possible types. This is supported by the data previously presented on the correlation between the two measurements (fig. 20).

GENERAL DISCUSSION

THIS HAS BEEN AN EFFORT to determine some of the genic differences between two species of fishes, *Platypoecilus maculatus* and *Xiphophorus hellerii*. Since species are usually defined by morphological differences, and since the structural details of the genitalia have been highly regarded as basic species characters, the gonopodia of the platyfish and swordtail were compared and recorded. Certain differential and critical gonopodial structures were studied genetically in various kinds of hybrids between these species.

The historic fact that the platyfish and swordtail are placed in separate genera might overemphasize the distinctions between them. By modern standards of taxonomic procedures, as expressed by Mayr (1942), the two genera could be joined, but it should be emphasized definitely that the fishes represent "good" species.

MORPHOLOGICAL DIFFERENTIATION

First, of course, there are many distinctive morphological differences between the platyfish and swordtail in addition to those of their gonopodia. They differ significantly in the number of dorsal fin rays, in the number of scales in the lateral line (Hubbs and Gordon, MS), in the extension of the lower caudal fin rays, in the structure of many details of their gonopodial suspensoria: gonactinosts and gonapophyses; and in the number of caudal vertebrae (Gordon and Benzer, 1945). In addition, they differ significantly in details of their embryonic development: embryonic rate of growth, rate of caudal fin development, and the time of initial pigment development (Tavolga, 1949). With respect to their germ cell development, they are quite different, according to Wolf (1931). The genetic mechanisms for sex determination vary in the two species, for in the platyfish it is quite stable (Gordon, 1937, 1947), whereas in the swordtail it is labile (Essenburg, 1923; Kosswig, 1939; Witschi, 1939; Regnier, 1939).

ECOLOGICAL CONDITIONS

Second, with regard to ecological criteria, Gordon (1947b) indicated that the swordtail

is generally a head-water species, whereas the platyfish is a lowland one (fig. 23). Nevertheless in 25 per cent of their habitats they are sympatric. Apparently in some lowland water areas swordtails either migrate or are swept downstream into platyfish territory, and both species may be taken in the same seine haul. No hybrids between them have as yet been collected in their natural habitats.

BEHAVIORAL DIFFERENTIATION

In an effort to analyze the sexual isolation mechanism, which apparently inhibits hybridization under natural conditions, Clark, Aronson, and Gordon (1948) found no single item of behavior that alone would account for failure of the species to hybridize. The fishes do differ in the frequency of a series of particular behavioral details. The effectiveness of the two distinct patterns of behavior seems to depend upon the total of the combined items of behavior. Under laboratory conditions, where freedom of action and choice is limited, these two balanced systems of behavior pattern are upset. It is merely necessary to place virgin platyfish and swordtail of opposite sexes together in a small (five-gallon) aquarium, and cross-mating will eventually be effective in most instances.

HYBRID STERILITY AND SEX CHROMOSOMES

The degree of sterility in the platyfish-swordtail hybrids depends primarily upon the type of mating, the genotype of the hybrid, and particularly the presence or absence of the Y chromosome of the platyfish. For example, if the wild platyfish from the Rio Jamapa is the female parent and the wild swordtail from the Rio Papaloapan (Zacatispan population) is the male parent, the sex ratio among their hybrids is approximately as follows: 90 per cent female, 10 per cent male, according to Gordon (1948a). This mating can be expressed genetically by indicating the various sex chromosomes "marked" by appropriate genes: *S_p* represents irregular macromelanophore spotting along the sides of the platyfish, and *S_d* represents macromelanophore spotting on the

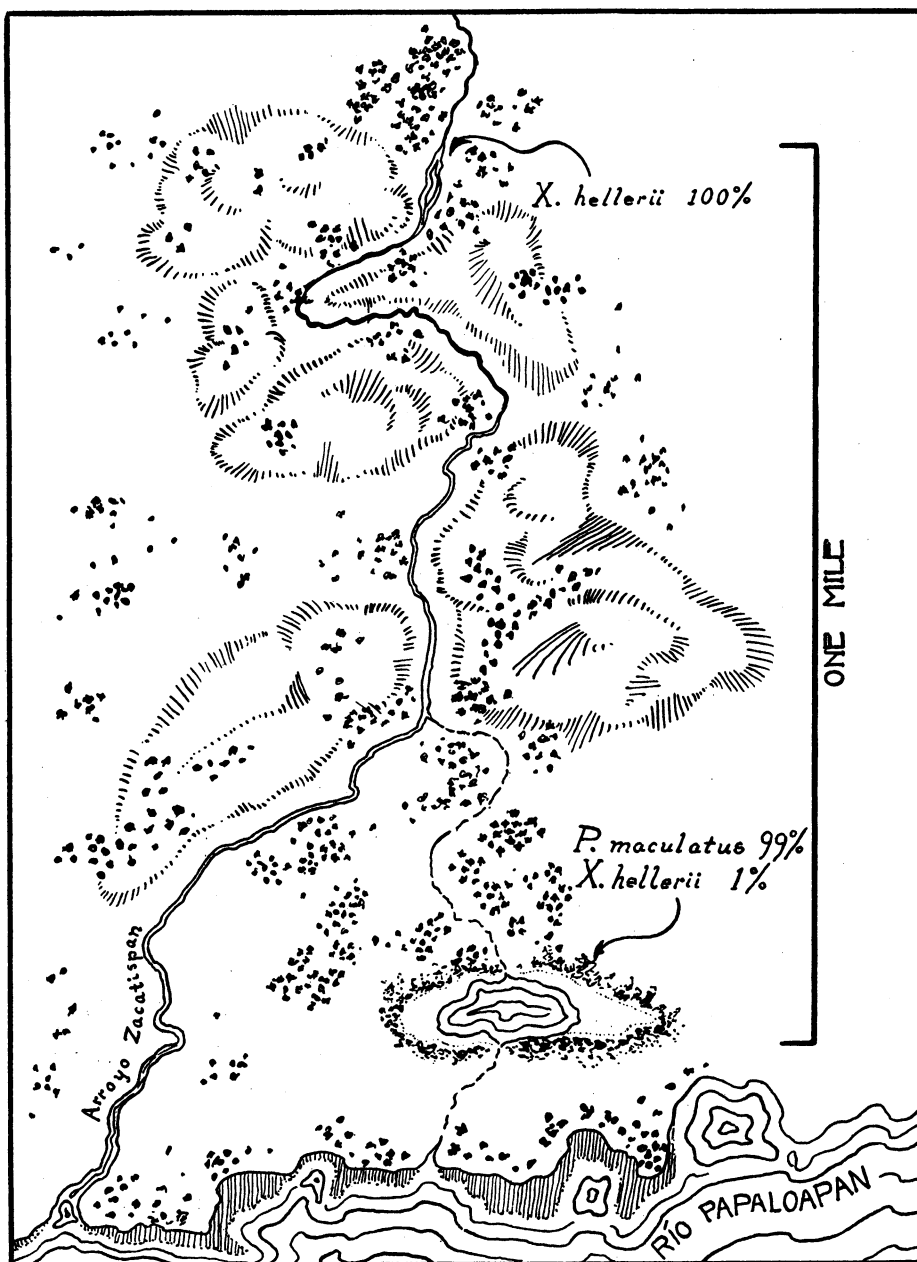


FIG. 23. Reconstruction of the habitats of *Platypoecilus maculatus* and *Xiphophorus hellerii*, indicating the point where these species are sympatric. The Arroyo Zacatispan arises in the hills of San Bartolo 10 kilometers south of Papaloapan, Oaxaca, Mexico. Here the water is clear and free of vegetation and here swordtails (*X. hellerii*) are found exclusively, as indicated in the figure. Downhill one of the temporary tributaries of the Arroyo Zacatispan fills the shallow depressions along the valley of the main Rio Papaloapan. At this point, where the water is less clear and contains vegetation, the platyfish (*P. maculatus*) are found in abundance. Here the platyfish are sympatric with swordtails, but the former are much more numerous than the latter. In the pool indicated in the figure, 5148 platyfish were taken to only 60 swordtails.

dorsal fin. The platyfish sex chromosomes may be identified by XY, while those of the swordtail are indicated by X'X'.

Platyfish Female	P ₁	Swordtail Male
(X)Sp/(X)Sd		(X')+/ (X')+
F ₁		
(X)Sp/(X')+: spotted hybrids:	45% females;	
	5% males	
(X)Sd/(X')+: spotted dorsal hybrids:	45% females;	
	5% males	

Tavolga (1949) showed that 33 (X)Sp/(X') + platyfish-swordtail female hybrids tested for fertility revealed only two Sp hybrids which were completely sterile; and three Sd hybrids were of low productivity, producing aberrant broods, each of which contained over 30 per cent anomalous embryos.

Of 34 (X)Sd/(X') + female hybrids, 15 were completely sterile, 11 possessed aberrant broods, and eight produced normal broods. Four out of five (X)Sd/(X') + male hybrids and four out of five (X)Sp/(X') + male hybrids, when tested with various virgin platyfish, swordtails, or hybrids, were fertile.

If the wild swordtail from Rio Papaloapan (Zacatispan) is the female parent and the wild, stripe-sided, spotted-dorsal platyfish from the Rio Jamapa is the male, the sex ratios are quite different, as follows:

Swordtail Female	P ₁	Platyfish Male
(X')+/ (X')+		(X)Sr/(Y)Sd
F ₁		
(X)Sr/(X')+: 50% stripe-sided hybrid females;		
mostly fertile; (no males)		
(Y)Sd/(X')+: 2% spotted-dorsal hybrid males;		
fertile		
3% spotted-dorsal hybrid females; fertile		
45% spotted-dorsal hybrids; sterile		

The first 10 (X)Sr/(X') + hybrid females tested proved to be fertile, and the assumption was made that the rest were probably also fertile.

Two (Y)Sd/(X') + hybrid males proved to be fertile. It was surprising that three hybrid females presumably of the same genetic constitution, (Y)Sd/(X') +, were also fertile, at least partially so, for they produced a few living young. None of these females approached the high degree of fecundity of the (X)Sd/(X') + female hybrids. Most of the

(Y)Sd/(X') + hybrids, amounting to approximately 45 per cent of the entire brood, had no visible gonads, and they attained a body size greater than that of the other hybrids.

From these details it appears that the X chromosome of the platyfish is fairly compatible with its theoretical counterpart, the X' chromosome of the swordtail, but the platyfish Y chromosome, for the most part, is incompatible with the X'. The degree of compatibility of the platyfish X chromosome with the swordtail X' chromosome varies with the type of gene the platyfish X carries. Sr and Sp genes on the X in combination with X' produce fertile hybrids, but the Sd on the X in combination with X' carries with it some sterility. When Sd is carried on the Y chromosome, most of the platyfish-swordtail hybrids are sterile. Thus it would be incorrect to state without qualification that the F₁ platyfish-swordtail hybrids are fertile.

Hybridization in many instances leads to abnormal cellular behavior not only in the reproductive cells but also in the pigment cells, particularly the macromelanophores, as indicated by Gordon (1948a). Many fertile hybrids develop melanotic tumors that are lethal. An attempt is being made to discover the relationship, if any, between the atypical growth of the reproductive cells and those of the pigmentary system.

FACTORS IN MORPHOGENESIS OF GONOPodium

In the course of this work some apparently normal male platyfish, male swordtails, and male hybrids could not be used in genetical analyses because their gonopodia were aberrant in the fine structural elements. Clark, Aronson, and Gordon (1949) have shown by experiment that the distal tip of the gonopodium containing these critical details must be intact to be effective in copulation. Anomalous gonopodia were found among the males of all the hybrid types analyzed.

There is a definite relationship between the development of the testes and the morphogenesis of the gonopodium in *Gambusia*, according to Turner (1941, 1947a). In line with this evidence the senior author (1937), in a study of a collection of *X. hellerii* taken in 1930 in its native environment at Jalapa,

Vera Cruz, found that there were two clearly defined size groups in the male-like members of the population. In the group of smaller males the gonopodia were well differentiated, and the sword-like tail extensions were well developed (being as long as the body), but in the second group of larger males most had imperfect gonopodia and their swords were relatively short. The anal fins of the larger swordtails had external contours of gonopodia, yet they were undifferentiated, being flabby and without definite segmentation. All the large swordtail males revealed upon dissection extremely small gonads or had none that could be detected, whereas the small swordtail males had large, firm, normal, fused testes.

The strong influence of sex hormones upon growth and differentiation of the poeciliid gonopodium has been indicated by many workers whose views and results have been conveniently summarized by Atz (MS). Grobstein (1948), for example, has shown by carefully designed experiments in which the aquarium water chemistry as well as the dosage of the androgen was rigidly controlled that, when an optimum concentration of methyl testosterone was applied at a constant rate to castrated immature platyfish males or to females with excised anal fins, their anal fins transformed to gonopodia. These fins resembled closely the gonopodia of normal males. When concentrations above the optimum were applied, the anal fins transformed more rapidly but were atypical, the 3-4-5 complex being shorter. Grobstein suggests that the proper morphogenesis of the gonopodium may be influenced by one or more specific androgens in proper combinations and concentrations together with some non-androgenic "modulators." Possibly the latter term might include genetic factors that may operate in other ways than through the androgens. In this connection, Cohen, Gordon, and Nigrelli (1941) reported the spontaneous development of gonopodia in two apparently normal female platyfish, both of which produced normal young and were gravid again at the time of observation.

Gabriel (1944) showed experimentally that when eggs from a single pair of *Fundulus* were hatched and allowed to develop in varying water temperatures the embryos had

varying numbers of vertebrae. In general, lower temperatures produced a higher number of vertebrae. This relation had previously been known and had been applied to the number of fin rays (Hubbs, 1922). Gabriel, however, found some batches of eggs that were temperature stable, and the embryos developing from such eggs did not vary markedly with reference to their vertebral counts. He concluded that variations in the number of vertebrae are dependent both on the temperature relationships between processes controlling growth and processes concerned with differentiation at certain critical stages of development.

In some preliminary observations for a forthcoming study on the inheritance of numbers of fin rays in platyfish, swordtails, and their hybrids, it was discovered that the frequency of dorsal fin rays was consistently lower in laboratory reared platyfish than in their wild progenitors. This is attributed to the probability that for most of the year the temperatures maintained in the laboratory are generally slightly higher than those of the natural habitats of the platyfish, according to Gordon (1948b). Slightly higher temperature may cause an earlier maturation of the gonads in the males, which may then release the sex hormones that cause an earlier differentiation of the gonopodium. This in effect would produce gonopodia that are smaller and that contain fewer segments. Lower temperatures would have an opposite effect, delaying the development of the testes. Two platyfish-swordtail male hybrids having an X'Y chromosome complex matured at a slower rate and grew to a larger size than X'X male hybrids. Most X'Y hybrids were sterile and without gonopodia, but those that were fertile were large and their gonopodia were correspondingly larger than those of ordinary males. In addition, their gonopodia had a greater number of segments.

In view of the many environmental influences upon the definitive characters of the gonopodia, only laboratory reared parental stocks and their various hybrids were used in this study. Under laboratory conditions, gonopodial characters remained fairly uniform and were therefore regarded trustworthy for genetical analysis.

Undoubtedly similar difficulties were en-

countered in previous attempts to study the genetics of species differences in the morphology of genitalic characters in other animals, especially among insects.

GENETICS OF GENITALIC CHARACTERS IN INSECTS

The genetics of the genitalia in insects was studied by Foot and Strobell (1914) who crossed two hemipterans of the genus *Euschistus*, *E. varolarius* and *E. serous*. They found that the length of the penis, a differentiating specific character, was intermediate in the F_1 hybrids. Since the parental types of genitalia were rarely recoverable in the F_2 hybrid offspring, they concluded that more than one genetic factor for penis length was involved.

Shull (1946) studied the inheritance of the form of the chitinous male genitalia in crosses of the lady beetle *Hippodamia*, *H. quinquesignata* and *H. convergens*. He obtained 13 F_2 beetle hybrids from two matings. He made two backcrosses to one of the parental species and a number of other types of matings. His analysis of the hybrids led him to conclude that some specific genital structure such as the siphonic flaps and the aedeagal keel were differentiated by two pairs of genes and the width of the siphon by possibly five pairs. Shull also reviewed the earlier work of Timberlake (1919) who crossed two *Hippodamia* species, *H. parenthesis* and *H. lunatamaculata*, and who found that the genitalia of the F_1 hybrids were intermediate. However divergent the genital structures were, Shull declared, they did not prevent successful copulation between the species or their various hybrids.

SPECIES DIFFERENCES IN GENITALIA AS ISOLATING MECHANISM

Robson and Richards (1936) indicated that considerable difference in genitalia in some pairs of distinct insect species did not prevent hybridization. Goldschmidt (1940), Dobzhansky (1941), and Mayr (1942) agree that genitalic differences in species are not

necessarily effective isolating mechanisms. This is supported by study of the biology of xiphophorin fishes.

In discussing the "lock and key" theory in part, Robson and Richards suggested that any change in one sex directly connected with pairing appears to necessitate a correlated change in the other sex. Satisfactory explanation of the mode of origin of these paired differences is wanting. Clark, Aronson, and Gordon (MS) will indicate that the genital orifice of the female *Xiphophorus hellerii* differs from that of *Platypoecilus maculatus*. These differences are not apparently so great as those in the gonopodia of the corresponding males, yet despite differences in the genitalia of both sexes, hybridization between these species is easily consummated in an aquarium, especially when homotypic pairing is prevented. In nature, opportunities are open for hybridization between a number of xiphophorin species. For example, *Platypoecilus variatus* and *Xiphophorus montezumae* are frequently found in the same pool and are caught with the same sweep of the seine; sometimes these two species are found in company with a third xiphophorin species, *X. pygmaeus*. In other localities *X. hellerii* is most often found together with *P. maculatus* (Gordon, 1947b), but this swordtail was found in 1948 by Gordon (unpublished) living with *P. variatus* in the Rio Nautla. It is most probable that the xiphophorin fishes, in the early stages of their speciation, were geographically isolated. Although their habitats were joined subsequently, effective sexual isolating mechanisms were established in the interval. This may explain why no hybrids are found in nature.

In summary, the deciding factors in the successful production of fertile hybrids in xiphophorin fishes under laboratory conditions depend, first, upon overriding the behavioral differences between the species and, second, upon the fortunate combination of compatible chromosomes. The small differences of structure that exist in the two pairs of apparently non-corresponding genitalia appear to be of minor importance.

MODE OF INHERITANCE AND EVOLUTION OF GENITAL- IC ELEMENTS IN XIPHOPHORINS

To estimate the number of gene pairs involved in the hereditary processes influencing structures of xiphophorin gonopodia, many more F_2 and other types of platyfish-swordtail male hybrids would be required than we have bred and reared. One observation may be brought into focus. Since 10 parental types were recovered in 105 F_2 hybrids, relatively few genetic factors were indicated. On the other hand, the recovered parental types were indicated by their mathematical equivalents and did not necessarily represent identical morphological counterparts. Thus our estimate of the actual P_1 types recovered in F_2 is perhaps too high, and there are probably many genetic factors involved.

Breider (1936) analyzed 14 differences in the body proportions and frequencies of vertebrae and fin rays in three poeciliid species of *Limia*: *L. vittata* of Cuba, *L. caudofasciata* of Jamaica, and *L. nigrofasciata* of Haiti. He attributed the present differentiation of the three *Limia* species to geographical isolation. Partial hybrid fertility made a number of genetic studies possible, and Breider suggested that the small differences between these species were genetic and controlled by multiple factors. Goldschmidt (1940) questioned the specific rank of these three forms. He denied Breider's conclusion that the genetic behavior of species hybrids is in principle similar to genetic behavior of racial hybrids in that both involve multiple factors. Goldschmidt claimed that these isolated forms represent no more than "diversified blind alleys within a species," and that species are separated by unbridgeable genetic gaps. The weight of opinion on genetic mechanisms leading to speciation and evolution is contrary to Goldschmidt's point of view. However, his assertion that many isolated forms do not interbreed solely for lack of opportunity is highly suggestive. It suggests that experiments can be designed to test for reproductive isolation in geographically isolated groups. With regard to the platyfish and the swordtail, it has already been indicated that these species are sympatric in 25 per cent of

their known habitats, and no hybrids between them have been found in thousands of specimens collected.

In accordance with the generally accepted genetical theory of variation and natural selection, Mather (1943) suggested that species differences are polygenic, i.e., depend on quantitative characters whose variation is controlled by many genes. Polygenes have individual effects which are both similar to one another and small when compared with non-heritable fluctuations. According to Mather, other kinds of heritable differences are secondary in importance to polygenic variation in speciation. Hubbs (1940), in his discussion of "Speciation of fishes," called attention to the differences in the apparent mode of inheritance "between the systematic characters showing blending inheritance on one hand and the phase characters with a simple genetic basis on the other." He contrasted the inheritance of a color phase which showed simple Mendelian segregation with the blending type of inheritance of such systematic traits as fin ray frequencies and other meristic characters. In Mather's terms color genes are "oligogenes," while systematic genes are "polygenes"; both, of course, are Mendelian. Often they interact with each other; for example, the sex-linked oligogene, *Sp*, for macromelanophores in the platyfish has a series of polygenic modifiers which keep the growth of these large black pigment cells in balance. When the spotted, *Sp*, platyfish is mated to a swordtail (or to a member of its own species but from another long-isolated population) the *Sp* polygene modifiers interact differently with the *Sp* oligogene. As a result the *Sp*, the spotted, hybrids develop melanomas or severe melanoses (Gordon, 1946, 1948a, 1949, 1950). These changes in the growth properties of macromelanophores, some of which are pathological, some malignant, are brought about by the new combinations of polygene modifiers. The probability is that polygene modifiers are somewhat different in each geographically or reproductively isolated xiphophorin population, each of which represents a different genetically balanced system. The genetic balance in the pigmentary complex is upset when the geographical or reproduc-

tive isolation barriers are removed or voided under conditions of domestication.

The differences in the gonopodia of the seven xiphophorin species are by no means so important phylogenetically as their similarities. To generalize further, the gonopodia of all Poeciliidae are quite uniform in that the third, fourth, and fifth rays of the anal fins of all of them are specialized. These structures may indicate another example of parallel evolution. Kosswig (1948) believed that the gonopodial structures in the various poeciliids are independently evolved and depend upon analogous rather than homologous genes. However, Mayr (1949) suggested that there is often a common genetic background which pre-adapts for a given evolutionary trend. He referred to the work on the saturniid moths by Michener (1949) who studied the variation in their genital structure among other characters. Michener utilized the concept that parallel morphological details in related saturniids depend upon homologous chromosomes and genes.

In view of the over-all similarities in the gonopodia of the seven platyfishes and swordtails, one might account for the presence of the small but consistent distinguishing characters in these organs by assuming that each species of this compact group has drawn differentially upon a common stockpile of accumulated polygenes which delimit the generalized xiphophorin gonopodium. This has been accomplished, most probably, through natural selection operating upon each discrete population. Recombinations of polygenes controlling gonopodial structures in xiphophorin species are apparently not injurious to the hybrid individuals, nor do they in themselves prevent reproduction. On the other hand, some recombinations of polygenes controlling macromelanophore growth are definitely injurious to the hybrid individual. Apparently the large-black-pigmented cell genes of the common platyfish like *Sp*, *Sb*, *Sd*, *Sr*, and *N* have not yet attained equilibrium with all their polygene modifiers that have accumulated in the xiphophorin fishes. Gordon (1949) suggested that the macromelanophore genes have appeared relatively recently in xiphophorin evolution.

TAXONOMIC IMPLICATIONS

The study of the common platyfish, *P. maculatus*, and common swordtail, *X. hellerii*, as well as the other five xiphophorins clearly demonstrates a compact group of seven species having a fairly uniform set of characters not only in their gonopodia but in their gonopodial suspensoria and other details of taxonomic importance. The historical record of the separation of this compact group into two genera has served its purpose during a period when only a few xiphophorin species were known. Now, however, there are intermediates known for every important taxonomic character, as follows:

The claw, while strong in *X. hellerii*, is weak, almost wanting, in *X. montezumae*. While the claw is not present in any of the platyfishes used, claws do appear in many of the hybrids of two platyfish species, according to unpublished data of Rosen and Gordon. This would indicate that the formation of the claw may depend upon complementary genes in platyfishes which became separated in some of the xiphophorin fishes during the speciation process.

The sword-like extension of the ventral rays of the caudal fin is poorly expressed in the pygmy swordtail, *X. pygmaeus*. In striking contrast, it is far better represented in the Rio Soto la Marina platyfish, *P. xiphidium*. The "sword" has been induced experimentally in *P. maculatus* by treatment with pregnenolone, according to Gordon, Cohen, and Nigrelli (1943) and Cohen (1946). This suggests that even the ordinarily swordless *P. maculatus* has genetic factors for sword formation but requires more or different androgenic hormones for the expression of this character.

The unpublished data of Hubbs and Gordon (MS) indicate that the frequencies of the dorsal fin rays in the four platyfish species range from nine to 12, while those of the three swordtails vary from 10 to 13; thus this taxonomic character is not critical for generic distinction. In addition, according to Gordon and Benzer (1945), the numbers of vertebrae and of ribs on the caudal vertebrae in the two groups of xiphophorins overlap, as follows:

	AVERAGE NUMBERS OF VERTEBRAE	NUMBERS OF PAIRS OF RIBS ON CAUDAL VERTEBRAE
1. <i>P. maculatus</i>	25.9	2
2. <i>P. xiphidium</i>	26.6	2
3. <i>X. pygmaeus</i>	26.9	2
4. <i>P. couchianus</i>	27.2	2
5. <i>P. variatus</i>	27.3	2
6. <i>X. hellerii</i>	27.9	4
7. <i>X. montezumae</i>	28.0	3

In view of the genetic continuity within the seven xiphophorin species, it is recommended that a single generic term be applied to all of them. Since *Xiphophorus* of Heckel (1848) is much the earlier, *Platypoecilus* being established by Gunther in 1866, the genus *Xiphophorus* should represent the group as a whole. It is suggested that the name *Platy-poecilus* be retained as a subgeneric grouping for the platyfishes, especially in view of its wide usage in genetical and other publications. The retention of *Platypoecilus* as a subgenus would serve to reflect the real but subtle difference between the platyfishes and swordtails which experienced ichthyologists and aquarists can detect at a glance, although statistical techniques and taxonomic terms fail to denote the line of demarcation between them.

According to Mayr (1942), the four platyfishes represent subspecies of a polytypic species, while Dobzhansky (1941) suggests that they may possibly be only races. Studies of reproductive isolation between the species as well as the dominance-subordination relations between members have been reported in xiphophorin fishes by Braddock (1945), Gordon (1947b), Clark, Aronson, and Gordon

(1948), and by Schlosberg, Duncan, and Daitch (1949). Unpublished preliminary data indicate that *P. couchianus* is sexually isolated from *P. xiphidium*. In view of the relatively small quantitative differences that have been found in the delicately balanced behavioral patterns between the sympatric (and most structurally divergent) species such as *P. maculatus* and *X. hellerii*, and of the difficulty of carrying on adequate experiments in sexual isolation, it is doubtful that data of these kinds can in themselves be of critical importance in further revision of the taxonomy of the xiphophorins. Such studies, however, are of definite value in animal behavior and comparative psychology, comparative genetics, and evolution. On the other hand, some systematists have neglected, some underrated, the use of genetic color patterns. Geneticists Vavilov (1922), Haldane (1932), and particularly Harland (1936), and others, have shown the usefulness of intensive studies of parallel color mutations in related organisms. Frequently the genetic differences lie in the polygenic modifiers of the principal color genes. These details have aided greatly in the interpretation of the genetic differences between species. They have been particularly helpful in study of the xiphophorin fishes as outlined by Gordon (1946, 1949).

From the analyses of the genetic color patterns, standard taxonomic characters, ecological conditions, geographic distributions, and an over-all general knowledge of the living and reproducing fishes, under uniform laboratory conditions, it is our present opinion that each of the seven xiphophorins is a "good" species.

SUMMARY AND CONCLUSIONS

IN THE XIPHOPHORIN FISHES the transfer of spermatophores from male to female is accomplished by the gonopodium, which is the male's elongated, stiffened, and highly modified anal fin. Each of the seven xiphophorin species has distinctive structures in the gonopodia by which it can be identified. These differences of *Xiphophorus* (*X. hellerii*, *X. montezumae*, and *X. pygmaeus*) and of *Platypoecilus* (*P. maculatus*, *P. variatus*, *P. xiphidium*, and *P. couchianus*) were measured and compared.

The common platyfish (*P. maculatus*) and swordtail (*X. hellerii*) are sympatric. They were found living together in many natural habitats, yet no hybrids were found there. The sexual behavior of these two species is on the whole quite similar. There are, however, sufficient small differences which when combined produce a distinctive pattern of behavior in each. Under laboratory conditions, where no choice of mates is offered, hybridization takes place.

The fertility of the hybrid is determined to a considerable degree by the type of chromosomal balance it chances to receive. The wild male Mexican platyfish is heterogametic (XY) in its genetic (chromosomal) mechanism for sex determination. No definite genetic mechanism for sex determination is known in the swordtail. Platyfish-swordtail hybrids receiving the Y chromosome are usually sterile. Fertile male and female hybrids have the chromosomal formula of $X'X$, where X' represents the swordtail homologue of the X chromosome of the platyfish. The second-generation hybrids have XX , $X'X$, or $X'X'$ chromosome combinations. Second-generation hybrids with $X'X'$ are generally male.

The deciding factors in the successful production of fertile hybrids in xiphophorin fishes depends, first, upon the overriding of the behavioral differences between the species and, second, upon the fortunate combination of compatible chromosomes. The small differences of structure that exist in the two pairs of apparently non-corresponding genitalia (in male and female platyfish and swordtails) appear to be of minor importance.

An intensive study was made of the inheritance of a number of key gonopodial characters in the hybrids between the common platyfish (*Platypoecilus maculatus*) and the swordtail (*Xiphophorus hellerii*). An analysis of the data obtained from the first- and second-generation hybrids, and in addition a variety of backcross hybrids, revealed that the characters within the gonopodia were controlled by polygenes, the number of which, however, could not be closely approximated.

The differences in the gonopodia of the seven xiphophorin species are by no means so important phylogenetically as their similarities. In consideration of genitalic characters, as well as many others of taxonomic importance, it appears that these species form a compact group which may be considered as forming a single genus. This is *Xiphophorus*, which is the older name.

The small dissimilarities in the gonopodia of the seven xiphophorin species can be accounted for by assuming that each species has drawn differentially upon a common stockpile of accumulated polygenes which delimits their generalized gonopodium. This has been accomplished, most probably, through natural selection operating upon each discrete population.

Recombinations of polygenes controlling gonopodial structures in xiphophorin fishes are not injurious to the hybrid individual nor do they in themselves prevent reproduction. On the other hand, some recombinations of the oligogenes that influence macromelanophore growth and their polygene growth modifiers are definitely injurious to the hybrid individual. Apparently the genes for gonopodial characters of the common platyfish (*P. maculatus*) have attained, while the large-black-pigmented cell genes have not yet attained, equilibrium with all their respective polygene modifiers that have appeared in non-*P. maculatus* xiphophorin fishes. It is suggested that perhaps the *P. maculatus* genes for macromelanophores have appeared more recently in the evolution of these fishes.

POSTSCRIPT

While the present article was in press, a paper entitled "Beiträge zur Kenntnis der erblichen Bedingtheit von Formunterschieden der Gonopodien lebendgebärender Zahnkarpfen" by Atif Sengün was published in Istanbul Üniversitesi Fen Fakültesi Mecmuası (1950, ser. B, vol. 15, no. 2, pp. 110-133).

Sengün studied the inheritance of the complex elements of the distal tip of the gonopodium, particularly the claw on ray 5a, in an effort to determine their genetic behavior. He utilized hybrids obtained from a mating of domesticated swordtail females (*X. hellerii*) and domesticated, homogametic platyfish males (*P. maculatus*). He examined a total of 283 hybrids: 59 F₁, 54 F₂, and 170 backcross hybrids.

Sengün selected the claw on ray 5a for analysis, for he found it to be present in the swordtail gonopodium and wanting in the platyfish. The claw he thought would be more useful for genetic analysis because the other segments have overlapping properties. The results of his analysis were as follows:

A. In the gonopodium of the F₁ hybrids the claw was present but intermediate.

B. In the F₂ hybrids some individuals had gonopodia with, and some without, claws. The majority of the fish had gonopodia with claws of intermediate size.

C. No claws were present in the gonopodia of the hybrids obtained by mating an F₁ hybrid with a platyfish.

D. Claws were present in all the gonopodia of the hybrids backcrossed to *X. hellerii*.

Sengün observed that there was a high variability in the form and frequency of the claw in the various hybrids, particularly in the second-generation hybrids. He found this to be true of other gonopodial elements as well.

He was unable to detect any Mendelian segregation of the character, claw, as against no claw. He suggested that the appearance of the claw was controlled by multiple factors which he estimated to be few in number.

Sengün was able to estimate only roughly the properties of the various gonopodial elements, since he did not utilize actual measurements that might lend themselves to statistical methods.

In our analysis of the gonopodial elements we pointed out some of the difficulties of accurately measuring the form of the claw. Recently a method has been devised by the present authors for evaluating the form of this segment. We are completing a series of analyses of the claw in the hybrids of the seven xiphophorin fishes.

Sengün utilized domesticated varieties of the platyfish and swordtail, while we employed hybrids from matings of fishes obtained from natural populations. Our unpublished data indicate that certain critical elements in the gonopodia of wild fishes differ significantly from those of domesticated stocks. Thus, the results obtained by Sengün and those presented in this paper may not be strictly comparable.

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