MATING BEHAVIOR PATTERNS IN TWO SYMPATRIC SPECIES OF XIPHOPHORIN FISHES: THEIR INHERITANCE AND SIGNIF- ICANCE IN SEXUAL ISOLATION

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INTRODUCTION

SEXUAL REPRODUCTION provides the means for an interchange of genes between freely interbreeding members of natural populations. The reassortment and recombination of genes create, in part, a reservoir of genetic variability in a population, which is necessary for the continuing evolution of a species in a changing environment. Through sexual reproduction, however, an interchange of genes between members of sympatric species may break down some successful, well-balanced, genetic combinations. This may happen when the natural barriers against hybridization between two closely allied species are overridden. Under these conditions, as Muller (1942) aptly phrased it, "sexual reproduction is not an unmixed blessing."

The free flow of genes between members of different species or between members of genetically different natural populations is either prevented or radically reduced by physical circumstances and biological forces to which Dobzhansky (1937) has given the generic term "isolating mechanisms." These have been variously classified by him and by others as being either geographical or biological, that is, in general, extrinsic or intrinsic, with a variety of subdivisions, some of which are overlapping.

When an interchange of genes between the spotted platyfish, *Xiphophorus (Platypoecilus) maculatus* (pl. 2, fig. 1), and the swordtail, *Xiphophorus helleri* (pl. 2, fig. 2), is induced under laboratory conditions, the deleterious effects of new genic combinations are quite evident in the development of pigment cell tumors in the species hybrids (Kosswig, 1927; Häussler, 1928; Gordon, 1931). Gordon and Smith (1938) and Gordon (1951a) pointed out the importance of these genetically induced tumors in comparative oncology because the neoplastic cells in fish, mouse, and human melanomas are histologically quite similar.

As a clear demonstration of the influence of genes on abnormal cell growth, the melanomas in platyfish-swordtail hybrids were of significance both to the oncologist and to the geneticist. Geneticists were interested in the evolutionary implications of the evidence. Crew (1940) and Dobzhansky (1941a),

for example, suggested that the development of the genetically induced tumors in these hybrids served as an isolating mechanism between the species. Hogben (1940) and Huxley (1942) referred to the lethal effects of the tumors in platyfish-swordtail hybrids as evidence of the dissimilarities in the genetic constitutions of related species. Muller (1942) used the evidence of tumor development in the fish hybrids to illustrate the reality of some genetic mechanisms that served to incapacitate hybrids between species. He referred to it as a type of physiological isolating mechanism.

It is true that the abnormal growth of pigment cells (macromelanophores) may incapacitate the playfish-swordtail hybrids. This kind of isolating mechanism, however, could only be effective, if at all, in nature in an insignificant proportion of the individuals which might make up a potential hybrid population. This is because (1) a macromelanophore gene must be present in a hybrid before a melanotic tumor can develop (Gordon, 1948), and (2) only 20 per cent of the platyfish collected in nature carry one or more macromelanophore genes (Gordon, 1934, 1949). Thus if the development of melanomas were the only isolating mechanism between the platyfish and swordtail species, one might expect to find many tumor-free platyfish-swordtail hybrids in nature.

It is significant that platyfish hybridize with swordtails only under special conditions in laboratory or home aquaria. Not a single hybrid between these species has ever been discovered in nature, although approximately 7000 specimens of platyfish and swordtails have been collected under conditions of sympatricity in the Atlantic coastal freshwater areas of Mexico and Guatemala. Moreover, because of our extensive knowledge of the hybrids based on laboratory experiments, there is no possibility, as suggested by Anderson (1949), that the scarcity of naturally occurring hybrids may be due to the inability of the collector to recognize the mongrel descendants of the backcrosses. Therefore Gordon (1947) concluded that extrinsic isolating mechanisms alone are not critical at the present time in preventing an inter-

change in genes between the species. He reviewed a number of possible intrinsic isolating mechanisms based on both morphological and physiological differences between the platyfish and swordtail which might be involved in the problem and suggested that, above all others, psychological factors were probably the most important.

This suggestion stems from the work of several previous investigators, especially Dobzhansky and his collaborators who have advanced the hypothesis that in certain instances psychological phenomena must form the crucial barriers to hybridization. Psychological factors most probably apply in those situations where geographical and ecological isolation are not of importance and where seasonal or temporal factors (differences in reproductive periods), mechanical factors (incompatibility of genital parts), failure of sperm to reach or penetrate the egg, inviability or weakness of the zygote, and hybrid sterility are not critical to the isolation of the species. To categorize this phenomenon, the term "sexual or psychological isolating mechanism" has been proposed by Dobzhansky (1941a), while Mayr (1942) has recommended the expression "ethological isolating mechanism." The implication relevant to this hypothesis is that because of differences in sensory, perceptual, and motor processes, the opposite sexes of the two strains or species do not behave towards each other in a properly coordinated manner, and effective cross matings occur less frequently or fail to materialize entirely. Although this hypothesis has met with general approval, attempts to elucidate such psychological barriers by observation and experimentation have been limited largely to (1) the demonstration that in laboratory situations interspecies matings often do not occur nearly so frequently as intraspecies matings; (2) the observation of sensory and motor differences between members of some of the strains and species studied and the conclusion that these sensory and motor differences bear a causal relationship to the reduced frequency of interspecies matings; and (3) observation and measurement of differential interactions of male and female in homospecific and heterospecific pairs which are evaluated in terms of "drives" and discriminatory reactions.

This background served as incentive for the present study, with the anticipation that a thorough knowledge of mating behavior and the related genetics of the two xiphophorin species and their various hybrids would throw additional light on the mode of operation of the supposed psychological isolating mechanism and hence on some of the intrinsic factors that may inhibit gene interchange between the members of these sympatric species.

ZOOGEOGRAPHY OF XIPHOPHORIN FISHES

The genus *Xiphophorus* consists of seven species, four of which (commonly known as the platyfishes) were formerly placed in a separate genus, *Platypoecilus*, by Günther (1866). Gordon (1951b), in a recent systematic evaluation based on a study by Gordon and Rosen (1951), has reduced *Platypoecilus* to the status of a subgenus in the genus *Xiphophorus*. All seven species are restricted geographically to the Atlantic coastal drainage areas of the Gulf of Mexico and the Caribbean Sea. The northernmost member, *Xiphophorus couchianus*, a platyfish, is found in one of the tributaries of the Río Grande near Monterrey, Mexico (Gordon, 1940a, 1951a). The southernmost member, *X. helleri*, a swordtail, is found just east of Ceiba, at Belfate, Honduras, according to Gordon (1951a).

The swordtail, *X. helleri*, has a more northern and a more southern distribution than its sometime sympatric species, the platyfish, *X. maculatus*, for the swordtail is found from the Río Nautla about midway between Tampico and Veracruz, Mexico, south to the streams near Belfate, Honduras. Just how much farther east its habitat extends along the coast of the Gulf of Honduras beyond Belfate has not been determined.

The range of the platyfish, *Xiphophorus maculatus*, is well within that of the swordtail, *X. helleri*. At its northernmost point the platyfish is found in the Río Jamapa at the city of Veracruz, and at its southernmost locality it has been collected in a tributary of the Belize River just north of Belize, British Honduras (Gordon, 1951a). In each of the

seven river systems where platyfish have been found, swordtails have also been collected, but not always in the same local habitat or exactly the same ecological niche. Gordon (1940b, 1947) summarized the data on the distribution of the two species in Mexico and Guatemala and indicated that in 10 localities swordtails were found alone, in five localities platyfish were found alone, and in 10 they were found living together. Many times they were caught together in a single seine haul. A closer analysis of the data indicated that often when the swordtail and platyfish were taken from the same river system, the swordtails were represented in the headwaters (pl. 1, fig. 1), and the platyfish in the lower reaches, of the streams (pl. 1, fig. 2). To some degree then the species are isolated geographically and ecologically. However, both species have the same breeding season, or rather the females of both species have been taken together and were carrying developing embryos at the same time of the year, which happened to be in March and April. Under laboratory conditions, both species breed and produce young throughout the year, although during the heat of the summer, when the water temperature rises above 28° C., they are less likely to produce young.

INHERITANCE OF BEHAVIORAL DIFFERENCES BETWEEN THE SYMPATRIC SPECIES

The minimum requirement for a genetic analysis of any divergent trait is an adequate number of hybrids of the second generation as well as those of the first generation and those of several backcross generations. Obtaining species hybrids of these various categories ordinarily is quite difficult, because "good" species rarely interbreed. When they do, they seldom produce many viable and fertile hybrids. This generally limits the number of second-generation and backcross individuals that are available for a genetic analysis. Under adequate laboratory conditions, however, many platyfish, swordtails, and their various hybrid combinations have been reared to maturity in connection with a project designed to study the genetics of normal and atypical pigment cell growth (Gordon, 1948, 1951a). As part of the correlated studies of this project many of the specimens were made available for a genetic analysis of their mating behavior patterns.

Some tests of the latter kind were made previously with platyfish-swordtail hybrids by Gerschler (1914), Kosswig (1936) and Breider (1939). Their results indicated that a morphological and a behavioral trait of a male swordtail, namely, the presence of a "sword" and his swimming backward towards a female and touching her with this structure, segregates in some of the platyfish-swordtail hybrids of the second generation. Some F_2 individuals, however, while lacking the "sword," back up towards the female, while others having "swords" do not. Actually, "backing up" is not exclusively a swordtail characteristic, since platyfish do it also, but to a much less degree. The long "sword" of the wild swordtail (which is about as long as the body) is rarely attained by any of the F_2 platyfish-swordtail hybrids.

Braddock (1945) described a number of basic behavioral characteristics in the platyfish in connection with his study of their dominance-subordination responses. Activities more directly related to mating behavior and copulation in the platyfish and swordtail were described briefly by Clark, Aronson, and Gordon (1948). Independently, Schlosberg, Duncan, and Daitch (1949) also described and analyzed the significant behavioral patterns in these species and in some of their hybrids.

Preliminary observations on mating patterns in wild stocks of platyfish and swordtails were undertaken in this laboratory in 1946 by Beach and Holz-Tucker (unpublished). Later Aronson and Landman (unpublished) developed a gonaductal smear technique which permitted, after a particular behavioral sequence, an examination of the female genital tract for the presence of sperm. This technique, which was perfected by the present authors, was then used in testing the significance of courtship and copulatory behavior in the two species and in the various hybrid generations.

ACKNOWLEDGMENTS

LITERATURE

REPRODUCTIVE BEHAVIOR STUDIES

THE NUMBER OF STUDIES on reproductive behavior in animals in general is quite extensive. Concerning fishes alone, Charles M. Breder, Jr., tells us that he has reviewed more than 3000 papers on the subject. While many of these contain incidental observations, others represent definitive studies.

In the viviparous poeciliid fishes there is considerable sexual dimorphism. This was reflected in an early taxonomic study in which the males and the females of one species were regarded as representing separate species.

Our knowledge of sexual behavior in the poeciliids begins with Agassiz (1853), who observed mating in *Mollienesia latipinna* and through this observation was able to relate the male to the appropriate female of this species. FitzGerald (1872), however, was one of the first to describe mating behavior of a poeciliid species (*Poecilia* sp.) in some detail, as follows: "The anal fin of the male consists principally of a long spine. In its normal position this spine lies close under the body and reaches 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."

Since these early studies, numerous descriptions of poeciliid courtship activities have appeared in popular articles in aquarium journals (for example, Milewski, 1920; Fraser-Brunner, 1947), and a number of casual observations have been reported in the scientific literature. The only detailed studies on poeciliid sexual behavior are those of Breder and Coates (1935), Noble (1938), Noble and Curtis (1935), Haskins and Haskins (1949), and Clark and Aronson (1951) on the common guppy *Lebistes reticulatus*, and Kosswig (1936), Noble and Borne (1941), Clark, Aronson, and Gordon (1948,

1949), and Schlosberg, Duncan, and Daitch (1949) on *Xiphophorus maculatus* and *Xiphophorus helleri*. A number of short reports on reproductive behavior in the platyfish, swordtail, and guppy have been reviewed by Clark and Aronson (1951) and Rosenthal (1952).

The copulatory organ or gonopodium of the male, developed by modification of the anal fin and used for internal fertilization, is one of the most characteristic features of the poeciliid fish. The gonopodium has been used as a diagnostic character for taxonomic differences in the Poeciliidae (Regan, 1913; Hubbs, 1924, 1926), and its highly modified structure has stimulated numerous detailed studies on its morphology and development (see review by Gordon and Rosen, 1951).

The action of the gonopodium during courtship and copulation is one of the most striking features in the sexual behavior of these fishes. Following the early note by FitzGerald mentioned above, Ryder (1885) described copulation in *Gambusia* during which act the head of the male was turned in the direction of the tail of the female, but the accuracy of this observation was questioned by Seal (1911), Henn (1916), and Collier (1936). According to Seal (1911), the mating activities of *Gambusia holbrooki* and *Heterandria formosa* are exactly alike. His rather anthropomorphic account states that, "The male follows incessantly and warily after the female, . . . watching for a moment when her attention will be distracted, when he will make a sudden dash, sometimes succeeding in inserting the intromittent organ into the genital pore, but oftener apparently missing, because of a quick turn of the female from which he flees in apparent terror. The contact is so sudden and brief that it required many observations to verify it. In these movements the male organ is thrust forward and to the right toward the female." Zolotnisky (1901) noted the varied movements of the gonopodium during courtship in *Giradinus*, and Collier (1936) studied the structure and function of the gonopodium and associated parts in *Gambusia*, correlating peculiarities of these structures with the method of

insemination believed to be employed, and studying especially the mechanism for reversal of the gonopodium. The studies on the mechanism of gonopodial movements have been expanded by Rosen and Gordon (1953).

In a popular article on the guppy for pet-fish hobbyists, Fraser-Brunner (1947) described breeding behavior and sexual dimorphism of the anal and pelvic fins. He illustrated by means of diagrams the synchronous movements of the gonopodium and pelvic fins which together form a tube through which spermatophores "are fired machine-gun fashion at the genital opening of the female." Fraser-Brunner believed that the gonopodium is never actually introduced into the female and no act of coitus occurs. Stepanek (1928), however, was the first to note a lasting contact between the male and female of *Lebistes reticulatus* and *Xiphophorus maculatus*—an act that he witnessed only a few times but that he considered to be copulation. His findings are supported by the more extensive studies on the sexual behavior of the guppy (Clark and Aronson, 1951) and the two species used in the present study.

Sexual discrimination (sex recognition) has been investigated in the guppy by Breder and Coates (1935), who concluded on a basis of isolated males that it is feeble, if present at all. Sexually active males, according to these investigators, will attempt to fertilize a variety of objects showing the characteristic motions of a living fish. The female is described as playing an entirely passive role. A similar conclusion was reached by Fraser-Brunner (1947) and by most aquarists. Noble and Curtis (1934), however, found that experienced males learned to avoid other males, their bright colors serving as "intimidating" devices. In a related study, Haskins and Haskins (1949) found that discrimination by the *Lebistes* male of the closely related and similar appearing females of three sympatric poeciliid species is based essentially on the behavior of the male. Again there was considerable evidence that much of this discriminatory behavior was learned.

While the above studies emphasize the passive role of the female guppy in the mating procedure, Stepanek (1928) and, more recently, Clark and Aronson (1951) indicated a definite response of the female to the courting activities of the male. The latter authors noted that the degree of receptivity of the female fluctuates markedly. Jaski (1939) reported for *Lebistes* an estrous cycle of four to six days at 28° C. characterized by an elevation of the position in which the female swims. Jaski claimed that a hormone "copulin" is secreted into the water by male fish and causes an elevation of the swimming position in virgin females. Jaski's findings have not been verified by other investigators and have been questioned by Clark and Aronson (1951). Bretschneider and Duyvené de Wit (1947) have described ovarian changes in the pregnant female guppy, and Rosenthal (1952) has reported evidence for cyclical production of ova in the virgin female guppy. However, Rosenthal states that this cycle is "as yet ill defined."

It should be noted that in almost all descriptions of copulation in poeciliids, the actual transfer of sperm during these acts was not verified. Preliminary reports of detailed studies on the sexual behavior of the platyfish and swordtails were presented by Clark, Aronson, and Gordon (1948, 1949), who furnished the first descriptions of copulations in these fishes where actual proof of insemination was established.

PSYCHOLOGICAL ISOLATING MECHANISMS

Biologists have reported many instances of almost complete absence of hybridization in nature between members of closely related sympatric species of mammals, birds, amphibians, fishes, and many invertebrates. Yet many of these sympatric species interbreed under artificial conditions. Blair (1951) in his review of the problem indicated that interbreeding under natural conditions is prevented by a complex of isolating mechanisms that are associated with various modes of reproduction. They may involve habitat preference, breeding season, call, mating preference, courtship patterns, and sexual dimorphism.

During experiments designed for the collection of semen for artificial insemination, stallions, bulls, boars, and rams were trained

to mount and copulate with stuffed effigies of species other than their own. Dobzhansky (1941a) says these experiments show that barriers to hybridization can be surmounted even in those species that never hybridize in nature. According to Dice (1942) attempts to secure matings between male coyotes and female dogs failed. However, after many trials, he reported that a female coyote accepted a male hound dog, and the pair produced one male and two female hybrids. Some of the structural features of the hybrids resembled the coyote, others the hound dog. Their walk was always clumsy, and they did not have the quick, agile movements of the coyote.

Numerous subspecies and species of the mouse genus *Peromyscus*, which are fully interfertile under laboratory conditions, fail to interbreed in nature, even when they at times occupy the same habitats. Dice (1940) suggests in explanation that a difference of some sort in the mating psychology of these forms probably exists, for otherwise their members would almost certainly interbreed in nature and thus their identities would merge. Also in *Peromyscus maniculatus*, three forms of which are geographically isolated and which hybridize readily in "no choice situations," have been shown to demonstrate strong sexual isolation when males were presented a choice of females (Blair and Howard, 1944).

Some bird species are closely similar in appearance and overlap in range, yet retain their specific identity owing probably to the striking differences in their calls and songs, which may be regarded as behavioral isolating mechanisms (Huxley, 1942). Thus, in the region between the Vistula and the Oder rivers, the true nightingale and its allied species, the sprosser, meet but remain distinct in nature. Under conditions of domestication, they are known to cross. In the United States the ranges of eastern and western species of meadowlarks overlap, but they rarely hybridize; the birds are quite similar except for their calls and songs. Huxley (1942) also reveals a similar situation among the British birds of the genus *Phylloscopus*—the chiffchaff, the willow warbler, and the wood warbler. The song, according to Huxley, has a dual function. Because its primary func-

tion is to advertise the possession of territory, it must be striking, but since the secondary function is to advertise the fact only to members of the same group, the song of related and overlapping forms must be markedly different. Mayr (1940) sums up the subject concerning allied species in nature by saying aptly that the birds are not deceived, though the taxonomists sometimes are. He uses the term "ethological factors" in reference to various courtship patterns that precede pairing or copulation, including the display of special colors, scents, sound (and songs), and movements. These traits may have more than one function, according to Mayr, since they may serve as: warning or threatening traits or actions, or both, to deter competing or rival individuals of the same species (these are probably most important in establishing dominance-subordination relationships within a group); as recognition marks to promote the mating of heterosexual members of the same species; and as stimulators to raise the physiological readiness of a pair, particularly their level of sexual excitation during the precopulatory period, and to synchronize copulatory behaviors.

Mayr (1942) also observes that in certain species of birds a long "engagement period" may be broken if the behavioral patterns of the two mates do not fit exactly. He gives this as the reason for the rarity of hybrids in those species of birds with definite pair formation and engagement periods but fairly common in genera and families without pair formation.

A psychological isolating mechanism is indicated between the sympatric mallard and pintail ducks. Natural hybrids are found in a ratio of less than one per 50,000 killed, according to Dobzhansky and Mayr (1944), but the F_1, F_2, and backcross hybrids produced under domestic conditions are completely fertile (Phillips, 1915). Although "no choice" hybridization of domesticated vertebrates is widely practiced by animal breeders, few studies have been made on the behavioral barriers which this procedure helps to overcome.

Natural hybrids between the golden-winged and blue-winged warblers were reported by Allee and others (1949) to occur in river valleys where the breeding areas of the

two species overlap. According to these authors the most conspicuous color characters of the two species seem to be Mendelian. These are the whitish under parts of the golden-winged which are dominant to the yellow under parts of the blue-winged warbler; in contrast is the plain throat of the blue-winged which is dominant to the black throat of the golden-winged warbler. The hybrid with both dominant characters is known as Brewster's warbler. The one with double recessive traits is known as Lawrence's warbler. Parkes (1951) indicated that Brewster's warbler is an F_1 hybrid between the golden-winged and the blue-winged species. Since the expected varieties among F_2 hybrids are rare, Parkes suggested that Brewster's warblers fail to mate together frequently. The F_2 hybrids may be inviable or, more likely, most of them are not able to adapt themselves to the available habitats.

Four species of the toad *Bufo* are distributed over the northern two-thirds of the eastern United States. Blair (1942) found that the toads occasionally interbreed at points where their ranges overlap or where their habitats are the same at certain seasons of the year. On the whole, however, the species retain their identities by ecological, seasonal, and certain sexual isolating mechanisms. Blair explains that toads find water-breeding areas apparently by chance. The males precede females to ponds by several days. Males usually call in choruses at night; the call of one stimulates that of another. The droning mating calls of *B. fowleri* and *B. woodhousi* are extremely similar, but that of the latter is louder. The calls of *B. americanus* and *B. terrestris* are trills, high-pitched in the former, much lower in the latter. Both male and female toads respond preferentially in a limited manner to the calls of their own species. However, they clasp each other at random, but at this level the different sizes of the various toad species become a factor in the final mating act.

In a pond formed by damming a tributary of the Pocantico River, which flows into the Hudson River in New York, Bailey and Lagler (1938) discovered three species of sunfish (some native, some introduced) which, owing to the limited spawning areas, produced a considerable number of hybrids. Intense crowding and competition for nesting sites was held responsible for the large hybrid population, which amounted to 11.7 per cent of the total. The species involved were the green sunfish, *Apomotis cyanellus*, the common sunfish, *Eupomotis gibbosus*, and the blue-gill, *Helioperca macrocira*. Hybridization was indiscriminate among the three species. The authors suggested therefore that the barrier to hybridization was neither a differential choice of mates nor a differential incompatibility of their germ cells.

The Centrarchidae form a rather closely related group of fishes. Frequently, Breder (1936) says, several sunfish species live side by side, resort to the same places for spawning purposes at about the same season, and often usurp each other's nests. Adams and Hankinson (1928) described the spawning activities of the common sunfish, *Eupomotis*, saying that the female and male, with their ventral surfaces close together, swim about in a circle, exuding eggs and sperm over a circular bottom nest 12 inches in diameter. Adams and Hankinson noted "clouds of sperms" at the time of spawning during which the female made quick tail movements. These activities could well send virile *Eupomotis* sperm short distances away from their nest which might readily penetrate ova of other species that happened to be spawning in an adjacent nest. It may have been in this sense that Bailey and Lagler referred to the lack of germ cell compatibility in sunfish hybrids. But there must have been some germ cell incompatibility, because in these hybrids the sex ratio of four or more males to one female was abnormal. Furthermore, the atypical sex ratio might indicate that sunfishes have a type of genetic mechanism for sex determination in which the females are heterogametic, the males homogametic. This would be in accordance with Haldane's rule (1922), which states that "when in the first generation hybrids between two species, one sex is absent, rare, or sterile, that sex is always the heterogametic sex."

In studying similar sunfish hybrids, Hubbs and Hubbs (1933) found all the hybrids sterile. One of the *Apomotis-Eupomotis* male hybrids played the part of a female in an abortive mating. A female hybrid dug a nest and otherwise played the part of the male in

nesting and mating behavior, its "female" mating partner on this occasion being a male *Apomotis-Helioperca* hybrid. Breder (1936) characterized these acts as instances of homsexuality in fishes, and Aronson (1949) described other examples of bisexual behavior in cichlid fishes.

Preliminary observations in this laboratory (reported by Gordon, 1947) indicated that differences in reproductive behavior between platyfish and swordtails could be recognized. In a multiple-choice experiment in which a male swordtail was placed with six adult virgin female platyfish and six similar swordtails, the male definitely selected females of his own species as evidenced by the type of young produced. Other preliminary tests in smaller aquaria revealed that no hybridization took place when there was a free choice. Clark, Aronson, and Gordon (1948) and Schlosberg, Duncan, and Daitch (1949) in more detailed studies of the sexual behavior of these two species have pointed out specific differences which may be factors in their sexual isolation. Haskins and Haskins (1949) studied the sexual isolating mechanism among three sympatric species of poeciliid fishes and found by observational methods that when a male *Lebistes* was given a choice among *Lebistes* females and *Micropoecilia* and *Poecilia* females, discrimination was poor at the beginning of the experiment but that a gradual change in behavior towards homospecific courtship gave some evidence that this is a learned reaction. Seitz (1948), studying the sexual behavior of two species of African cichlids of the genus *Tilapia*, mentioned briefly what he considers the lack of complete psychological isolation. This he based on his findings that only homospecific courting occurred when a choice of mates was presented, but when no choice was offered, heterospecific courtship and probably mating took place.

Physiological isolating mechanisms have been described for morphologically distinct populations of the three-spined stickleback, *Gasterosteus aculeatus* (Heuts, 1947). In Belgium and neighboring countries two populations (types A and B) are found: one in freshwater and the other in salt-water habitats. These populations differ especially in mean body size, in the mean number of body plates, and in their ability to retain chlorine as related to osmoregulatory function. Although the breeding grounds are generally separate, occasional individuals of type B are found in the spawning areas of type A, and vice versa. By means of artificial fertilization Heuts found that embryonic survival is closely correlated with salinity and that the two populations have distinctly different salt tolerance peaks. The optimum salt tolerance for hybrid embryos is not intermediate but is identical with that of the mother. This situation minimizes but does not prevent gene flow. Heuts suggests that possibly some form of sexual isolation may also operate in mixed populations, but the data are as yet inconclusive.

Crane (1949) found that under laboratory conditions, heterosexual pairs of closely related salticid spiders courted each other regularly when the tactile, chemical, or visual differences between the species were small. When a heterospecific display was initiated, however, it was rarely carried far. When occasionally it was continued by a particular male in high sexual excitation, the females always broke away well before actual mating. An interspecific courtship was interrupted when the constantly changing stimulus configuration became too weak and fell below the response threshold of one partner. When individuals of their own species were introduced to a pair of mismatched displaying spiders, attention was quite promptly turned to the appropriate newcomer. Crane indicated that display in the salticid spiders is particulate, but in order to be effective the parts must form a whole pattern of activity.

The males of certain species of moths become so sexually excited by the scent of females of their own species that they copulate with females of other species (Federley, 1932). Kaston (1936) revealed that in the precopulatory behavior of some vagabond spiders, the males must see and palpate the females before initiation of courtship. He also found that an ether-soluble substance removed from the cuticle of the female contained the essence that attracted the males.

An extensive series of studies on sexual isolation in *Drosophila* has been conducted by various investigators. In 1915 Sturtevant reported species differences in courtship and mating habits, and in 1920 he cited the first

case of interspecific hybridization in *Drosophila*. Today more than 60 different interspecific crosses are known in this genus (see review by Wharton, 1944). The numerous stocks of various species and strains of *Drosophila* that are available have provided abundant material for such investigations.

It has been evident from earliest studies of sexual isolation in *Drosophila* that among various species and among strains within a species there is in general the ability to discriminate in the selection of mates, with a strong tendency for homogamic rather than heterogamic matings (see review in Patterson, McDonald, and Stone, 1947). This tendency results in varying degrees of sexual isolation, and according to Spieth (1951) is dependent on the interaction of two factors which he calls courtship discrimination and sexual drive.

It has been pointed out by Dobzhansky and Koller (1938) that the early investigations on *Drosophila* showed that in addition to the full-fledged isolating mechanisms operating between species, rudimentary isolating factors also operate between races. There have been a number of attempts to correlate the geographic distribution of species and geographic races of *Drosophila* with the degree of their sexual isolation, and in some instances positive correlations have been made (Stalker, 1942; Dobzhansky and Streisinger, 1944).

Among the various methods developed in the course of investigations of sexual isolating mechanisms in *Drosophila*, three are in general practice:

1. The procedure of direct observation has been used by numerous investigators (Sturtevant, 1915; Stalker, 1942; Mayr, 1946, 1950; Wallace and Dobzhansky, 1946; Spieth, 1947, 1949; and Streisinger, 1948). Here the sexual behavior patterns of one or several pairs of flies are studied in homogamic and heterogamic associations. These visual observations furnish clues as to which behavioral factors limit heterospecific inseminations. In the fruit fly the "tapping" behavior of the male (Spieth, 1949), the "wing flutter" of the male (Mayr and Dobzhansky, 1945), the position of the female's wings when mounted (Stalker, 1942), and the overall activity of the female (Streisinger, 1948)

have been considered as behavior patterns possibly related to the success of sperm transfer. In some cases, however, no species differences in mating behavior could be detected that would account for species discrimination. Miller (1950), for example, could find no difference between the mating behavior of *D. affinis* and that of *D. algonquin* except for the duration of copulation. Males courted heterogamic females only briefly, if at all, and did not attempt to copulate with them. Miller suggests that in such cases male discrimination may be due to stimuli, possibly chemical, received at a distance.

2. In "no choice" situations, males and females of different strains or species are used. These situations encourage cross matings by lowering the excitation threshold (Speith, 1949) and can be used in testing sympatric forms that are completely sexually isolated in nature. Variations of this method for studying sexual isolation have been used. For example, a number of males and females of different strains or species were placed together, and later the females were dissected to check the percentage that had been inseminated (Dobzhansky and Koller, 1938). Patterson and his co-workers (1947) placed together single pairs of a male and female of different strains and recorded the failure to produce offspring. With the use of a "no choice" situation combined with the observational procedures noted above, fewer quantitative but more informative behavioral data have been obtained (Stalker, 1942; Spieth, 1949; Miller, 1950).

3. "Multiple choice" experiments developed by Dobzhansky and Koller (1938) and used extensively by Dobzhansky and his co-workers offer valuable quantitative data on varying degrees of isolation. An important contribution to these experiments was made by Stalker (1942) who introduced a quantitative measure of isolation which he called the "isolation index." This index is determined by enclosing males of one strain with females of the same (homogamic) strain and with an equal number of females of a related (heterogamic) strain. After approximately 50 per cent of the females are inseminated, the distribution of the inseminations among the females is determined. The isolation index is equal to the percentage of heterogamic

inseminations minus the percentage of homo-gamic inseminations divided by the sum of these two percentages. Accordingly, the in-dex can range from +1 for complete isola-tion, through zero for no isolation (random matings) to −1 for complete negative isola-tion.

Bateman (1949) observed that an index derived in this manner may lead to the con-clusion that the observed isolation is due to discrimination on the part of the males, whereas in his estimation the females may play the all-important role by discriminating between homogamic and heterogamic males. Therefore, Bateman proposed a system of complementary tests (male A enclosed with females A and B; male B enclosed with fe-males A and B), and his joint isolation index is equal to one-half of the sum of the recip-rocal indices.

In the use of the above indices it is nec-essary to stop each test when approximately 50 per cent of the females are inseminated. If this figure is exceeded, the isolation index ap-proaches zero and is no longer an informative measure. Levene (1949) discussed this limi-tation, particularly in reference to Bateman's combined index, where the total number of females inseminated may differ in the recip-rocal tests even when considerable care is taken. Under such circumstances the comple-mentary indices are not equivalent, and the joint isolation index may yield misleading results. As an alternative, Levene recom-mends a new measure, the coefficient of iso-lation, which is less sensitive to changes in the length of time that the males are en-closed with the females. The changes in the manner in which sexual isolation has been measured reflect the way male and female dis-crimination has been emphasized in the course of these studies in *Drosophila*. There was at first a tendency to give prime con-sideration to the discriminatory ability of the male. However, it was soon demonstrated that the female also played an important part in sexual selection. By using etherized females in multiple choice experiments, Streisinger (1948) found that random mat-ings took place between some species that ordinarily showed strong homogamic prefer-ences. The important role of the female has also been demonstrated in the studies of Bateman (1949) and Merrell (1949) who re-ported cases where males courted homogamic and heterogamic females at random. Here discrimination was exercised by the females who were much more receptive to homogamic males. Mayr (1950) found that the antennae of the female function as organs of species discrimination and that removal of these an-tennae resulted in the breakdown of the sex-ual isolation between *S. pseudoobscura* and *D. persimilis*. According to Spieth (1949) both sexes are involved in species discrimina-tion among the *willistoni* group, and it is probable that in many cases both males and females exercise discrimination between ho-mogamic and heterogamic mates.

It is of interest to note that there is con-siderable variation in the degree of sexual isolation between various species as well as between various strains of *Drosophila*, al-though there is a somewhat greater tendency towards homogamic matings on the species level. However, heterogamic preferences have been demonstrated at both levels, as for example between certain strains of *D. willis-toni* (Dobzhansky and Mayr, 1944) and be-tween different *Drosophila* species (Patterson and Stone, 1949). However, Spieth (1951) points out that these cases are always "one-sided," i.e., males of A may inseminate more females of B than their own females, thus producing a negative isolation index, but the reciprocal cross, using males of B, always produces a positive index.

MATERIAL AND METHODS

THE PLATYFISH, *Xiphophorus* (*Platypoecilus*) *maculatus* (Günther), and the swordtail, *Xiphophorus helleri* Heckel, used in these studies were derived from pure wild strains, original stocks of which were obtained from Mexico. The platyfish is designated as P (pl. 2, fig. 1) and the swordtail as X (pl. 2, fig. 2). The laboratory-produced, first-generation hybrids are PX (pl. 3, fig. 1), and their second-generation hybrids, PX² (pl. 3, figs. 2, 3). The F_1 fish, PX, were the product of matings between platyfish females and swordtail males. The platyfish backcross hybrids, PX-P (pl. 4, fig. 1), were obtained from matings between F_1 female hybrids and platyfish males. The swordtail backcross hybrids, X-PX (pl. 4, fig. 2), were obtained from the mating of swordtail females to F_1 males. (Other types of backcross hybrids were not available when this study was undertaken.) The methods used for maintaining the fish were similar to those described by Gordon (1950).

BACKGROUND AND TRAINING OF INDIVIDUALS

All the female fish that were tested had previously been isolated from mature males. With the use of virgin females it was possible to determine when and under what conditions insemination was accomplished. To obtain an adequate supply of virgin female fish, young animals in stock tanks were examined daily. Immature males showing any sexual differentiation of their anal fins were removed. (A completely differentiated anal fin or gonopodium is required by the male before copulation can be effective in sperm transfer.) All the males were removed well before the gonopodium was completely transformed, while the virgin females were retained in the aquarium. Females were considered sufficiently mature for sexual behavior studies when the yellow color of the ova was visible through the translucent body walls.

Many of the males used had some previous sexual experience with females of their own type. In a number of instances, however, sexually inexperienced males and males with special previous training were available for particular studies.

Prior to all behavioral tests, the males were isolated in an aquarium for at least 20 hours, and then the female was introduced into the male's tank at the beginning of the recorded observation. Our preliminary trials indicated that this procedure facilitated the initiation of sexual behavior, and in this detail our observations are in line with the findings of other investigators who have studied sexual behavior in mammals. This procedure also made possible shorter observation periods.

TYPES OF OBSERVATIONS AND CONDITIONS

Two-gallon aquaria (25.0 cm. long by 16.5 cm. high by 18.0 cm. wide) were used for all observations. The tanks were painted on the exterior with an opaque blue-white enamel on two sides and one end, while the other end was left transparent. Each tank was covered by an unpainted glass. A shaded 25-watt lamp placed directly above the aquaria provided adequate illumination for the observer who sat about 2 feet away. The observation tanks had a one-half inch layer of gravel on the bottom, but contained no plants because preliminary trials in tanks with vegetation showed that the plants formed a hiding place for the female and this interrupted and delayed

sexual activities. Within the confines of these aquaria all activities of the fishes could be followed easily. Many of these tanks were used for isolating individual males. Sometimes as many as 30 males were tested in one day.

All observations were conducted for a standard 10-minute interval. Since the male initiates sexual activity, all observations were made in the aquarium in which the male had been maintained previously. The female never appeared to be seriously disturbed by the transfer, and many pairs of fish copulated successfully a few seconds after the female was placed in the male's tank. Trial obser-

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vations showed that the reciprocal method (introducing the male into the female's home tank), or the technique often used in other studies of transferring both sexes to a third location, seriously delayed the onset of sexual behavior. By use of this simple expedient it was possible to give each male repetitive tests and to use much larger samples than would have been feasible if appreciably longer testing periods had been adopted. Moreover, it is extremely doubtful whether prolonged testing periods would have added materially to the number of copulations observed or to the over-all picture of sexual behavior in these species proportional to the time involved. In mammals (e.g., minks) the situation is somewhat similar, and the practical advantages of introducing the female into the male's cage are recognized by commercial breeders (see discussion in Beach, 1947a, p. 264).

Over 1700 10-minute observations and records were made on mating behavior between paired fish. Ninety-nine observations were completed on the activities of single males given a choice of two females, one of its own species and one of another. In the latter experiments, both females were placed in the male's aquarium simultaneously.

METHODS OF RECORDING OBSERVATIONS

The activities seen in the first several hundred 10-minute periods were recorded on paper, with the use of a tally system. The observer jotted down a specific mark each time a particular behavioral act occurred and kept a record of the minute in which it was enacted. This method was not entirely satisfactory because the observer was compelled to cease observing the fish too frequently while glancing at the clock or while recording a behavioral item on the tally sheet. This manual method of recording was replaced later by the use of an electrically operated polygraph (pl. 6, fig. 1) assembled specifically for the purpose of recording behavioral observations rapidly without loss of some of them. This apparatus is composed of two recorders[1] and a keyboard which is somewhat similar to that of a typewriter. It may be held in the lap of the operator, who may use a technique similar to the touch system in typewriting. The keyboard contains 40 separate leaf-actuated microswitches,[2] which may be pressed separately or simultaneously in recording every item of behavior. Each recorder contains an unwinding roll of graph paper connected to a synchronous electric motor by gears of variable ratios. On each of the two recorders a series of 20 pens is hooked up independently to the 40 switches on the keyboard. Switches for controlling independent or simultaneous starting and stopping of these recorders are located on the keyboard. One machine, manipulated by the left hand, was operated at a slow speed. The recording during a 10-minute observation period produced notes covering 15 inches of graph paper. The records indicated the exact duration, sequence, and time of occurrence for each behavioral item. A second, higher speed recorder in which 1.25 minutes of observations ran to 15 inches of graph paper was turned on just prior to copulations (or whenever copulations were expected) for the purpose of obtaining an accurate measurement of the duration of copulation.

The accuracy of timing the durations of copulation by this method was tested in the following manner. A pilot lamp controlled by a sensitive automatic time clock[3] was set up in front of the operator. The timing device was operated by a second person in an adjacent room who determined the exact interval that the light flashed and who varied the intervals at random between 0.25 and 2.50 seconds. The operator recorded these random durations of illumination on the polygraph. The results of 100 comparisons with the timing device showed that at speeds comparable to the copulation tests, the records on the graphic recorder did not differ significantly from those of the Westinghouse timer. The mean deviation equals -0.2 second ± 0.009 second; σ equals ± 0.089 second; t equals 1.91; p equals 0.05.

[1] The Esterline-Angus Company, Inc., P. O. Box 596, Indianapolis 6, Indiana.

[2] MicroSwitch, First Industrial Corp., Freeport, Illinois.

[3] Westinghouse Electric Corp., 40 Wall Street, New York 5, New York.

SMEAR TECHNIQUE FOR SPERM DETECTION

In order to determine whether or not a particular act in the sexual behavior pattern resulted in insemination, a technique was developed to reveal the presence of sperm in the genital tract of the female. This was accomplished by inserting a very fine pipette containing a drop of 0.8 per cent saline solution well into the female gonaduct. By means of a rubber tube held in the experimenter's mouth at one end and attached to the pipette at the other, the drop of solution was gently expelled into the gonaduct and then the fluid was sucked back into the pipette. The redrawn drop was placed on a glass slide without a cover slip and examined immediately under a compound microscope.

In the testes of mature males, the sperm aggregate in relatively large compact clusters, 37 to 54μ in diameter known as spermatophores (Chavin and Gordon, 1951). It is in this form that the sperm are transmitted to the female. As soon as the spermatophores are deposited in the female oviduct they begin to disintegrate as the individual sperm become motile. If a smear is taken immediately after copulation partially intact spermatophores can sometimes be recovered, and the process of disintegration can be followed under the microscope. This process can also be studied in spermatophores taken directly from males (pl. 7, fig. 2) by means of a collecting technique described by Clark (1950a) for *Xiphophorus*.

In addition to sperm (pl. 7, figs. 3, 4; text fig. 1C) three other types of cells are frequently seen in smears:

1. ERYTHROCYTES (FIG. 1B): In young virgins, because of the small size of the genital opening, the pipette often must be forced slightly before it will enter the gonaduct for the first time. This may result in minor injury. Females injured in this manner can be easily detected by the presence of large numbers of erythrocytes in the smear. Such injury does not ordinarily occur in mature fish.

2. VACUOLATED EPITHELIAL CELLS (FIG. 1D): These cells are always present in a smear that has been taken correctly. They are easily recognized by the presence of one large or several medium-sized vacuoles. They are often amoeboid in shape. Large numbers are usually present in smears from old females and females that have just had a brood. These cells apparently slough off from the lining of the ovary. In *Glaridichthys*, similar cells have been found in the dorsal wall of the
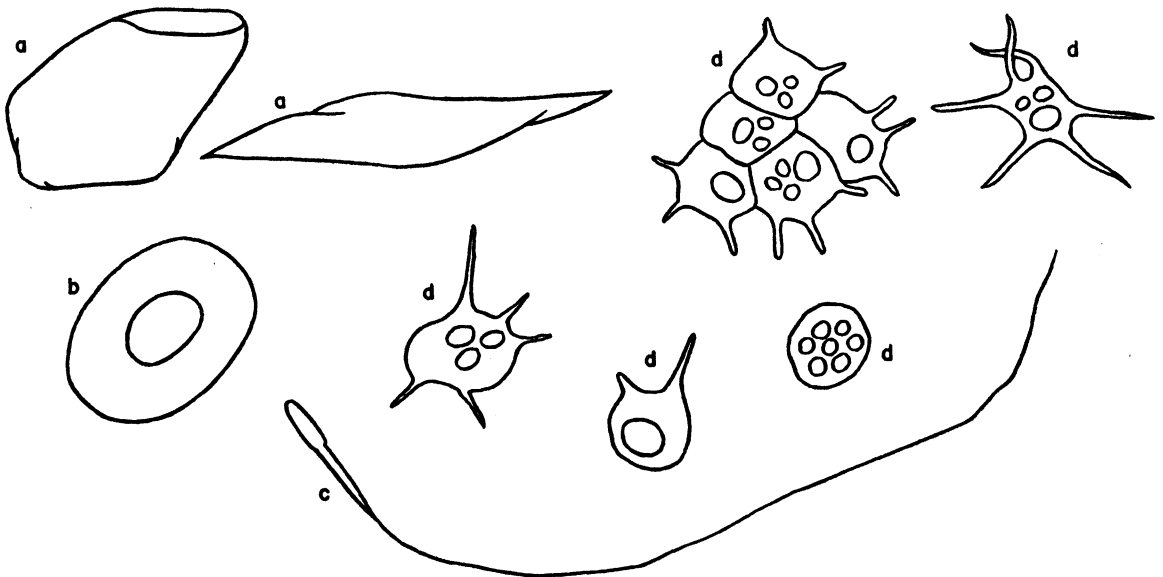
FIG. 1. Sketches showing types of cells frequently seen in smears taken from female gonaduct. ×2200. A. Squamous epithelial cells. B. Erythrocyte. C. Sperm. D. Vacuolated epithelial cells.

ovary (Philippi, 1909), and it has been suggested by this investigator that excess sperm are caught in the amoeboid extensions and are digested in the vacuoles.

3. SQUAMOUS EPITHELIAL CELLS (FIG. 1A): Occasionally a few cells of this type are seen in a smear. They may come from the region of the genital aperture.

Figure 1 of plate 7 is a photomicrograph of a stained smear taken from the gonaduct of a female platyfish 20 minutes after insemination.

If the smear was negative (i.e., without sperm) the female was checked two additional times.

With the use of this technique, motile sperm have been recovered from the gonaducts of females one week, and in some cases several weeks, after a single insemination (p. 162). The females were usually uninjured after being checked by this technique, and those that were not inseminated were used again in further studies. The reliability and limitations of this technique are given on page 160. Similar procedures were followed in the study of sexual behavior in the guppy by Clark and Aronson (1951) and Clark and Kamrin (1951).

RESULTS
RÉSUMÉ OF THE NORMAL MATING PATTERN

BEFORE A DETAILED QUANTITATIVE and qualitative analysis of the sexual behavior of *Xiphophorus maculatus*, *X. helleri*, and their hybrids is reported, it is desirable to present a general description of the courtship and mating activities of these fishes. The purpose of this section is to delineate briefly the general sexual behavior in each of the two species and to define the terms used for the various categories of behavioral activities.

Basically the sexual behavior patterns of the males of the platyfish, the swordtail, and the hybrids are similar. Shortly after the female is placed in the aquarium containing a male, the latter usually approaches, and starts to follow her about the aquarium. Sometimes the male does no more than this for an entire 10-minute period. More often, however, he approaches the female, pursues her, and thrusts the tip of his gonopodium towards her genital region. Less frequently, a thrust-like gonopodial contact lasting a few seconds is observed. This prolonged thrust often results in insemination, and we have therefore termed this relatively prolonged contact a "copulation."

During the time that the male is pursuing the female, other behavior patterns are usually observed. These are presented in the order in which they often occur during a typical observation period.

MALE BEHAVIOR PATTERNS

1. GONOPODIAL SWINGING: This refers to the forward movements of the male gonopodium, an action that is always made in conjunction with one of the pelvic fins (fig. 2A). The male moves the gonopodium and pelvic fin into such a position that the distal end of the gonopodium appears to be placed between the lappet (the modified first pelvic ray) and the second ray of the pelvic fin. To attain this position the male swings the gonopodium first laterally and downward and then forward, still somewhat to one side, anterior to the homolateral pelvic fin; the latter is brought forward to meet it. The male then returns the gonopodium and pelvic fin to their normal positions, usually completing the entire swing in less than a second. During the swing the male may also arch or quickly jerk his body, and during a series of swings there is a tendency to rotate the gonopodium to alternate sides (Aronson and Clark, 1952). Simultaneously, with the forward swing of the gonopodium, the male erects his dorsal fin if this has not been erected previously. (Unlike thrusting, see below, gonapodial swinging is not directed towards the female.)

In the presence of a female, a sexually excited male may swing his gonopodium several times within one minute, but this behavior has also been seen in isolated males. In some pairings, however, when the female is easily approached by the first advances of the male, he may succeed in copulating and transferring his sperm without any preliminary swinging of his gonopodium. This indicates that swinging in the presence of the female is not a necessary prelude to copulation and insemination.

2. SIDLING: This refers to the first direct approach of the male to the female, in which he moves alongside the female, sometimes twisting his head and tail away from her and bringing his mid-region quite close to her body. The male swordtail usually sidles on one side of the female for a short time and then interposes other courtship acts, and then again sidles on the opposite side of her. The male platyfish sidles for a longer period of time than the swordtail and quite often passes from one side to the other, crisscrossing the dorsal region of the female closely. In this act the ventral surface of the male frequently brushes against the female's dorsal fin.

3. THRUSTING: After a male sidles towards the female he usually thrusts his gonopodium towards her. The male, in the sidling position, brings the gonopodium forward and to one side, directing the tip of the gonopodium towards the genital area of the female. The male's pelvic fin is brought forward in the same manner as in swinging (fig. 2B). The male may make one quick thrust or a series of thrusts with his gonopodium. Superficially a thrust resembles a copulation, but it is a
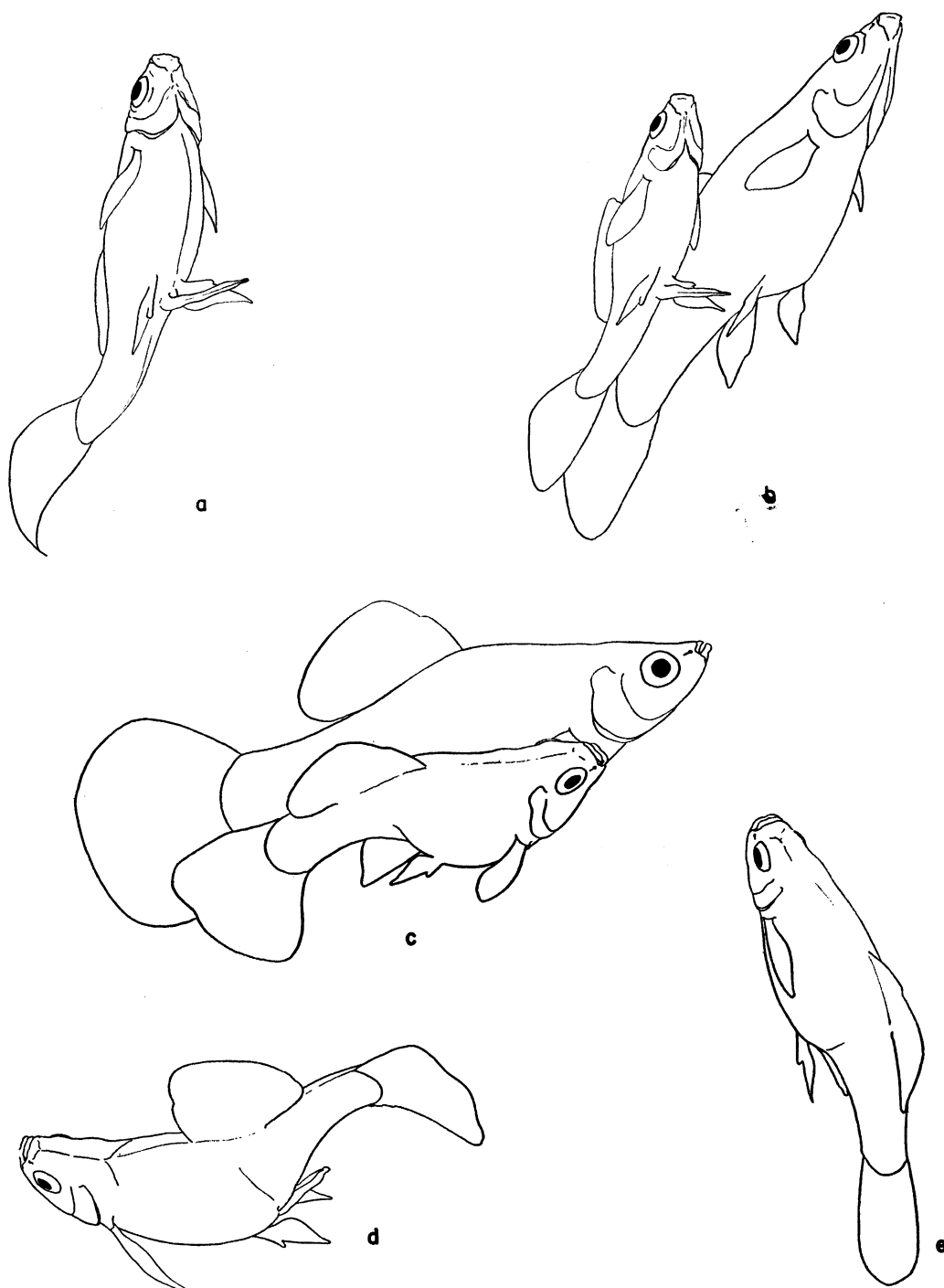
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FIG. 2. Diagrammatic sketches of male and female platyfish illustrating various aspects of sexual behavior. A. Male at the peak of a gonopodial swing, ventral view. B. Male platyfish (left) thrusting at a female, ventral view. C. Male and female during copulation. The male is on the right side of the female. D. Male showing S-curving behavior. E. Male showing retiring behavior.

much quicker movement and insemination of the female is never effected.

4. NIPPING AND NIBBLING: Nipping refers to a number of closely associated acts. Braddock (1945) defined a nip in the platyfish as a "quick bite involving no noticeable harm to the fish that is bitten." He called attention to the importance of this behavior in the establishment of a social hierarchy or "nip-order" in a community group. In our use of the term, the act refers to one fish lunging at, or biting, quickly and lightly, the body of another, but it is difficult sometimes to determine whether actual contact is made. Braddock did not describe the non-contact, nip-like lunge in his study of "nip-order," but our observations indicate that these lunges are equally as important as the contact nip in the establishment of social hierarchies in these fishes. Somewhat similar behavior acts were described for the sunfish by Greenberg (1947) and for the filefish by Clark (1950b). During an exchange of nips between the male and female in the platyfish and swordtail, activities such as thrusting of the gonopodium and copulation rarely occur. Occasionally the male will nip a female very gently. The swordtail in particular may nip the anal-genital region of a female gently and repeatedly. It is sometimes difficult for the observer to differentiate between typical aggressive nipping and this gentle nipping. The latter is referred to here as "nibbling." In the swordtail male nibbling appears to be directly concerned in courtship.

5. BACKING: Here the male approaches the female by backing towards her, tail first, usually touching her body with the tip of his tail, and this is followed by sidling. The male often backs towards the female after he comes to a stop near and usually slightly in front of her. During backing the male platyfish may curve his body in an S-shape (see 6 below) and then he may quiver (see 7 below). In the swordtail male, backing appears exaggerated owing to the long sword-like extension of the tail, and the movement is more pronounced because this maneuver is often started at some distance, as much as 7 inches, from the female. The platyfish, on the other hand, rarely backs into a female unless he is within 1 or 2 inches of her. In the quantitative study that follows, these short backward movements

of the platyfish were not placed in the category of backing behavior.

6. S-CURVING: The male confronts the female and stops momentarily before her with his body curved and with his dorsal and caudal fins usually spread rigidly (fig. 2D). In this attitude the body of the male is tilted to one side, with the head generally higher than the tail. S-curving by males and females appears in heterosexual and homosexual groupings of both sexes. Rival males may S-curve to each other, particularly when one has been courting a female and the other has interfered. Braddock (1945) suggested that this behavior may be important in the establishment of a hierarchical system in these fish. S-curving is common among platyfish. Among swordtails it is relatively rare. When it is performed, the curve of the body is much less pronounced and hence would not be accurately distinguished for quantitative purposes.

7. QUIVERING: This refers to the rapid up and down movement of the male's whole body while his dorsal and caudal fins are folded and the body is in an S-curve. Quivering suggests a highly excited state. Females of both species and male swordtails rarely quiver. However, male platyfish do, particularly during pecking (see 9 below) and just before sidling and thrusting.

8. RETIRING: This refers to the male's sudden backing away from the female with his body limp and his dorsal and caudal fins folded (fig. 2E). The male may retire after he has thrust his gonopodium at a female a number of times without effecting a copulation or when the female has chased and nipped him, whether or not he has made any advances. After retiring, the male may sink to a bottom corner of the aquarium, with his tail curved to one side. He may remain in this position for a few seconds or longer, during which time he usually makes one or many gonopodial swings, and then he may approach the female again. In his courtship the male platyfish often exhibits retiring, but the male swordtail does not.

9. PECKING: This consists of a series of biting movements on the bottom gravel or sides of the aquarium. It is similar to the method in which a fish picks at an object when feeding, but in pecking behavior it is

TABLE 1

BEHAVIORAL TERMS USED IN THIS PAPER, WITH THEIR SYNONYMS

This Paper	Schlosberg *et al.* (1949)	Braddock (1945)
Copulation[a]	Lasting contact	
Thrusting[b]	Thrusting	
Swinging[a]	Isolated flexion of the gonopodium	
Sidling	Jockeying for position	
Pecking	Periods of grazing	
Backing	Arcing; backward swim	
Quivering (new)		
Nibbling (included with nipping)		
Retiring		Submission
S-curving		Challenge
Nipping	Nipping	Nipping

[a] Used also by Clark, Aronson, and Gordon (1948) and by Clark and Aronson (1951).
[b] Used by Clark and Aronson (1951); equivalent to "jabbing," Clark, Aronson, and Gordon (1948).

obvious that no food is obtained. Often the male quickens the rate of his pecking, so much so that his mouth barely comes in contact with the object pecked at, and finally the fish begins to quiver violently, shaking up and down. After pecking, the male almost invariably sidles over to the female. Pecking is characteristic of the sexually active male platyfish but not of the male swordtail nor of the females of either species.

A similar kind of behavior has been observed in the stickleback and domestic cocks when, according to Tinbergen and Van Iersel (1947) and Tinbergen (1951), the animals are in a state of strong sexual excitation but at the same time are in some way prevented from consummating their activities in the appropriate way. This has been termed a "displacement reaction."

10. COPULATION: This is the close, comparatively long contact between a male and female in which the tip of the gonopodium is held against, or possibly inserted slightly into, the genital orifice of the female (fig. 2C); this kind of contact is maintained for about one second or longer. During the copulatory period the pelvic fin of the male, on the side of contact, is in a forward position, similar to that in swinging and thrusting. The act of copulation is easily distinguished from that of the thrust, since the latter is momentary and in most cases is quickly and often repeated. On the other hand, copulation is a more prolonged contact which is accomplished comparatively rarely. During copula-

tion the male and female seem to be physically hooked together. The gonopodial tip, illustrated and explained by Gordon and Rosen (1951) and Rosen and Gordon (1953) contains a holdfast mechanism that could account for this lasting contact. During the more prolonged copulations the fishes may swim about the aquarium joined together. The copulatory act is terminated when the fishes pull apart in a rather violent separation. Immediately after copulation the male platyfish usually swims about the aquarium in quick dashing movements. The male may occasionally rush back to the female and court her again, which may then result in another copulation. More frequently, both fishes may become relatively inactive sexually. Females are inseminated only during copulations, never during a thrust or series of thrusts. This has been checked repeatedly by the use of the smear technique to check the presence or absence of sperm (see p. 160). Under our laboratory conditions, platyfish copulate more frequently than swordtails, but in swordtails copulations are of longer duration.

A number of the behavioral activities defined here have been described previously by other authors, and table 1 contains a list of synonymous terms.

BEHAVIOR OF THE FEMALE

The female platyfish and swordtails exhibit fewer recognizable acts of courtship than the corresponding males. A female

sometimes swims close to a male with her anal fin folded or she may back up to a male. She may swim up and down the glass wall, pressing her mouth against the glass, and at this time she may be successfully courted. This has previously been described by Schlosberg, Duncan, and Daitch (1949). The over-all picture one obtains, however, is that most females are courted without exhibiting any special behavioral activity or response. When a female is paired with a male, she occasionally shows nipping, S-curving, or backing behavior. In addition, she sometimes performs tail slapping, an action in which the female twists her body and flips her tail in the direction of the male. Tail slapping, nipping, and S-curving appear to be avoidance responses on the part of the female and seem to indicate a low level of sexual receptivity. A female may avoid a courting male by swimming away from him, or by slapping him with her tail when he gets close. In some cases, such avoidance behavior on the part of the female delays or prevents copulation, though the male may be highly active sexually. On the other hand, a highly aroused and persistent male may copulate with a seemingly non-receptive female and one that had previously successfully rebuffed a less persistent male on the same day. In general, the

situation appears to be comparable to that described in the behavior of rats as reported by Beach (1942), who indicated that whether a copulation occurs or not depends both on the degree of sexual arousal on the part of the male and on the degree of receptivity on the part of the female.

Our limited data show no evidence for cycles of receptivity in female platyfish (see p. 173) as have been reported in the guppy by Jaski (1939). (See also Clark and Aronson, 1951.) Females appeared to be most receptive to male advances when they were taken directly from a stock tank of mature virgin females and placed directly into the male's tank. On these occasions they exhibited the least amount of nipping, S-curving, and tail slapping, and they did not swim away from the males.

Schlosberg, Duncan, and Daitch (1949) describe a "courting run" in swordtails (but not in platyfish) in which the female swordtail moves forward during the interval between male thrusts, flutters the caudal fin, and flexes the pelvic fins. We have not been able to recognize the courting run as a clearly defined pattern of the swordtail courtship behavior, possibly due to the fact that all our experiments were conducted in smaller aquaria than those used by the above authors.

MECHANISM OF INSEMINATION

RELATION OF THRUST AND COPULATION TO INSEMINATION

Early in the present study it became apparent that it was important to know the conditions under which insemination was effected. Apparently it had been assumed by many previous observers that the transfer of sperm from male to female was accomplished during the momentary thrusts of the gonopodium. Until recently this assumption was never seriously questioned, except for a brief statement by Stepanek (1928) who suspected that in *Lebistes*, spermatophores are ejaculated only during a prolonged contact of several seconds' duration. Evidence that effective insemination results from copulation rather than from the thrust was reported first by Clark, Aronson, and Gordon (1948).

The following critical experiments were designed to determine whether the thrust, copulation, or both, produce insemination. In

two series of tests, gonaduct smears were taken from females immediately after thrusts, and after copulations. The data are presented in table 2. From these results it can be concluded that the thrust alone (as many as 102 in the platyfish and 38 in the swordtail) does not result in the insemination of the female. On the other hand, an appreciable number of females are inseminated after one or more copulations. These data also reveal that in the platyfish insemination is less likely to result after a single copulation than after several copulations have taken place during the observation period. In the swordtail the data on the effectiveness of multiple copulations are too few for any conclusions to be drawn.

RELIABILITY AND LIMITATIONS OF SPERM SMEAR TECHNIQUE

In the above experiment 16 female platyfish and 23 female swordtails that had copu-

TABLE 2

FREQUENCY OF INSEMINATION AFTER THRUSTS AND AFTER COPULATIONS
IN PLATYFISH AND SWORDTAILS

	Platyfish	Swordtails
Observation periods with thrusts recorded but no copulations		
Number of observation periods	12	46
Average number of thrusts per observation period	39.6	8.8
Range of thrusts per observation period	3–102	1–38
Number of observation periods during which insemination occurred[a]	0	0
Observation periods with both copulations and thrusts recorded[b]		
Number of observation periods with one copulation recorded	64	30
Number of observation periods during which insemination occurred	50	12
Per cent of observation periods during which insemination occurred	78%	40%
Number of observation periods with more than one copulation recorded	47	6
Number of observation periods during which insemination occurred	45	1
Per cent of observation periods during which insemination occurred	96%	17%

[a] To verify the reliability of these negative smears, the 12 female platyfish were dissected after 14 to 18 days. No embryos were found. Owing to a shortage of virgin swordtails at the time, only the first 10 tested were dissected, and the others were reused in other experiments. In those dissected no embryos were seen, and in those reused no offspring were ever recovered.

[b] Pairs of swordtails copulated two times in some of the 10-minute observation periods, but platyfish copulated as many as six times in this same period.

lated revealed no sperm when tested by the smear method. Some of these females were isolated for two months in aquaria that were heavily planted with *Nitella*. The rest were sacrificed and dissected after two or three weeks. No young were born nor were embryos recovered. The smear technique, therefore, is a reliable test for the presence of sperm. In these cases, however, the smear tests were conducted within a day after the female had copulated. Then this question arose: How many days after an effective copulation can sperm be recovered by the smear technique? Table 3 contains the results of taking sperm smears on varying numbers of days after effective copulation from 42 platyfish, five swordtails, and 10 F_1 hybrid females. The results indicate that the smear technique is reliable for the detection of sperm up to seven days after insemination and that good positive smears can be obtained sometimes as late as 20 days after copulation.

It is well known that sperm often remain viable in the genital tract of the female for six months or more. Hence the reduction and eventual disappearance of sperm from the smears indicate that they are no longer free in the lumen but have moved into folds of the ovary and genital duct, as described for

Glaridichthys by Philippi (1909) and for *Lebistes* by Winge (1922) and Stepanek (1928). In this connection, the genital tract of one female was fixed for histological examination just four hours after her first insemination. Most of the sperm were found in the lumen of the genital duct, but some had already penetrated among the epithelial cells lining the wall of the duct.

ROLE OF TIP OF GONOPODIUM
DURING COPULATION

The distal tip of the gonopodium in the mature male of the platyfish and the swordtail forms a rounded arrowhead-like structure formed by the terminal segments of the third, fourth, and fifth anal fin rays, some of which are differentiated to form terminal hooks and a series of serrae (pl. 6, fig. 2; text figs. 3, 4). From morphological studies of Langer (1913), Regan (1913), and Gordon and Rosen (1951), and occasional observations of courtship behavior of the male, it is easy to understand why the gonopodium has been called an intromittent or contact organ by many workers. However, its actual function, as an organ of insertion during copulation, has never been established experimentally.

It should be noted that in these fishes the

TABLE 3

SPERM RECOVERY IN FEMALE PLATYFISH, SWORDTAILS, AND THEIR HYBRIDS ON VARIOUS DAYS AFTER INSEMINATION

Number of Females Tested	Number of Days after Copulations															
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	19	20
Platyfish																
1	M						M	M				F	F	X		
1	M				M		M	M						X		
1	M				M		M							X		
1	M							M			F	X	X			
1	M	M														
8	M					M										
4	M						M									
9	M							M								
1[a]	M								X							
6	M								M							
4	M									M						
4	M										M					
1	M											F				
Swordtails																
1[a]	M				X											
3	M							M								
1	M							M								
F₁ hybrids																
2		M														
2			M													M
1			M													M
1[a]	F		X												X	
1				M												
3	M															M

M, many sperm in the smear.
F, few sperm in the smear.
X, no sperm in the smear.

[a] These females had no broods.

gonopodium is not a closed tube. However, in the forward position taken during swinging, thrusting, and copulation, rays 4 and 5 rotate either to the right or left on their axes, to form a groove. In the forward position, the flange part of ray 5 meets ray 3, so that in effect a tube-like structure is formed near the distal end. The mechanical aspects of this maneuver have been analyzed by Rosen and Gordon (1953). Proximally this transitory groove appears to be partially enclosed when the pelvic fin has moved to the forward position. This enclosure is supplemented by contact of the gonopodium with the ventral surface of the male's body.

From direct observation and study of photographs of the relative positions of the bodies of the male and female fish during copulation (pl. 8, fig. 2), it is apparent that only the distal end of the gonopodium is in contact with the female's genital aperture. It is highly improbable that more than a small part of the gonopodial tip is inserted into the female. In the forward position of the gonopodium the tip folds on itself so that the serrae (and claw in the swordtails) reënforce the hook, and the structure thus formed (Rosen and Gordon, 1953) suggests an effective holdfast mechanism for attachment to the genital aperture of the female. In addition to this morphological evidence for a holdfast mechanism, the following observa-

FIG. 3. Gonopodium of swordtail. ×50. Fin rays 3, 4, and 5 form the major supporting structures. Rays 4 and 5 are bifurcate, and the two parts are designated as A (anterior) and P (posterior).

tional items support this hypothesis: (1) close contact of the male and female during copulation is maintained for as long as 2.9 seconds in the platyfish and 5.6 seconds in the swordtail (pp. 168 and 175); (2) the end of copulation is marked by a sharp, quick breakaway which suggests the sudden release of a tight hold; (3) two cases of bleeding in the region of the genital opening in females have been observed immediately after the fish had copulated.

The following tests were designed to study further the role of the tip of the gonopodium in the copulatory act. This was done by removing the tip in an attempt to destroy the holdfast mechanism.

Mature males and mature virgin platyfish females about 10 to 14 months old were used in these experiments. The gonopodium and pelvic fins of a number of males were examined. Only males with typically normal fins were chosen for the following experi-
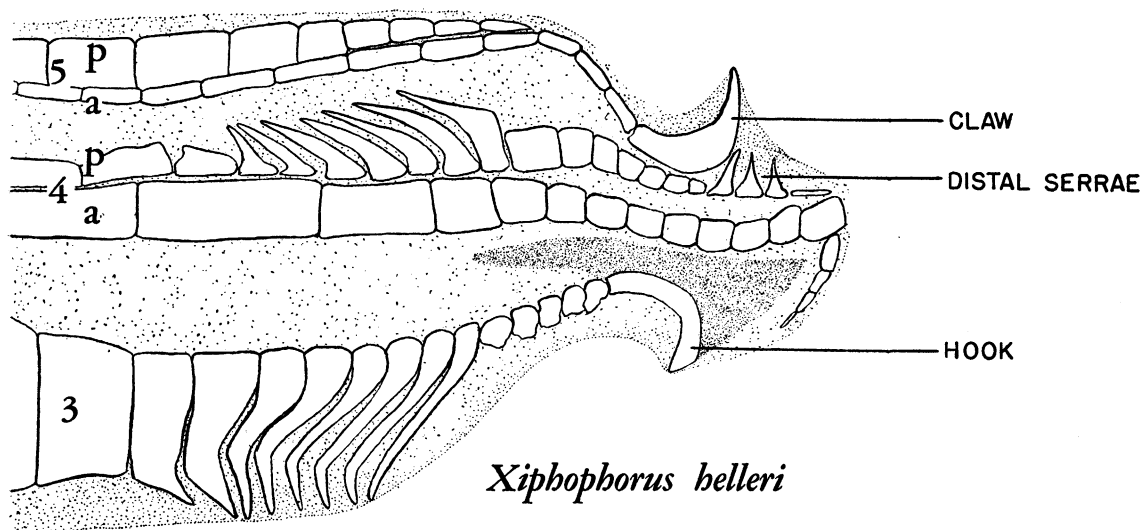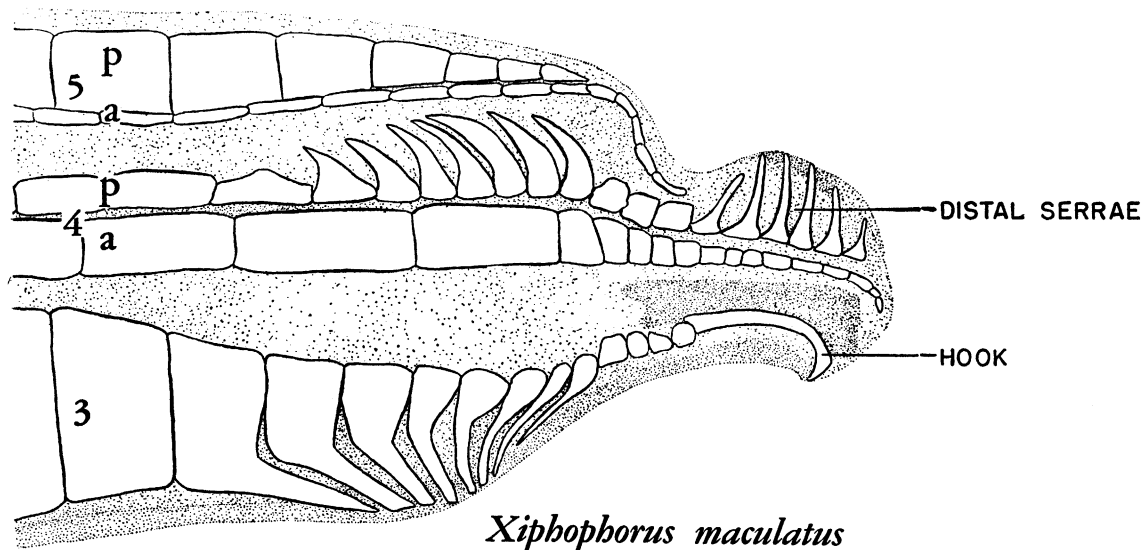

FIG. 4. Gonopodium of platyfish. ×50. Fin rays 3, 4, and 5 form the major supporting structures. Rays 4 and 5 are bifurcate, and the two parts are designated as A (anterior) and P (posterior).

FIG. 5. Outline of gonopodium showing level at which tips were amputated (broken line).

ments. The male fish were held fast in wet absorbent cotton wrappings, and the tip of the gonopodium was amputated with a fine pair of scissors at the line of constriction at the base of the expanded tip (fig. 5). During the days between the operation and the test, each male was isolated. As usual, the female was introduced into the male's tank. The test pairs were observed in two-gallon aquaria.

PRELIMINARY TEST: A virile male that had copulated with and had inseminated 13 females was selected. The tip of his gonopodium was cut off, and the male was tested in seven tests, each with a different virgin female. In these tests the experimental male, as before, showed all the typical courtship patterns, and he thrust at all females placed with him. However, the frequency of thrusts was noticeably higher, and he did not copulate. Not a single female, of the seven tested, was inseminated.

EXPERIMENT 1: Eleven males with gonopodial tips removed were individually paired with virgin females for 55 days. All the females were checked for sperm at various intervals, with the use of the smear technique. Tests were made on the third and fifty-fifth day, and random checks were made on two or three females on the ninth, fourteenth, eighteenth, and forty-third days. No sperm were recovered from any of the females. In a control group of six normal males with virgin females, five of the six females were inseminated at the end of the third day. These results are summarized in table 4. At the end of 55 days the amputated gonopodia of the experimental males showed no regeneration whatever.

The results of the above experiment indi-

TABLE 4

OVIDUCT SMEARS[a] TAKEN FROM FEMALES INSEMINATED BY PLATYFISH MALES AFTER REMOVAL OF THE GONOPODIAL TIPS OF THE MALES

Male's Number	No. of Days after Insemination						
	3	9	14	18	27	43	55
Gonopodial tip removed							
31	−	−				−	−
32	−		−				−
33	−		−				−
34	−			−			−
35	−		−				−
36	−	−				−	−
37	−	−				−	−
38	−			−			−
39	−			−			−
40	−				−		−
41	−				−		−
Intact controls							
1	+						
2	−						
3	+						
4	+						
5	+						
6	+						

[a] Positive, +; negative, −.

TABLE 5

NUMBER OF COPULATIONS PER TEST BEFORE AND AFTER REMOVAL
OF GONOPOIDAL TIP IN MALE PLATYFISH

	Control Males						Totals	Experimental Males						Totals
	1	2	5	6	7	8		3	4	9	10	11	12	
Preoperative tests														
Dates														
10/13/49	0	1+	0	2+	1+	1+	5	2+	3+	2+	0	0	1+	8
10/14	0	3+	1−	0	0	2+	6	0	1+	0	1+	1+	4+	7
10/15	0	1−	1−	0	0	0	2	2+	1−	0	0	2+	1−	6
10/19	0	5+	0	0	0	0	5	0	2+	0	0	0	0	2
10/20	0	3+	1−	0	1+	1+	6	1+	5+	0	0	0	0	6
10/21	1−	0	0	0	1−	0	2	0	0	0	0	0	1−	1
10/22	0	0	0	0	0	0	0	0	2+	0	0	0	0	·2
10/25	0	0	0	0	1−	3+	4	0	0	0	0	0	0	0
11/4	0	2+	0	0	0	0	2	0	1−	0	1+	0	0	2
11/5	0	0	0	2+	0	0	2	0	1+	0	1−	0	0	2
Totals	1	15	3	4	4	7	34	5	16	2	3	3	7	36
Postoperative tests (control males untreated)														
12/8	2+	1+	0	2+	1+	—[a]	6	0	0	0	0	0	0	0
12/9	0	3+	0	1−	0	—	4	0	0	0	0	0	0	0
12/13	3+	4+	1−	4+	1+	—	13	0	0	0	0	0	0	0
12/14	0	6+	0	2−	1+	—	9	0	0	0	0	0	0	0
12/15	1+	0	0	0	0	—	1	0	0	0	0	0	0	0
12/21	2+	1−	0	4+	3−	—	10	0	0	0	0	0	0	0
12/22	3+	3+	0	1−	2+	—	9	0	0	0	0	0	0	0
12/23	0	1+	0	2+	0	—	3	0	0	0	0	0	0	0
12/28	0	0	0	2+	0	—	2	0	0	0	0	0	0	0
1/3/50	0	0	0	0	0	—	0	0	0	0	0	0	0	0
Totals	11	19	1	18	8		57	0	0	0	0	0	0	0

+, Female was inseminated.
−, No sperms were recovered in the smear.

[a] Male died.

cate that the gonopodial tip is essential in the insemination process. However, it was still not known whether insemination was blocked during copulation or the copulatory act was actually prevented by the removal of the tip. To investigate this problem, the following observational type of experiment was designed.

EXPERIMENT 2: Ten observations were made of each of 12 normal males. A virgin female was used in each observation. The behavioral details were observed and recorded, and each female was checked for sperm after it had copulated. The males were then divided into two groups of six each. One group

of six males were kept as controls. In the other six the gonopodial tip of each male was removed. All 12 males were isolated for 33 days (during this interval one of the control males died), then each was tested again with virgin females over a period of 27 days. The frequency of copulations in the control group and in the experimental group of males before and after gonopodial tips were removed is shown in table 5. At the close of the experiment the entire gonopodium of each experimental male was removed, mounted on a glass slide, and studied under a microscope. Examination showed no signs of regeneration in the distal tip of the gonopodia during the

TABLE 6

TOTAL NUMBER OF THRUSTS PER MALE IN MALE PLATYFISH BEFORE
AND AFTER REMOVAL OF GONOPODIAL TIP

| | Control Males | | | | | | Totals | Experimental males | | | | | | Totals |
	1	2	5	6	7	8		3	4	9	10	11	12	
Preoperative tests	37	122	128	77	90	(46)[a]	454	19	204	50	95	34	103	505
Postoperative tests (control males untreated)	228	176	228	341	213		1186	499	232	439	312	160	753	2345
Totals							1640							2850

[a] This figure was not used because the male died before the second 10 observations were taken.

experiment. (See pl. 9.)

All the males used in the first part of this experiment, with the exception of male 5, copulated with and inseminated at least one female. Although male 5 copulated four times, he did not inseminate any of the four females with which he was tested. This exceptional male was killed, sectioned, and the testis and gonaduct were examined histologically. The testis was abnormally large and packed with mature spermatophores. The gonaduct was greatly swollen and filled with spermatophores proximal to the testis. It had no spermatophores at the end of the duct, which was closed off instead of open as in normal males. Apparently this closure prevented normal transfer of sperm.

The results of these experiments show that males with gonopodial tips removed do not copulate with females although their sexual activity, as measured by the frequency of thrusting, increased considerably (table 6). It may be noted also that interference with the copulatory pattern had the decided effect of prolonging the courtship process. This was indicated especially by increased gonopodial swinging, pecking, and sidling. A comparable increase in courtship activity when copulation is prevented has also been noted in rodents (Beach, 1947a).

A few somewhat similar experiments were conducted on eight swordtail males. From four fish, the claw of the gonopodial tip was carefully dissected, and from the other four

only the hook was removed.[1] Each male was paired two days with a virgin female swordtail, after which the latter were tested for the presence of sperm. Three of the four males without a hook and one of the four males without a claw had inseminated the female. These latter experiments indicate that neither the hook nor the claw is absolutely essential for successful copulation, but a much more elaborate experiment is needed to determine whether or not these small deprivations may not act as partial impediments to copulation. In this connection the conclusions of Rosen and Gordon (1953) are pertinent. In their study of the functional anatomy of the gonopodia of the poeciliid fishes they found a compensatory mechanism in the arrangement of the terminal elements. Thus in the platyfish that have no claws in the gonopodia the distal serrae are enlarged. In the swordtail, the distal serrae are small and ineffectual as holdfast organs.

ROLE OF PELVIC FINS DURING COPULATION

In a number of poeciliid fishes the pelvic fins of the males differ from those of the females by modification and specialization of the first and second fin rays. Henn (1916) suggested that in the males of such genera as Poecilia, Mollienisia, Limia, and Xiphophorus, the elongated pelvic fins if pressed against the folded anal fin "would form a

[1] Unpublished data of Clark and Rosen.

tube extending nearly to the end of the anal, through which the spermatozoa might pass." Fraser-Brunner (1947) said that in *Lebistes* the male's two pelvic fins rotate forward with the anal during thrusting behavior and in so doing the tips of the modified second pelvic rays slip into the so-called "prepuce" hood of the gonopodium and thus form a tube. In such a tube, he added, the stretched hood then serves as the floor, and the remaining pelvic rays form the roof. However, Clark and Aronson (1951) observed that only one pelvic fin of *Lebistes* is brought forward at a time, and the hood is not engaged, for it dangles loosely. This was verified by means of electronic flash photographs.

In the male platyfish and swordtail the pelvic fins are more elongated than in the female, and the first ray is modified to form a palp-like structure termed a lappet. When the gonopodium rotates to the right during swinging, thrusting, or copulatory behavior, the right pelvic fin then rotates forward. Conversely, if the gonopodium is directed to the left, the left pelvic fin rotates forward to meet the gonopodium (pl. 8, fig. 1; text fig. 2B). With this in mind, preliminary experiments were made to test the role of the male's pelvic fins during copulation in the platyfish, as follows.

AMPUTATION OF BOTH PELVIC FINS: After both pelvic fins were removed, five platyfish males (previously mated and known to be fertile) were paired each with a virgin female. The females were tested by the smear technique after two days and again after 35 days. None of the smears contained sperm except one taken on the thirty-fifth day. In this ex-

ceptional case the female had been paired with a male that showed almost complete regeneration of both pelvic fins. Three of the other males showed partial regeneration of one or both pelvic fins of not more than one-third of a normal sized fin. In the remaining male there was no indication of fin regeneration. The female with the positive sperm smear had a brood of young on the fifty-ninth day after pairing; the other test females were kept for two additional months but yielded no broods. These results are summarized in table 7.

AMPUTATION OF LAPPETS ONLY: After removal of the distal two-thirds (the expanded portion) of the lappets of both pelvic fins, another set of five platyfish males were paired each with a normal virgin female. On the following day all five females were examined by means of gonaductal smears, and sperm were found in three of them. Five days later sperm were found in a fourth female. The remaining female, for unknown reasons, did not become inseminated at this time. About 10 days later there were signs of regeneration of the pelvic fins in all five males; two months later some lappets had partially, others almost completely, regenerated.

These experiments suggest that the pelvic fins of the male platyfish are important in the transfer of sperm to the female but that the pelvic fin lappet, a secondary sex character of the male, is not an essential part of the apparatus. A study by Clark and Kamrin (1951) of the platyfish, swordtail, and guppy confirm this preliminary conclusion, namely, that the pelvic fins are important in the mechanism of insemination.

TABLE 7

TESTS OF INSEMINATION IN PLATYFISH AFTER AMPUTATION OF
THE PELVIC FINS OF THE MALE

Pair Number	Sperm Smears from Females after 2 Days	Sperm Smears from Females after 35 Days	Regeneration of Male Pelvic Fins	Broods
1	—[a]	—	None	None after 119 days
2	—	—	Slight on right fin	None after 119 days
3	—	+	Considerable on both fins	9 young on 59th day
4	—	+	Slight on both fins	None after 119 days
5	—	+	Slight on both fins	None after 119 days

[a] Symbols: —, negative; +, positive.

Three alternative hypotheses are presented in the study by Clark and Kamrin concerning the function of the pelvic fins during copulation: the pelvic fin serves as a support for the gonopodium while it is in the copulatory (forward) position; the pelvic fin covers the transitory groove of the gonopodium, thus further enclosing the passageway for the spermatophores; or the forward movement of the pelvic fin is a supplementary part of the ejaculatory mechanism.

CONCLUSIONS

From these observations and experiments it can be concluded that:

1. Gonopodial thrusts can be distinguished behaviorally from copulations.

2. Thrusts alone do not result in insemination of the female.

3. Insemination of the female results from some copulations but not from all.

4. Insemination is more likely to result from multiple copulations.

5. The sperm smear technique is a reliable test of insemination up to seven days after copulation, and positive smears can sometimes be obtained up to 20 days after copulation.

6. The gonopodium is a holdfast organ of the male which serves to direct spermatophores to the genital aperture of the female. As such it may be considered an intromittent organ.

7. The serrae, hook, and claw on the tip of the gonopodium form an effective holdfast mechanism.

8. In the absence of this holdfast mechanism, copulation does not occur, and males so deprived cannot inseminate females.

9. In the forward position, the gonopodium forms a partially enclosed transitory groove which forms an effective path for the passage of the spermatophores from male to female during copulation.

10. The pelvic fin on the side to which the gonopodium is swung also moves forward, and this is an integral part of the copulatory mechanism. In the absence of both pelvic fins, the ability to transfer sperm to the female is greatly reduced.

ANALYSES OF SEXUAL BEHAVIOR

SEXUAL BEHAVIOR IN THE PLATYFISH

Thirty-three males and 127 females were studied during a total of 416 10-minute observation periods. However, the most detailed and inclusive records were made on the last 12 males (150 observations). The graphic recorder was not available when these experiments were begun, so that the data on the first 21 males and on the first three or four observations of the last 12 males were recorded manually. In the analysis of the data on copulation and thrusting, it was possible to utilize all or part of the 416 observations. Elsewhere the analysis was based on 150 observations of the last 11 males.

The analysis that follows is based chiefly on courtship behavior of the platyfish male and the copulatory behavior of both sexes.

COPULATION (TABLES 8, 9): For the 33 males the copulatory act was recorded during 111 of 416 observation periods (26.7%). If, however, the copulatory frequency of the last 12 males tested is considered separately, the figure is 42.0 per cent (table 14). This difference is accounted for by the fact that during the early part of the project optimum testing conditions were not established. In addition, the last 12 males were particularly vigorous. In an experiment by Kamrin and Aronson (in preparation), using 29 males, each tested 10 times with the same testing techniques, a copulatory frequency of 30.9 per cent was obtained.

The number of females that were inseminated after one or more copulations was relatively high (78%). A higher percentage of females were inseminated after two or three copulations (96% and 93%), and 100 per cent were inseminated after four to six copulations. Kamrin and Aronson also find a higher rate of insemination after multiple copulations. They record as many as 12 copulations in a single observation period.

The duration of 106 copulations of 12 males was timed on the mechanical recorder and was found to range from 0.3 second to 2.9 seconds, with an average of 1.36 seconds ± 0.05 second; Σ equals ± 0.47 second. In

TABLE 8

FREQUENCIES OF COPULATIONS AND INSEMINATIONS IN PLATYFISH

(For comparisons, see tables 16, 21, 25, and 30.)

Number of Copulations Recorded During an Observation Period	Number of Observation Periods	Observation Periods after Which Insemination Was Recorded	
		Number	Per Cent
1	64	50	78%
2	26	25	96
3	14	13	93
4	4	4	100
5	2	2	100
6	1	1	100
1–6	111	95	86

Total number of observation periods	416
Number of observation periods with copulations recorded	111
Per cent of observation periods with copulations recorded	26.7%
Total number of copulations recorded	190

order to determine whether or not the 12 males involved in the test were random samples from the same population, the data were subjected to an analysis of variance. The results are summarized in table 9. F equals 1.78, whereas, as the 0.05 level with 11/94 degrees of freedom, F equals 1.89. Indications are that the variance between males is not significantly greater than the variance within males. We are therefore dealing with a homogeneous group.

The time during the 10-minute observation period at which the first copulation occurred was recorded in 111 10-minute observation periods. No copulations occurred during the first minute. The average time required for the first copulatory act was 5.2 minutes ± 0.19 minute; Σ equals 2.0 minutes. These results are represented graphically in

TABLE 9

ANALYSIS OF VARIANCE OF DURATIONS OF COPULATIONS (IN SECONDS) IN PLATYFISH

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Total	105	25.88	
Between males	11	4.55	0.41
Within males	94	21.33	0.23

$F = 1.78$. For df 11/94, $F_{0.05} = 1.89$; $F_{0.01} = 2.45$

figure 6, in which comparisons are also made between the time of first copulation in the swordtail and in the F_1 and F_2 platyfish-swordtail hybrids.

THRUSTING (TABLE 10): Thrusting of the gonopodium was observed in platyfish males in 77.9 per cent of a total of 276 10-minute periods (for the last 150 observation periods this figure was 76.3% ; see table 14). The average number of thrusts per period in the 215 observations in which thrusts occurred was 15.3 (table 10, section C). In 106 of these 215 periods the fish copulated one or more times. The analysis in table 10, section B, and figure 7 indicates that the frequency of copulations is positively correlated with the frequency of thrusting. Additional evidence of the relation of thrust to copulation may be obtained by comparing the average frequency of thrusts in observations where copulations occurred with periods in which no copulations were recorded but in which thrusts occurred (table 10, section A). In the 106 observations in which copulations were recorded, the average number of thrusts per observation period was 15.4. In the other 109 observations with thrusts but no copulations, the average number of thrusts per observation was only 9.7. It appears from these data that the frequency of thrusts bears a direct relation to the occurrence and frequency of copulations.
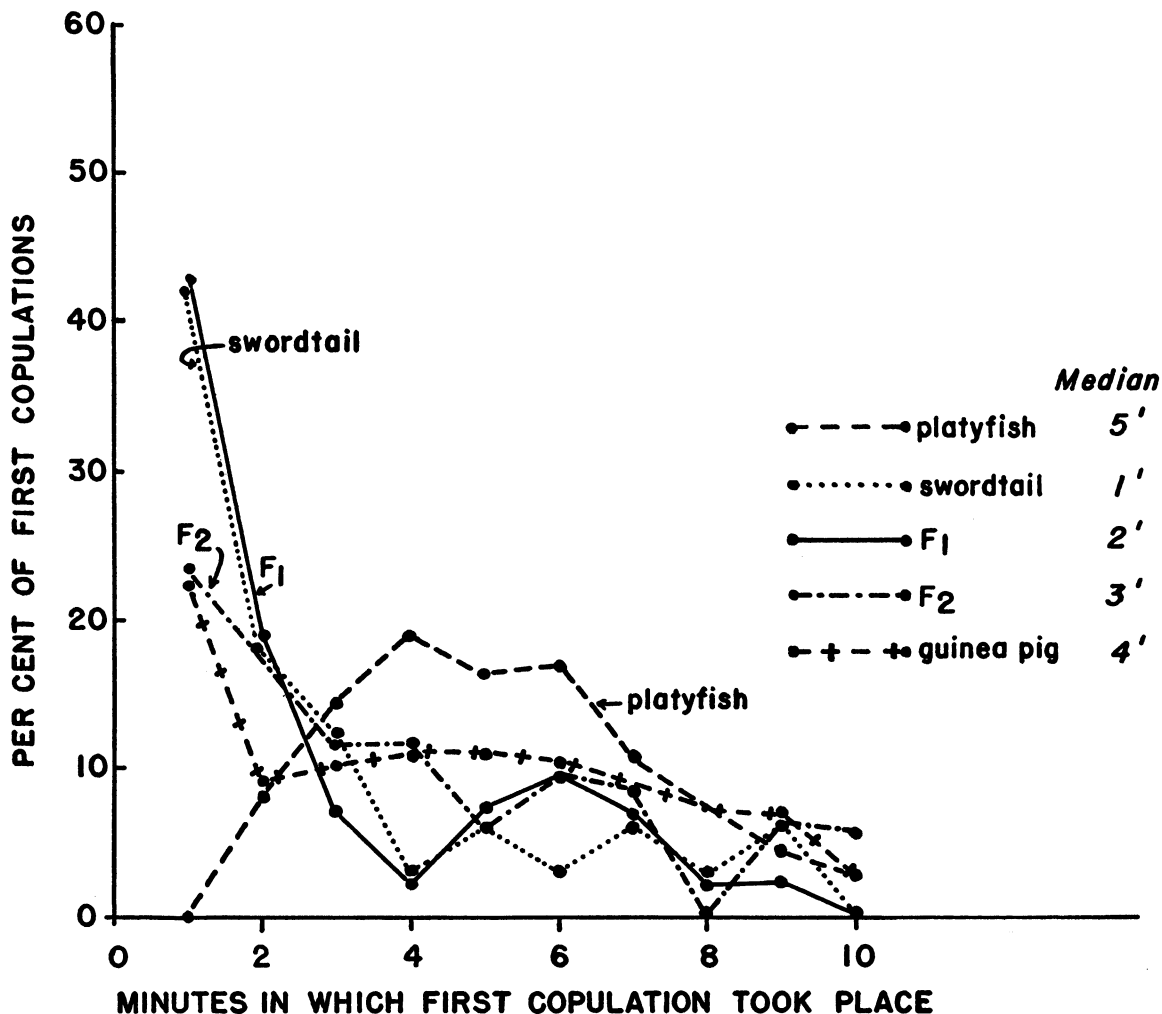
FIG. 6. Distribution of the time of occurrence of first copulations in platyfish, swordtails, and F_1 and F_2 hybrids. For comparison with mammals, data from Young and Grunt (1951) for the guinea pig are included.

GONOPODIAL SWINGING (TABLE 11): Gonopodial swinging was observed at least once in 92.2 per cent of the test periods. The average frequency of swinging per observation period was highest during prolonged courtship, particularly during periods in which the males thrust but did not copulate. It was second highest during those periods in which the male copulated only once. Its frequency was lowest when the males were very active and copulated repeatedly or, on the other hand, when the males were inactive and had not thrust or copulated.

PECKING (TABLE 12): If a male pecked five times or more within a second, the act of pecking was recorded. Males pecked in a considerable proportion of observation periods in which they also thrust and copulated. Pecking was most frequently observed during prolonged courtship when thrustings did not result in copulation. The next highest frequency of pecking was found during those observation periods in which the male copulated only once. Where several copulations were recorded, pecking was relatively infrequent, almost as infrequent as in inactive males.

NIPPING (TABLE 13): Nipping behavior was
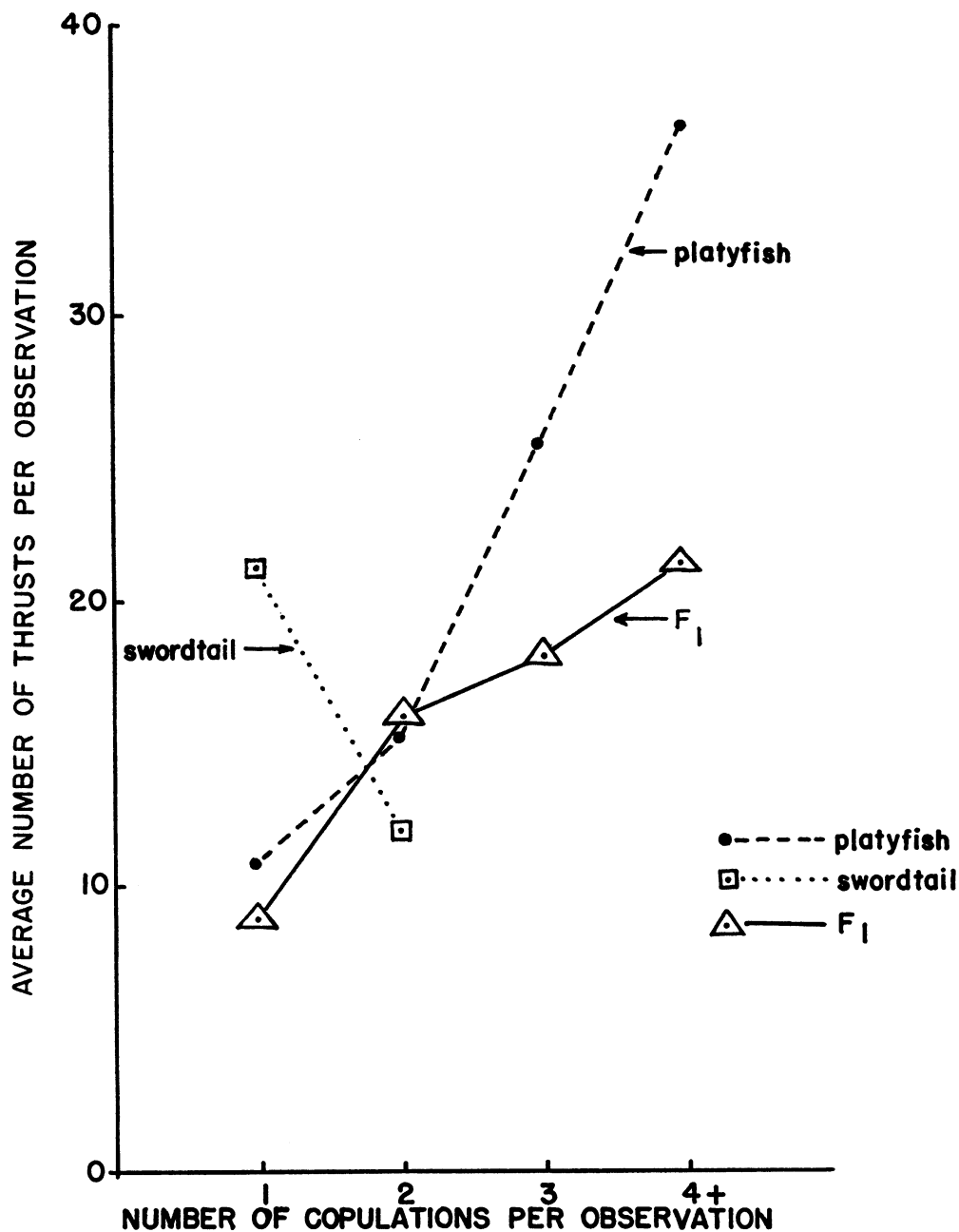
FIG. 7. Relation of thrusts to multiple copulations in platyfish, swordtails, and F_1 hybrids.

recorded only when a male nipped three or more times during a 10-minute observation. (Fish occasionally nip at another fish when it happens to swim close by, but this appears to have little significance unless it nips repeatedly.) Nipping was recorded in 16.6 per cent of the observation periods. Non-copu-

lating fish nip more frequently than those that copulate.

The swordtail has a related behavior called nibbling which is directed towards the genital opening. This action is always rather gentle. Although some of the nips of the platyfish were quite mild, they never seemed to be

TABLE 10

RELATION OF GONOPODIAL THRUSTS TO COPULATIONS IN PLATYFISH

	Number of Copulations Recorded During an Observation Period	Number of Observation Periods	Average Number of Thrusts During an Observation Period
A	0	170	9.7
	1–6	106	15.4
B	1	60	10.7
	2	25	15.3
	3	14	25.5
C	4–6	7	36.4
	0–6	276	11.9

Total number of observation periods	276
Number of observation periods with thrusts recorded	215
Per cent of observation periods with thrusts recorded	77.9%

directed at the genital region of the female.

OTHER BEHAVIOR PATTERNS (TABLE 14): Sidling, quivering, retiring, and S-curving are other recognizable behavior patterns exhibited by the platyfish. In table 14 all items of behavior that could be analyzed quantitatively are listed, and their occurrence in relation to copulation and thrusting is given. These data are based on the same 150 observation periods as those on swinging, pecking, and nipping. It is indicated in the table that sidling always preceded thrusts and copulations. The high percentage of observations in

which sidling occurred, but thrusts and copulations did not, is indicative of the fact that males reach an intermediate level of sexual excitability. Quivering behavior was also seen more often when thrusts and copulations occurred, and this is related to the observation that quivering often preceded sidling.

Braddock (1945) reported that S-curving is the initial behavior observed when two strange platyfish approached each other for the first time. He considered this behavior instrumental in the establishment of social hierarchies and termed it the "challenge." It

TABLE 11

RELATION OF GONOPODIAL SWINGS TO COPULATIONS AND THRUSTS IN PLATYFISH

(For comparison, see tables 18, 23, 27, and 32.)

Number of Copulations Recorded During an Observation Period	Thrusts	Number of Observation Periods	Average Number of Swings During an Observation Period
0	Absent	30	5.4
0	Present	57	11.6
1	Present	30	8.2
2–6	Present	33	5.2
1–6	Present	63	6.6
0–6	Present or absent	150	8.3

Total number of observation periods	150
Number of observation periods with swings recorded	138
Per cent of observation periods with swings recorded	92.2%

TABLE 12

FREQUENCY OF PECKING BEHAVIOR IN MALE PLATYFISH

Number of Copulations Recorded During an Observation Period	Thrusts	Number of Observation Periods	Observation Periods with Pecking Recorded	
			Number	Per Cent
0	Absent	30	11	36.7%
0	Present	57	43	75.4
1	Present	30	21	52.3
2–6	Present	33	12	70.0
1–6	Present	63	33	36.4
0–6	Present or absent	150	87	58.0

is of considerable interest, therefore, that in our experiments S-curving was recorded most frequently when the males were sexually active but did not copulate. This was true with respect not only to the number of observations in which S-curving occurred but also to the average number of S-curves per 10-minute period. The latter data are as follows (negative observations excluded):

	S-CURVES PER OBSERVATION
No thrusts and no copulations	1.4
Thrusts but no copulations	1.9
Thrusts and copulations	1.4

Also of interest is the low order of magnitude of S-curving frequency in these observations as compared with male-male pairs tested under our standard conditions. In a series of 12 such tests, S-curving was observed in 59 per cent. The average number of S-curves per male per test was 8.4 (negative tests excluded), ranging to 21.

Retiring, like nipping, is associated with difficulty in copulation, that is, poor behavioral synchronization between male and female. It was recorded least frequently during observations in which copulations occurred.

RECEPTIVITY OF FEMALE PLATYFISH: Jaski (1939) has reported an estrous cycle of about four to six days' duration in the guppy. According to Fraser-Brunner (1947) this has led some guppy breeders to believe that fertilization is more successful at the peak of such a cycle. No cycle of receptivity in female platyfish was evident in these studies, although it was obvious that females copulated more readily at certain times. Virgin females and non-virgins that had been separated from males for a week or longer were likely to be the most receptive.

To investigate this question of receptivity

TABLE 13

FREQUENCY OF NIPPING BEHAVIOR IN MALE PLATYFISH

(For comparisons, see table 19.)

Number of Copulations Recorded During an Observation Period	Thrusts	Number of Observation Periods	Observation Periods with Nipping Recorded	
			Number	Per Cent
0	Absent	30	8	26.7%
0	Present	57	13	22.8
1–6	Present	63	4	6.4
0–6	Present or absent	150	25	16.6

TABLE 14

RELATION OF COPULATIONS AND THRUSTS TO THE FREQUENCIES OF OTHER TYPES OF BEHAVIOR IN MALE PLATYFISH

(For comparisons, see tables 20, 24, 28, 29, 33, 37, and 41–44.)

Types of Behavior	Per Cent of Observation Periods in Which Behavior Was Recorded			Per Cent of Total Observation Periods with Behavior Recorded
	No Thrusts and No Copulations	Thrusts but No Copulations	Thrusts and Copulations	
Copulation	0.0%	0.0%	100.0%	42.0%[a]
Thrusting	0.0	100.0	100.0	76.3
Swinging	5.4[b]	11.6[b]	6.6[b]	92.2
Sidling	43.5	100.0	100.0	88.7
Pecking	36.7	75.4	52.3	58.0
Backing	0.0	0.0	0.0	0.0
Quivering	30.0	86.0	73.0	66.6
Nibbling	0.0	0.0	0.0	0.0
Retiring	33.3	49.1	25.3	34.0
S-curving	14.7	50.0	35.3	22.7
Nipping[c]	26.7	22.8	6.4	16.6
Total number of observation periods	35	52	63	150

[a] For comment on this figure, see text.
[b] These figures represent the average number of gonopodial swings per observation.
[c] Three or more nips.

TABLE 15

RECEPTIVITY OF FEMALE PLATYFISH ON VARIOUS DAYS AFTER THEIR FIRST INSEMINATION

Female's Number	Days after First Insemination																						Day of First Brood
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
2	C	C	C	T	C							T					—		T			—	30
45	C			C	—		T		T	T													26
48	C			C			T		T			—											25
47	C			—	C		T			T													31
49	C	T					C					—							—				27
34	C				—						C		C								T		27
53	C		T								C		C										a
43	C							—							—		C				C		b
36	C							—			—					T					C		47c
39	C	—																			C		46c
40	C																T			T	T		23
42	C					T																—	24

C, observation period resulting in copulation.
T, observation period in which male thrust at female but no copulation occurred.
—, observation period in which male neither thrust nor copulated.

[a] This female died 24 days after her first insemination. Dissection revealed well-developed embryos.
[b] No young after 60 days. Possibly this female was sterile.
[c] The brood recorded probably resulted from the second copulation.

TABLE 16

FREQUENCIES OF COPULATIONS AND INSEMINATIONS IN SWORDTAILS

(For comparisons, see tables 8, 21, 25, and 30.)

Number of Copulations Recorded During an Observation Period	Number of Observation Periods	Observation Periods after Which Insemination Was Recorded	
		Number	Per Cent
1	27	12	44.5%
2	6	1	16.7
1 or 2	33	13	39.4

Total number of observation periods	246
Number of observation periods with copulations recorded	33
Per cent of observation periods with copulations recorded	13.4%
Total number of copulations recorded	39

cycle, 12 virgin females were selected. These fish appeared highly receptive, copulated, and were inseminated during their first 10-minute experience with mature males. They were retested for receptivity on various days thereafter for a period of 21 days. The criterion used was whether or not thrusts of the male were followed by copulations. The results of these observations are presented in table 15. Although the data are limited, the scattered temporal distribution of copulations suggests that regular cyclical periods of receptivity in the female are not evident in this species.

SEXUAL BEHAVIOR IN THE SWORDTAIL

Twenty-one male and 84 female swordtails

were tested during 246 10-minute observations. All these observations, which include 52 that were taken on the polygraphic recorder, were used in the following analysis of sexual behavior.

COPULATION (TABLE 16): Copulation was witnessed 39 times in 33 of the 246 observation periods. Unfortunately, most of these observations were made before the polygraphic recorder became available, but nine copulations were accurately timed on the recording apparatus. These were found to range from 1.0 second to 5.6 seconds, with an average of 2.39 seconds ± 0.45 second; σ equals ± 1.34 seconds. The mean duration of copulation for the platyfish was 1.36 seconds (see p. 168). The difference between means is

TABLE 17

RELATION OF GONIPODIAL THRUSTS TO COPULATIONS IN SWORDTAILS

(For comparisons, see tables 10, 22, 26, and 31.)

	Number of Copulations Recorded During an Observation Period	Number of Observation Periods	Average Number of Thrusts During an Observation Period
A	0	144	6.7
	1 or 2	33	19.4
B	1	27	21.1
	2	6	11.9
C	0–2	177	9.1

Total number of observation periods	246
Number of observation periods with thrusts recorded	177
Per cent of observation periods with thrusts recorded	72.0%

TABLE 18

RELATION OF GONOPODIAL SWINGS TO COPULATIONS AND THRUSTS IN SWORDTAILS

(For comparisons, see tables 11, 23, 27, and 32.)

Number of Copulations Recorded During an Observation Period	Thrusts	Number of Observation Periods	Average Number of Swings During an Observation Period
0	Absent	69	0.7
0	Present	144	4.2
1	Present	27	3.6
2	Present	6	4.8
1 or 2	Present	33	3.8
0–2	Present or absent	246	3.2

Total number of observation periods	246
Number of observation periods with swings recorded	192
Per cent of observation periods with swings recorded	78.0%

1.03 seconds, with t equaling 5.1 and p less than 0.01, indicating a good probability that this difference in duration of the copulatory act between the platyfish and the swordtail is statistically significant.

The number of females inseminated after copulation took place (39.4%) was much lower than in the platyfish (86%). Also in contrast to the platyfish, fewer females were inseminated after two copulations than after one, but this quantitative relationship of insemination to copulations may not be reliable because of the limited data on the results of multiple copulations in the swordtail.

On the average the interval between the start of the observation and the first copulation was two minutes. However, the distribution of these data are strongly skewed to the right (fig. 6). In this circumstance the median interval, which is one minute, represents more adequately the central tendency of the distribution. For the same reason further statistical treatment of the data was not attempted, but it is evident nevertheless that the said interval was usually much shorter in the swordtail than in the platyfish in which the median is 5 minutes and the mean is 5.2 minutes.

THRUSTING (TABLE 17): Complete records of all thrusts of the gonopodium were taken for each of the 246 observation periods. In 177 observation periods the fish thrust one or more times. The average frequency of thrusts in observation periods in which copulations occurred was more than twice the average frequency of thrusts in those periods in which copulations did not occur (table 17, section A). However, the average number of thrusts during the 10-minute observation time was less for two copulations than for one

TABLE 19

FREQUENCIES OF NIPPING AND NIBBLING BEHAVIORS IN SWORDTAILS

(For comparisons, see table 13.)

Number of Copulations per Observation Period	Thrusts	Number of Observation Periods	Observation Periods with Nipping Recorded		Observation Periods with Nibbling Recorded	
			Number	Per Cent	Number	Per Cent
0	Absent	69	31	44.9%	13	18.8%
0	Present	144	67	46.5	74	51.4
1 and 2	Present	33	13	39.4	22	66.7
0–2	Present or absent	246	111	45.1	109	44.3

copulation (table 17, section B). This trend is opposite to that of the platyfish and the F₁ hybrid (fig. 7) but could not be critically evaluated because of high variability and the small number of observation periods in swordtails in which two copulations occurred. Nevertheless a somewhat different relationship of thrusts to copulations in swordtails and platyfish is suggested.

GONOPODIAL SWINGING (TABLE 18): This behavior was recorded at least once in 78.0 per cent of the 246 observation periods. The average frequency of swings was high (4.2 per 10-minute observation period) in sexually active males that thrust at the female whether or not copulation took place. The average frequency of gonopodial swings was lowest during those observation periods in which thrusts were not recorded (sexually inactive males).

NIPPING AND NIBBLING (TABLE 19): In the swordtail the distinction between the aggressive nip and the gentle nibble was well marked; therefore these two types of behavior were recorded separately. Nibbling usually lasts about one second, as the male swims along under the female making a series of oral contacts with her anus; nips are momentary. Three or more nips at any part of the female's body during a 10-minute observation period were counted as nipping behavior, whereas a single nibble at the genital region of the female was defined as nibbling behavior. On several occasions the male in the nibbling act engulfed and then rejected partly extruded feces which had been hanging from the female's anus. It can be seen from table 19 that while nipping appears to have little relation to sexual activity nibbling is generally associated with thrusts and copulation.

OTHER BEHAVIOR PATTERNS (TABLE 20): Pecking, retiring, S-curving, and quivering were never observed as distinct patterns in the swordtail. On the other hand, a marked backing behavior (frequently followed by sidling, quivering, and by a movement having some semblance to S-curving) occurred in 32.8 per cent of the 246 observation periods. Backing behavior was recorded in 42.4 per cent of the observation periods in which copulations were recorded, in 44.4 per cent of the

TABLE 20

RELATION OF COPULATIONS AND THRUSTS TO THE FREQUENCIES OF OTHER TYPES
OF BEHAVIOR IN MALE SWORDTAILS

(For comparisons, see tables 14, 24, 28, 29, 33, 37, and 41–44.)

Types of Behavior	Per Cent of Observation Periods in Which Behavior Was Recorded			Per Cent of Total Observation Periods with Behavior Recorded
	No Thrusts and No Copulations	Thrusts but No Copulations	Thrusts and Copulations	
Copulation	0.0%	0.0%	100.0%	13.4%
Thrusting	0.0	100.0	100.0	72.0
Swinging	0.72[a]	4.2[a]	3.8[a]	78.0
Sidling	3.0	100.0	100.0	72.6
Pecking	0.0	0.0	0.0	0.0
Backing	4.3	44.4	42.4	32.8
Quivering[b]	—	—	—	—
Nibbling	18.8	51.4	66.7	44.3
Retiring	0.0	0.0	0.0	0.0
S-curving[b]	—	—	—	—
Nipping[c]	44.9	46.5	39.4	45.1
Total number of observation periods	69	144	33	246

[a] These figures represent the average number of gonopodial swings per observation.
[b] Occurs rarely but could not be accurately quantified.
[c] Three or more nips.

TABLE 21

Frequencies of Copulations and Inseminations in Platyfish-Swordtail F₁ Hybrids

(For comparisons, see tables 8, 16, 25, and 30.)

Number of Copulations Recorded During an Observation Period	Number of Observation Periods	Observation Periods after Which Insemination Was Recorded	
		Number	Per Cent
1	13	7	53.8%
2	14	11	78.6
3	10 ⎫		
4	3 ⎬	9	64.4
5	2 ⎭		
1–5	42	27	64.3

Total number of observation periods	145
Number of observation periods with copulations recorded	42
Per cent of observation periods with copulations recorded	29.0%
Total number of copulations recorded	91

observation periods in which thrusts but no copulations were recorded, and in only 4.3 per cent of the observation periods in which no thrusts or copulations occurred. Hence backing appears to be a characteristic item of pre-copulatory procedure. Backing in the swordtail seems to be the counterpart of pecking in the platyfish in that both behaviors characteristically precede sidling.

In the swordtail, courtship activity (swinging, backing, and sidling) was markedly low in observation periods in which the threshold for thrusting was not reached. This may be constrasted with platyfish behavior, in which relatively high frequencies of courtship activity occurred even in the absence of thrusting.

SEXUAL BEHAVIOR IN PLATYFISH-SWORDTAIL F₁ HYBRIDS

A total of 145 10-minute observations were made on five male and 59 female platyfish-swordtail F₁ hybrids. At the time of our observational tests, only a small number of males was available owing to the atypical sex ratio of F₁ hybrids. Approximately 10 females to one male are obtained (Gordon, 1948). The analysis of sexual behavior in F₁ hybrids follows.

COPULATION (TABLE 21): Copulation was witnessed 91 times in 42 of the 145 observation periods. Insemination took place in 64.3 per cent of those observation periods in which

copulations were recorded. As in the pure platyfish, more inseminations occurred after two or more copulations than after only one. The first copulation was found to occur, on the average, during the second minute after the female was placed in the aquarium with the male. The frequency distribution of these first copulations in F₁ hybrids during the 10 minutes is very similar to that for the swordtail (fig. 6) with a median of two minutes.

An unusual item of behavior occurred during one of these observations which merits reporting. Copulatory contact had just started with the female on the right side of the male when a vigorous jerk was observed between the two fish. With this, the position of the female rotated about 150 degrees. The new position, with the head of the male pointed in the direction of the female's tail, was held for the remainder of the copulation, which lasted about two seconds. This unusual behavior was observed but once during all our observations. Ryder's report (1885) of head to tail position during copulation in *Gambusia* had been considered incorrect by some authors, but it is possible that his description was based on an exceptional case such as this one. On the other hand, in the oviparous catfish *Ameiurus nebulosus*, the customary spawning position is head to tail (Breder, 1936).

THRUSTING (TABLE 22): Thrusting of the gonopodia in F₁ hybrids occurred in 46.2 per

TABLE 22

RELATION OF GONOPODIAL THRUSTS TO COPULATIONS IN PLATYFISH-SWORDTAIL F_1 HYBRIDS

(For comparisons, see tables 10, 17, 26, and 31.)

	Number of Copulations Recorded During an Observation Period	Number of Observation Periods	Average Number of Thrusts During an Observation Period
A	0	25	4.3
	1–5	42	15.1
B	1	13	8.9
	2	14	16.1
	3	10	18.3
	4 and 5	5	21.3
C	0–5	67	11.0

Total number of observation periods	145
Number of observation periods with thrusts recorded	67
Per cent of observation periods with thrusts recorded	46.2%

cent of the 10-minute observations. The frequency of thrusts was higher in observations in which copulations occurred, and as in the platyfish the frequency increased with the number of copulations (fig. 7). However, the general level of thrusting frequency (table 22, column 3) was closer to that of the swordtail.

GONOPODIAL SWINGING (TABLE 23): This behavior in F_1 hybrids was exhibited at least once in 34.4 per cent of the observation periods. The average frequency of gonopodial swinging was highest (2.8 swings per 10-minute observation period) in observation periods in which thrusts occurred but copulations did not. The frequency of swings was lowest among sexually inactive males which did not thrust or copulate. It is interesting to note that the percentage of observations in which swinging was recorded, as well as the mean frequency of swings per observation, was lower in the F_1 hybrids than in either the platyfish or swordtails.

TABLE 23

RELATION OF GONOPODIAL SWINGS TO COPULATIONS AND THRUSTS IN PLATYFISH-SWORDTAIL F_1 HYBRIDS

(For comparisons, see tables 11, 18, 27, and 32.)

Number of Copulations Recorded During an Observation Period	Thrusts	Number of Observation Periods	Average Number of Swings During an Observation Period
0	Absent	78	1.0
0	Present	25	2.8
1	Present	13	3.1
2–5	Present	29	1.1
1–5	Present	42	1.7
0–5	Present or absent	145	1.5

Total number of observation periods	145
Number of observation periods with swings recorded	50
Per cent of observation periods with swings recorded	34.4%

TABLE 24

RELATION OF COPULATIONS AND THRUSTS TO FREQUENCIES OF OTHER TYPES OF
BEHAVIOR IN PLATYFISH-SWORDTAIL F_1 HYBRIDS

(For comparisons, see tables 14, 20, 28, 29, 33, 37, and 41–44.)

| Types of Behavior | Per Cent of Observation Periods in Which Behavior Was Recorded | | | Per Cent of Total Observation Periods with Behavior Recorded |
	No Thrusts and No Copulations	Thrusts but No Copulations	Thrusts and Copulations	
Copulation	0.0%	0.0%	100.0%	29.0%
Thrusting	0.0	100.0	100.0	46.2
Swinging	1.0[a]	2.8[a]	1.7[a]	34.4
Sidling	1.3	100.0	100.0	46.8
Pecking	0.0	0.0	0.0	0.0
Backing	3.8	28.0	9.5	9.7
Quivering	3.8	44.0	11.9	13.1
Nibbling	0.0	0.0	0.0	0.0
Retiring	3.8	8.0	0.0	3.4
S-curving	19.2	28.0	11.9	15.2
Nipping[b]	25.6	8.0	4.8	16.6
Total number of observation period	78	25	42	145

[a] These figures represent the average number of gonopodial swings per observation.
[b] Three or more nips.

OTHER BEHAVIOR PATTERNS (TABLE 24): Pecking behavior, which is characteristic of platyfish, and nibbling, a specific swordtail pattern, were not seen in the F_1 hybrids. Quivering and retiring, which are also restricted to platyfish, were observed in the F_1 hybrids but in a much smaller percentage of the total number of observation periods than for the parental platyfish. S-curving, another typical platyfish pattern, was recorded at a somewhat lower frequency in the F_1 hybrids than in the pure platyfish. Backing, which is a swordtail pattern, was observed in the F_1 generation in slightly fewer observations than in the pure swordtail. Nipping appeared in approximately the equivalent number of observations as in the platyfish, and here too was inversely related to sexual activity.

Quivering, backing, S-curving, and retiring were recorded mostly in prolonged courtships where thrusts of the gonopodium did not culminate in copulation.

SEXUAL BEHAVIOR IN BACKCROSS
HYBRID MALES

Tables 25–29

Observations were made on 10 male hy-

brids (PX-P) produced by the backcross of an F_1 hybrid female to a platyfish male, and on 10 male hybrids (X-PX) produced by backcrossing a swordtail female to an F_1 hybrid male. The 20 hybrid males were all tested with virgin females from pure platyfish stock. The reasons for testing with platyfish females rather than with hybrid females were threefold: (1) an inadequate number of backcross females was available as test animals; (2) backcross hybrids are very variable particularly as to size, whereas test females should be as uniform as possible; and (3) it was known that many backcross hybrid females have abnormal ovaries. Although the relationship of ovarian function to sex behavior has not been systematically investigated in any poeciliid species, we may expect from general knowledge of vertebrate endocrinology that deficient ovarian function may lead to a decrease in female receptivity. Unfortunately, overt signs of receptivity in poeciliid fishes are not adequately understood,[1] and also external secondary sex char-

[1] Jaski (1939) claimed that in *Lebistes* he could recognize receptive females by the angle at which they swim. This conclusion has been questioned by Clark and Aronson (1951).

TABLE 25

FREQUENCIES OF COPULATIONS AND INSEMINATIONS IN PLATYFISH-
SWORDTAIL BACKCROSS MALE HYBRIDS

(For comparisons, see tables 8, 16, 21, and 30.)

Number of Copulations Recorded During an Observation Period	Number of Observation Periods		Observation Periods after Which Insemination Was Recorded			
			Number		Per Cent	
	PX-P	X-PX	PX-P	X-PX	PX-P	X-PX
1	6	3	1	0	16.7%	0.0%
2	2	1	0	0	0.0	0.0
3	0	.1	—	0	—	0.0
1–3	8	5	1	0	12.5	0.0

PX-P, backcross hybrid obtained from mating an F_1 hybrid female with a platyfish male.
X-PX, backcross hybrid obtained from mating a swordtail female with an F_1 hybrid male.

	PX-P	X-PX
Total number of observation periods	108	102
Number of observation periods with copulations recorded	8	5
Per cent of observation periods with copulations recorded	7.4%	4.9%
Total number of copulations recorded	10	8

TABLE 26

RELATION OF GONOPODIAL THRUSTS TO COPULATIONS IN PLATYFISH-
SWORDTAIL BACKCROSS HYBRIDS

(For comparisons, see tables 10, 17, 22, and 31.)

Number of Copulations Recorded During an Observation Period	Number of Observation Periods		Average Number of Thrusts During an Observation Period	
	PX-P	X-PX	PX-P	X-PX
0	100	97	4.2	8.6
1–3	8	5	9.5	18.8
1	6	3	8.0	17.7
2	2	1	14.0	28.0
3	0	1	0.0	14.0
0–3	52	75	4.8	9.5

PX-P, backcross hybrid obtained from mating an F_1 hybrid female with a platyfish male.
X-PX, backcross hybrid obtained from mating a swordtail female with an F_1 hybrid male.

	PX-P	X-PX
Total number of observation periods	108	102
Number of observation periods with thrusts recorded	52	75
Per cent of observation periods with thrusts recorded	48.1%	73.5%

TABLE 27

Relation of Gonopodial Swings to Copulations and Thrusts in
Platyfish-Swordtail Backcross Hybrids

(For comparisons, see tables 11, 18, 23, and 32.)

Number of Copulations Recorded During an Observation Period	Thrusts	Number of Observation Periods		Average Number of Swings During an Observation Period	
		PX-P	X-PX	PX-P	X-PX
0	Absent	56	30	3.3	0.4
0	Present	44	70	8.3	4.0
1	Present	6	3	5.7	2.0
2	Present	2	1	11.0	6.0
3	Present	0	1	0	1.0
1–3	Present	8	5	6.8	2.6
0–3	Present or absent	108	105	5.5	2.9

PX-P, backcross hybrid obtained from mating an F_1 hybrid female with a platyfish male.
X-PX, backcross hybrid obtained from mating a swordtail female with an F_1 hybrid male.

	PX-P	X-PX
Total number of observation periods	108	105
Number of observation periods with swings recorded	85	74
Per cent of observation periods with swings recorded	78.7%	72.5%

acters are not available as indices of ovarian hormonal output. Hence it was considered inappropriate to use as test females fish with unknown or unpredictable behavioral characteristics.

The backcross hybrid males copulated infrequently (table 25). During 108 observations, only 10 copulations were recorded, and the female was inseminated during only one observation period. The male in this case was a platyfish backcross (PX-P). This result is not surprising since it has been shown by Berg and Gordon (1953) that almost 50 per cent of the backcross hybrids have markedly defective gonads. On the other hand, the frequencies of gonopodial thrusting and swinging were considerable. For the X-PX males, the order of magnitude of thrusting was comparable to that of the swordtails (tables 26 and 37). For the PX-P males the frequency was somewhat less and was definitely smaller than that of the pure platyfish. For gonopodial swinging (table 27) the scores were somewhat higher for the PX-P males than for the X-PX hybrids and

were roughly comparable to ratings for the parental species.

Concerning the remaining sexual behavior patterns (tables 28, 29, and 37) we find that backing and nibbling, which are characteristic swordtail patterns, were present at a low frequency in the PX-P males and appeared at a much higher frequency in the X-PX males. On the other hand, picking and retiring, which are platyfish patterns, were not seen in any of the X-PX males tested. Yet the platyfish patterns, quivering and S-curving, appeared in substantial amounts in both types of backcross hybrids.

SEXUAL BEHAVIOR IN F_2 HYBRID MALES
Tables 30–40

A total of 404 observation periods were devoted to the study of 61 F_2 males. Each hybrid was paired with a pure platyfish female for at least five observation periods. The reasons for using platyfish as test females were similar to those outlined above in the backcross experiments. During 34 observation periods, one or two copulations occurred,

TABLE 28

RELATION OF COPULATIONS AND THRUSTS TO FREQUENCIES OF OTHER TYPES
OF BEHAVIOR IN MALE BACKCROSS HYBRIDS
(F₁ HYBRID FEMALE ✕ PLATYFISH MALE)

(For comparisons, see tables 14, 20, 24, 29, 33, 37, and 41–44.)

Types of Behavior	Per Cent of Observation Periods in Which Behavior Was Recorded			Per Cent of Total Observation Periods with Behavior Recorded
	No Thrusts and No Copulations	Thrusts but No Copulations	Thrusts and Copulations	
Copulation	0.0%	0.0%	100.0%	9.3%
Thrusting	0.0	100.0	100.0	48.1
Swinging	3.3[a]	8.3[a]	6.8[a]	78.7
Sidling	3.6	100.0	100.0	50.0
Pecking	14.3	18.2	12.5	15.7
Backing	0.0	11.4	0.0	4.6
Quivering	0.0	52.2	50.0	25.0
Nibbling	0.0	2.2	0.0	0.9
Retiring	0.0	0.0	12.5	0.9
S-curving	5.3	0.0	12.5	3.7
Nipping	5.3	2.3	0.0	3.7
Total number of observation periods	56	44	8	108

[a] These figures represent the average number of gonopodial swings per observation.

TABLE 29

RELATION OF COPULATIONS AND THRUSTS TO FREQUENCIES OF OTHER TYPES
OF BEHAVIOR IN MALE BACKCROSS HYBRIDS
(SWORDTAIL FEMALE ✕ F₁ HYBRID MALE)

(For comparisons, see tables 14, 20, 24, 28, 33, 37, and 41–44.)

Types of Behavior	Per Cent of Observation Periods in Which Behavior Was Recorded			Per Cent of Total Observation Periods with Behavior Recorded
	No Thrusts and No Copulations	Thrusts but No Copulations	Thrusts and Copulations	
Copulation	0.0%	0.0%	100.0%	4.9%
Thrusting	0.0	100.0	100.0	73.5
Swinging	0.4[a]	4.0[a]	2.6[a]	72.5
Sidling	7.4	100.0	100.0	75.5
Pecking	0.0	0.0	0.0	0.0
Backing	7.4	67.4	20.0	49.0
Quivering	0.0	71.4	0.0	49.0
Nibbling	7.4	4.2	20.0	4.9
Retiring	0.0	0.0	0.0	0.0
S-curving	0.0	2.8	0.0	2.0
Nipping[b]	11.1	8.6	0.0	8.8
Total number of observation periods	27	70	5	102

[a] These figures represent the average number of gonopodial swings per observation.
[b] Three or more nips.

TABLE 30

FREQUENCIES OF COPULATIONS IN PLATYFISH-SWORDTAIL F_2 HYBRIDS

(For comparisons, see tables 8, 16, 21, and 25.)

Number of Copula-tions Recorded During an Observa-Period	Number of Observation Periods	Observation Periods after Which Insemination Was Recorded	
		Number	Per Cent
1	26	4	15.4%
2	8	4	50.0
1 and 2	34	8	23.5

Total number of observation periods	404
Number of observation periods with copulations recorded	34
Per cent of observation periods with copulations recorded	8.4%
Total number of copulations recorded	42

but in only eight of these observations did insemination take place (table 30). As in the platyfish and the F_1 hybrids, a higher percentage of females was inseminated when two copulations occurred during the observation period. It should be noted that copulatory frequency and effectiveness (insemination) were lower in the F_2 generation than in either parental species or F_1 hybrids.

The form of the frequency distribution of intervals between the start of the observation and the first copulation is similar to that for the swordtail and F_1 generation (fig. 6). The median interval is three minutes (mean = 4 minutes). This is midway between the values for platyfish and swordtails.

The duration of copulation was timed on the polygraphic recorder in 33 cases. These varied from 0.3 second to 2.8 seconds, with an average of 1.1 seconds ±0.09 second; σ equals ±0.52 second. To determine whether the F_2 males tested represented a sample of a single population, the data on eight of these F_2 males with records of multiple copulations were subjected to an analysis of variance (tables 38, 39). From this analysis F was found to equal 3.85. At 7/17 degrees of freedom $F_{.05}$ equals 2.62 and $F_{.01}$ equals 3.93. Hence the variance between males is significantly greater than within males, indicating that we are actually dealing with two or more sets of males with different means.

TABLE 31

RELATION OF GONOPODIAL THRUSTS TO COPULATIONS IN PLATYFISH-SWORDTAIL F_2 HYBRIDS

(For comparisons, see tables 10, 17, 22, and 26.)

Number of Copulations Recorded During an Observation Period	Number of Observation Periods	Average Number of Thrusts During an Observation Period
0	370	13.0
1 or 2	34	35.4
1	26	32.9
2	8	42.0
0–2	404	14.8

Total number of observation periods	404
Number of observation periods with thrusts recorded	268
Per cent of observation periods with thrusts recorded	66.3%

TABLE 32

RELATION OF GONOPODIAL SWINGS TO COPULATIONS AND THRUSTS IN
PLATYFISH-SWORDTAIL F$_2$ HYBRIDS

(For comparisons, see tables 11, 18, 23, and 27.)

	Number of Copulations Recorded During an Observation Period	Thrusts	Number of Observation Periods	Average Number of Swings During an Observation Period
A	0	Absent	136	2.2
	0	Present	234	7.5
B	1	Present	28	8.2
	2	Present	6	8.4
C	1 or 2	Present	34	8.2
	0–2	Present or absent	404	5.6

Total number of observation periods	404
Number of observation periods with swings recorded	329
Per cent of observation periods with swings recorded	81.4%

Thus while the mean duration of copulation in the F$_2$ males is close to the figure for the pure platyfish, an important difference is suggested. In the platyfish, we are dealing with a homogeneous group (see p. 168 and table 9), while in the F$_2$ fish, some males consistently copulate relatively briefly, others copulate for much longer periods, and still others fall into one or more intermediate groups. Moreover, the males with the highest scores fall well within the range of variability for pure swordtails.

Thrusts of the gonopodia (table 31) were recorded in 66.3 per cent of the observation periods; the frequency of thrusts was higher when copulations occurred. Gonopodial swinging (table 32) was recorded in 81.4 per cent of the observation periods. While copulatory activity is low, precopulatory thrusting and gonopodial swinging are notably high. The average number of thrusts per observation is higher in the F$_2$ generation than in either parental species or in the other hybrid types (table 37). This situation might be interpreted as indicating a high level of sexual arousal coupled with a partial block or lack of coordination which prevents, in many cases, the completion of the mating pattern. A similar situation is suggested by the data for the backcross

hybrids, but the distinctions are much less clear.

A detailed study of the structure of the tips of the gonopodia was made by Gordon and Rosen (1951) for most of the F$_2$ males used in the present study. In a number of these fish, defects were observed, especially small spines and serrae and abnormally shaped elements. It was anticipated that those F$_2$ males with high scores for thrusting (i.e., sexually active individuals) but with low or zero scores for copulation might have markedly defective gonopodial tips. However, no such correlation could be established.

Berg and Gordon (1953) conducted a histological analysis of the testes of 49 of the F$_2$ males used in our observations. They found varying degrees of abnormality in almost two-thirds of the testes. These data are correlated in tables 34 and 35, together with the sexual performance based on swings, thrusts, copulations, and inseminations. For purposes of analysis, the males were arranged into four groups based on the histological analyses of the testes as follows:

1. Normal testes.
2. Slightly abnormal testes in which a few sperm were not in spermatophores (i.e., free sperm) and/or a little more colloid than usual was present in the genital duct and tubules.

TABLE 33

RELATION OF COPULATION AND THRUSTS TO FREQUENCIES OF OTHER TYPES OF
BEHAVIOR IN PLATYFISH-SWORDTAIL F_2 HYBRIDS

(For comparisons, see tables 14, 20, 24, 28, 29, 37, and 41–44.)

Types of Behavior	Per Cent of Observation Periods in Which Behavior Was Recorded			Per Cent of Total Observation Periods with Behavior Recorded
	No Thrusts and No Copulations	Thrusts but No Copulations	Thrusts and Copulations	
Copulations	—	—	100.0%	8.4%
Thrusting	—	100.0%	100.0	66.3
	—	20.5[a]	35.0[a]	14.8[a]
Swinging	49.4%	98.0	97.0	81.4
	2.2[b]	7.5[b]	8.2[b]	5.6[b]
Sidling	6.6	100.0	100.0	68.7
Pecking	9.6	20.5	32.4	17.6
Backing	8.1	52.2	35.3	36.0
Quivering	11.8	68.3	50.0	47.7
Nibbling	0.7	3.4	5.9	0.3
Retiring	2.9	13.2	14.7	99.1
S-curving	8.8	15.8	8.8	12.9
Nipping	33.8	13.2	11.8	20.0
Total number of observation periods	136	234	34	404

[a] These figures represent the average number of thrusts per observation.
[b] These figures represent the average number of gonopodial swings per observation.

3. Testes in which one or more of the following major abnormalities were found in several combinations.

Considerable excess of colloid

Large numbers of free sperm filling genital duct and tubules

Scarcity of early stages of spermatogenesis

Very few sperm in genital duct

Immature testis without sperm

Minute gonad markedly atypical in structure.

4. Testes absent.

From tables 34 and 35 it may be seen that among the males with normal or only slightly atypical gonads, copulations were frequent and several females were inseminated. Among the males with major gonadal aberrations, copulations were very limited (four males copulated once each), and no inseminations were recorded. On the other hand, precopulatory swinging and thrusting behavior showed no such trend. It is of particular interest to

TABLE 34

COMPARISON OF THE SEXUAL BEHAVIOR WITH THE HISTOLOGY OF THE
TESTES IN PLATYFISH-SWORDTAIL F_2 HYBRIDS

Histological Appearance of Testes	No. of Males Analyzed	Swings[a]	Thrusts[a]	Copulations[a]	Inseminations[a]
Normal	20	38.9	102.7	1.1	0.3
Slightly abnormal[b]	7	51.1	75.0	1.6	0.1
Grossly abnormal[b]	20	29.7	91.4	0.2	0.0
Testes Absent	2	89.0	104.0	0.5	0.0

[a] Figures based on average number per male.
[b] For descriptions of abnormalities, see text.

TABLE 35

RECORDS OF FOUR MALES THAT COPULATED DESPITE MAJOR GONADAL ABNORMALITIES

Male's Number	No. of Observations	Abnormality	Swings[a]	Thrusts[a]	Copulations[a]
16	10	Immature testes without sperm	9	54	1
38	5	Considerable colloid; sperm in ducts scarce	26	256	1
49	5	Considerable colloid; sperm in ducts scarce	45	192	1
18	10	No gonad	134	144	1

[a] Figures based on average number per observation.

note that the scores for swinging and thrusting in the two males without testes were approximately the same as the scores for males with well-developed testes. These two exceptional males without testes had normal appearing gonopodia. According to the investigations of Turner (1947) on *Gambusia*, and Grobstein (1940) on platyfish, the proper differentiation of the gonopodium at sexual maturity depends upon the presence of testicular hormone, but once morphogenesis is completed, male hormone is no longer necessary for the maintenance of the differentiated gonopodium. Hence, we must conclude that, in these two males without gonads, either the testes degenerated and disappeared after sexual maturity, or the males had an extra gonadal source of androgenic hormone which may still have been present when the behavioral observations were made. Through an unfortunate accident, the viscera of one of these males was not preserved after dissection, and histological examination for microscopic testicular remnants was not made. Hence, as a third alternative explanation, it is conceivable, although not very probable, that in one case microscopic remnants of degenerative testes produced sufficient hormone to permit normal morphogenesis of the gonopodium and a high fre-

TABLE 36

RELATION OF PECKING, RETIRING, BACKING, AND NIBBLING BEHAVIOR TO THE
LENGTH OF THE SWORD AND TO THE PRESENCE OF PLATYFISH
COLOR PATTERNS IN F$_2$ HYBRID MALES

Types of Males	Number of Males	Platyfish Behavior				Swordtail Behavior			
		Pecking		Retiring		Backing[a]		Nibbling	
		Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
All	61	24	39.3%	14	23.0%	41	67.3%	9	14.8%
Medium sword[b]	4	0	0.0	0	0.0	4	100.0	2	50.0
Short sword[b]	40	14	35.0	11	27.5	27	67.5	7	17.5
Sword absent	17	10	58.8	3	17.6	10	58.8	0	0.0
Phenotype Swordtail type double recessive	41	14	34.0	13	31.7	32	78.1	9	22.0
Platyfish type spotted pattern	20	10	50.0	1	5.0	9	45.0	0	0.0

[a] The exaggerated type of backing shown by swordtails and not by platyfish.
[b] The length of the sword on F$_2$ males never approaches what is usually considered long in the pure swordtails which sometimes is equal to the length of the body. A sword is classified here as medium if it extends beyond the end of the central rays of the caudal fin for a distance equivalent to more than half of the length of the caudal fin. It is classified as short if it extends less than this distance.

TABLE 37

COMPARISONS OF SEXUAL BEHAVIOR IN THE MALE PLATYFISH,
SWORDTAILS, AND THEIR HYBRIDS

(For comparisons, see tables 14, 20, 24, 28, 29, 33, and 41–44.)

	P	X	F_1	F_2	Back-cross PX-P	Back cross X-PX
Total number of observation periods	150	246	145	404	108	102
Number of males tested	12	21	5	61	10	10
Copulation						
Per cent of observation periods with copulations recorded	42.0%	13.4%	29.0%	8.4%	7.4%	4.9%
Maximum number of copulations recorded per observation period	6	2	5	2	2	3
Per cent of observation periods with copulations recorded in which insemination occurred	79.1%[a]	39.4%	64.3%	23.5%	9.3%	0.0%
Thrusting						
Per cent of observation periods with thrusts recorded	76.3%	72.0%	46.2%	66.3%	48.1%	73.5%
Average number of thrusts recorded in observation periods with						
Copulations recorded	21.6	19.4	15.1	35.4	9.5	18.8
No copulations recorded	11.5	6.7	4.3	20.5	4.2	8.6
Swinging						
Per cent of observation periods with swinging recorded	92.2%	78.0%	34.4%	78.2%	79.0%	72.5%
Average number of swings per observation period						
Without thrusts or copulations	5.4	0.7	1.0	3.2	3.3	.04
With thrusts but no copulations	11.6	4.2	2.8	8.4	8.3	4.0
With thrusts and copulations	6.6	3.8	1.7	9.5	6.8	2.6
Per cent of observation periods with						
Sidling	88.7%	72.6%	46.8%	1.1%	50.0%	75.5%
Pecking	58.0	0.0	0.0	17.8	15.7	0.0
Backing	0.0	32.8	9.7	11.4	4.6	49.0
Quivering	66.6	—	13.1	50.2	25.0	49.0
Nibbling	0.0	44.3	0.0	4.5	0.9	4.9
Retiring	34.0	0.0	3.4	9.7	0.9	0.0
S-curving	22.7	—	15.2	2.3	3.7	2.0
Nipping	16.6	45.1	16.6	22.8	3.7	8.8

P, platyfish.
X, swordtail.
PX-P, backcross hybrid obtained from mating an F_1 hybrid female with a platyfish male.
X-PX, backcross hybrid obtained from mating a swordtail female with an F_1 hybrid male.

[a] Based on 11 males only since one male was found later to be sterile (see p. 166).

quency of precopulatory behavior (about six "gonad-less" hybrids in 100 have microscopic remnants according to Berg and Gordon, 1953).

Riddle (1937) reported normal masculine behavior in 16 congenitally gonad-less pigeons. He suggested that androgens of adrenal origin may account for the behavior. These may also be the explanation for the finding, reported by Cohen, Gordon, and Nigrelli (1941), that gonopodia developed spontaneously in two gravid female platyfish.
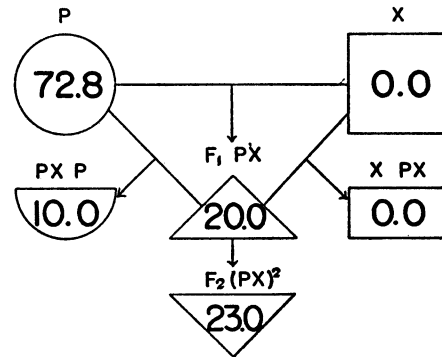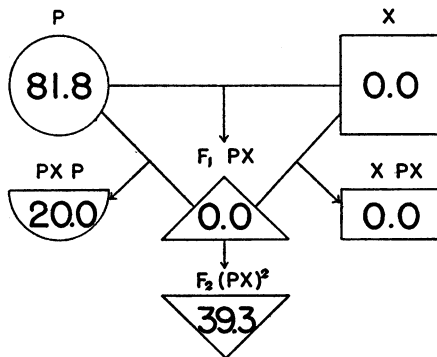
TOTAL NO. OF MALES
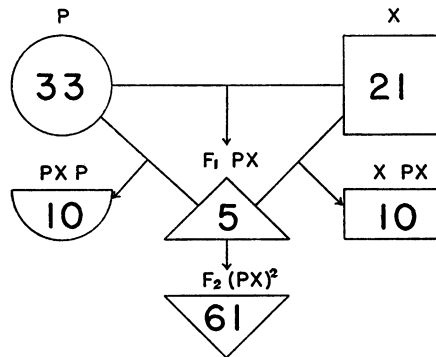
MALES TESTED WHICH SHOWED BEHAVIOR
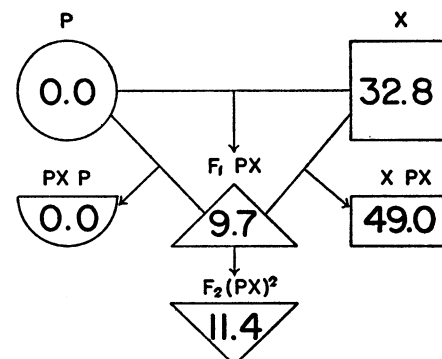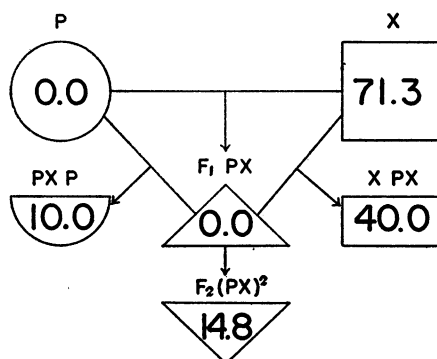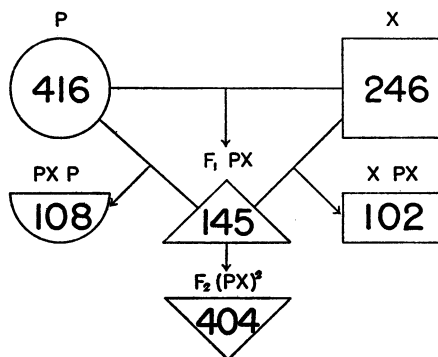### PECKING (IN PER CENT) RETIRING

NIBBLING BACKING

FIG. 8. Per cent of males tested in which pecking, retiring, nibbling, and backing behavior were recorded in platyfish, swordtails, F_1, F_2, and backcross hybrids.
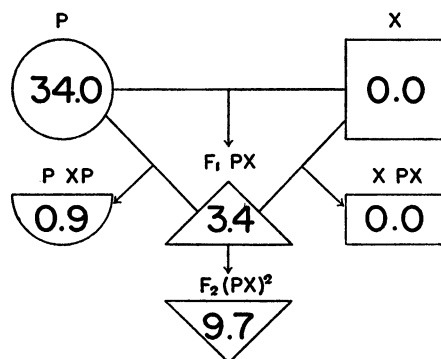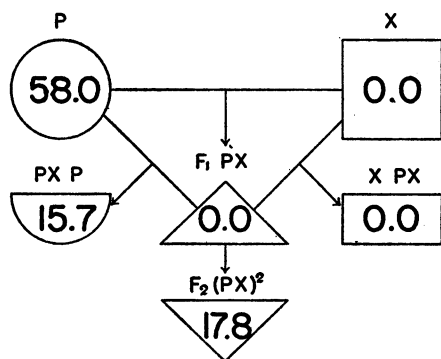
TOTAL NO. OF OBSERVATIONS

OBSERVATIONS WHERE BEHAVIOR OCCURRED
PECKING   (IN PER CENT)   RETIRING
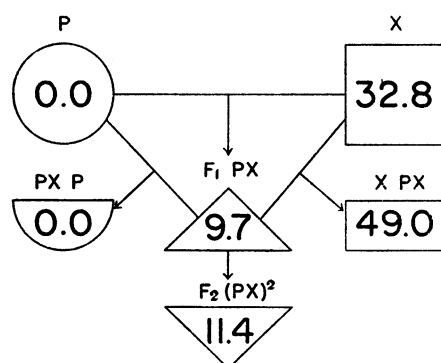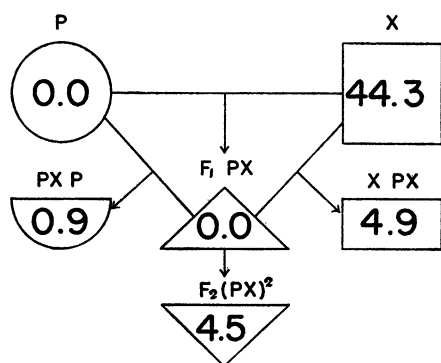
NIBBLING   BACKING

FIG. 9. Per cent of observations in which pecking, retiring, nibbling, and backing behavior appeared in platyfish, swordtails, F_1, F_2, and backcross hybrids.

The behavioral patterns in the platyfish, swordtails, and their hybrids that promised to lend themselves to genetic analysis are those found in only one of the two species used as parents. These are the pecking and retiring behavior of male platyfish and the exaggerated backing and nibbling behavior of male swordtails. Sidling, S-curving, and quivering which are also characteristic platyfish patterns were not used in this analysis since slight semblances of these acts can be recognized during backing behavior in the swordtail (see p. 177).

Figures 8 and 9 show the per cent of the total number of observation periods and the per cent of males tested which showed these four behavior patterns in platyfish, in swordtails, and in their various hybrid combinations. It should be pointed out that in our limited observations the absence of a behavior in a particular male is not conclusive evidence that this male did not possess the faculties for performing that behavior. It is possible that the sexual drive of the male was too low to result in courtship, or on the other hand too high to result in prolonged courtship where these behavior patterns show up most frequently. Eight of the F_2 male hybrids showed none of these four patterns, and 24 showed a combination of behavioral items of both platyfish and swordtails. No association was noted between the two typically platyfish behavior patterns or between the two swordtail patterns. A number of F_2 males exhibited one pattern without the other in each case. Pecking and retiring were associated to some extent with F_2 males having little or no "sword" developed on the tail, whereas there was a tendency for backing and nibbling to be exhibited by males having a small or well-developed "sword" on the tail.

It should be noted that the best-developed "swords" on F_2 males never exceeded in length the remaining caudal fin and hence are short in comparison with swords of pure swordtail males, which approximate the length of the body. Backing, nibbling, and retiring appeared in more F_2 males of the double recessive type (pl. 3, fig. 2) than in F_2 males showing the dominant platyfish genes for spotting (pl. 3, fig. 3). Pecking, on the other hand, showed up in more males with platyfish genes for spotting than in the

TABLE 38

COMPARISON OF THE DURATION OF COPULATION IN F_2 HYBRID MALES WITH OTHER PARENTAL CHARACTERISTICS

| | | | | Male's Number | | | | | |
	17	19	20	21	27	36	38	47	Totals
Length of sword on tail[a]	Short	Short	Short	Short	Absent	Short	Short	Short	
Platyfish pattern for spotting	Absent	Absent	Absent	Absent	Absent	Sd	Sd	Sd	
Behavior									
Platyfish type	Pk Rt	Pk	Pk	Pk	Pk Rt	Pk	—	Pk Rt	
Swordtail type	Nb Bk	Nb	Bk	Nb Bk	Bk		—	Bk	
Duration of copulation	0.50	1.75	0.90	1.95	0.60	2.80	1.10	1.50	
(in seconds)	0.60	1.20	0.95	0.30	1.60	2.10	0.75	0.90	
	0.55		1.00		1.30	2.40			
	0.95				0.95	1.35			
						2.30			
						1.95			
Totals	2.60	2.95	2.85	2.25	4.40	12.90	1.85	2.40	32.20
Number of Copulations	4	2	3	2	4	6	2	2	25
Means	*0.65*	*1.48*	*0.95*	*1.13*	*1.10*	*2.15*	*0.93*	*1.20*	*1.20*

Characteristic platyfish behavior: Pk, picking; Rt, retiring.
Characteristic swordtail behavior: Bk, exaggerated backing; Nb, nibbling.
Sd: gene for the pattern "spotted dorsal fin."

[a] A sword is classified here as medium if it extends beyond the end of the central rays of the caudal fin for a distance equivalent to more than one-half of the length of the caudal fin. It is classified as short if it extends less than this distance.

TABLE 39

ANALYSIS OF VARIANCE OF DURATION OF COPULATION IN F_2 HYBRID
MALES BASED ON DATA OF TABLE 38

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares (Variance)
Total	24	11.42	
Between males	7	6.98	1.00
Within males	17	4.44	0.26

$F = 1.00/0.26 = 3.85$. For df 7/17, $F_{0.05} = 2.62$; $F_{0.01} = 3.93$.

recessive forms. These comparisons are shown in table 36.

In table 33 the behavioral data of F_2 males are summarized, and in table 37 comparisons are made with the behavior of the parental species and the other hybrid combinations. In tables 38, 39, and 40 comparisons are also presented between the duration of copulation and (1) the length of the sword (the presence of a sword being characteristic of the swordtail); (2) the spot-sided (Sp) and spotted-dorsal (Sd) color pattern genes for two different kinds of macromelanophore spotting, which are characteristic of the platyfish; and (3) pecking (Pk) and retiring (Rt), which are platyfish behavior, and nibbling (Nb) and exaggerated backing (Bk), which are swordtail behavior. These comparisons reveal no special correlations between known genes and behavioral items characteristic of the parental species, between diagnostic morphological features and behavior, or even between precopulatory and copulatory behavior.

OBSERVATIONS ON INTERSPECIFIC MATINGS

ANALYSIS OF INTERSPECIFIC BEHAVIOR OF PAIRED FISH

After the behavior of the platyfish and swordtails was evaluated and quantified, an investigation of the behavior of heterosexual members in interspecific matings was undertaken. The behavior of interspecific and intraspecific pairs was compared. Two series of

TABLE 40

COMPARISONS OF CHARACTERISTICS OF ADDITIONAL F_2 HYBRID MALES WITH
RECORDS OF ONLY ONE COPULATION PER MALE

	Male's Number							
	18	22	24	34	37	49	55	59
Length of sword on tail[a]	Short	Short	Short	Short	Short	Short	Short	Long
Platyfish pattern for spotting	None	None	None	None	None	None	Sd	Sp
Behavior								
Platyfish type	Pk Rt	Pk			Rt	Rt	Pk	Bk
Swordtail type	Nb Bk	Bk	Bk	Bk		Bk		
Duration of Copulation								
(in seconds)	0.90	1.00	0.80	0.70	0.90	1.40	0.90	1.70

Characteristic platyfish behavior: Pk, picking; Rt, retiring.
Characteristic swordtail behavior: Bk, exaggerated backing; Nb, nibbling.
Sd: gene for the pattern "spotted dorsal fin."
Sp: gene for the pattern "spotted side."

[a] A sword is classified here as medium if it extends beyond the end of the central rays of the caudal fin for a distance equivalent to more than one-half of the length of the caudal fin. It is classified as short if it extends less than this distance.

TABLE 41

MALE BEHAVIOR IN INTERSPECIFIC PAIRS (PLATYFISH MALE AND A SWORDTAIL FEMALE),
INCLUDING COMPARISONS WITH INTRASPECIFIC PLATYFISH PAIRS

(For comparisons, see tables 14, 20, 24, 28, 29, 33, 37, and 42–44.)

Types of Behavior	Interspecific Pairs			Per Cent of Total Observation Periods with Behavior Recorded	Intraspecific Platyfish Pairs Per Cent of Total Observation Periods with Behavior Recorded
	Per Cent of Observation Periods in Which Behavior was Recorded				
	No Thrusts and No Copulations	Thrusts but No Copulations	Copulations		
Copulation	0.0%	0.0%	100.0%	11.6%	42.0%
Thrusting	0.0	100.0	100.0	37.9	76.3
Swinging	0.9^a	3.8^a	3.9^a	57.9	92.2
Sidling	3.4	100.0	100.0	40.0	88.7
Pecking	31.6	52.0	0.0	45.2	58.0
Backing	0.0	0.0	0.0	0.0	0.0
Quivering	3.4	31.9	0.0	10.5	66.6
Nibbling	0.0	0.0	0.0	0.0	0.0
Retiring	11.8	0.0	0.0	7.4	34.0
S-curving	15.3	20.0	0.0	14.8	22.7
Nipping^b	25.4	12.0	9.1	20.0	16.6
Total number of observation periods	59	25	11	95	150

^{a} These figures represent the average number of gonopodial swings per observation.
^{b} Three or more nips.

10-minute observations were made of (1) a male platyfish with a female swordtail and (2) the reciprocal—a male swordtail with a female platyfish. Plate 5 shows the relative difference in body sizes of the heterosexual platyfish and swordtail used in these observations. All the records were taken on the polygraphic recording apparatus.

MALE PLATYFISH AND FEMALE SWORDTAIL: Fifteen males and 20 females were tested in a total of 95 observation periods. One or more copulations occurred in 11 of these observations, but only two resulted in the insemination of the female. These two females were set aside and after 18 days were dissected and the ovaries examined for the presence of developing embryos. No embryos were found. Gonopodial thrusting, swinging, pecking, sidling, and quivering behavior, which are related to sexual activity in intraspecific platyfish pairs, were decidedly less frequent in the interspecific pairs. As in intraspecific pairs, the frequency of nipping

behavior showed an increase, especially in observation periods in which thrusts and copulations did not take place.

Table 41 contains a summary of the data on male behavior in these interspecific matings and comparisons are made with the behavior of members of intraspecific pairs.

MALE SWORDTAIL AND FEMALE PLATYFISH: Six males and 12 females were tested in 27 observation periods. No copulations or inseminations were recorded, and the percentages of observation periods in which the several items of male behavior appeared were decidedly low. Moreover, where the behavior was observed, the frequency of occurrence was less than with intraspecific swordtail pairs. (See table 42.)

BEHAVIOR OF MALES WITH INTERSPECIFIC
AND INTRASPECIFIC CHOICE OF
FEMALES

It was then of interest to learn what would happen if a male of one species was placed

TABLE 42

MALE BEHAVIOR IN INTERSPECIFIC PAIRS (SWORDTAIL MALE AND A PLATYFISH FEMALE),
INCLUDING COMPARISONS WITH INTRASPECIFIC SWORDTAIL PAIRS

(For comparisons, see tables 14, 20, 24, 28, 29, 33, 37, 41, 43, and 44.)

| Types of Behavior | Interspecific Pairs | | | | Intraspecific Swordtail Pairs |
| | Per Cent of Observation Periods in Which Behavior was Recorded | | | Per Cent of Total Observation Periods with Behavior Recorded | Per Cent of Total Observation Periods with Behavior Recorded |
	No Thrusts and No Copulations	Thrusts but No Copulations	Copulations		
Copulation	0.0%	0.0%	—	0.0%	13.4%
Thrusting	0.0	100.0	—	51.8	72.0
Swinging	0.3[a]	4.6[a]	—	55.7	78.0
Sidling	0.0	100.0	—	51.8	72.6
Pecking	0.0	0.0	—	0.0	0.0
Backing	0.0	50.0	—	25.8	32.8
Quivering[b]	—	—	—	—	—
Nibbling	0.0	7.1	—	3.7 .	44.3
Retiring	0.0	0.0	—	0.0	0.0
S-curving[b]	—	—	—	—	—
Nipping[c]	7.7	14.2	—	11.1	45.1
Total number of observation periods	13	14	0	27	246

[a] These figures represent the average number of gonopodial swings per observation.

[b] Occurs rarely but could not be accurately quantified.

[c] Three or more nips.

with two females, one of his own species, and the other of the related species. Two series of observations were made: one using platyfish males and one using swordtail males. For each observation, two virgin females (one platyfish and one swordtail) were introduced simultaneously into a tank containing a male. The behavior of the male with each female was recorded separately during a series of standard 10-minute observation periods. All records were taken with the aid of the polygraphic recording apparatus.

The total frequency of the several items of male behavior in these mixed groupings of three fish was notably low. When a male began following one of the females, the other frequently interrupted the beginning of courtship activity by swimming close by and distracting the male. Occasionally a male was observed courting one female to the point where thrusts were made and the frequency of gonopodial swinging, pecking,

sidling, and quivering behavior indicated a high level of sexual excitation on his part. If at this time the second female swam between the male and the first female, the male usually thrust at the second female. During all these observation periods only two copulations were observed although courtship was frequently initiated. The interruptions caused by the second female appeared to be a significant influence in preventing copulation.

MALE PLATYFISH, FEMALE PLATYFISH, AND FEMALE SWORDTAIL: Ten platyfish males were tested during 54 observation periods. Eight of the males had been raised with males and females of their own species only, whereas the remaining two males had no previous experience with mature female platyfish. These two platyfish had been taken from community tanks where they had lived with six female swordtails for a three-month period just prior to the beginning of this set of observations.

TABLE 43

BEHAVIOR OF A MALE PLATYFISH PLACED WITH BOTH A FEMALE PLATYFISH AND A FEMALE
SWORDTAIL IN TERMS OF PER CENT OF OBSERVATION PERIODS

(For comparisons, see tables 14, 20, 24, 28, 29, 33, 37, 41, 42, and 44.)

	Behavior Directed Towards Platyfish Female		Behavior Directed Towards Swordtail Female
44 observation periods on 8 males previously experienced with platyfish only			
Copulation	0.0%		0.0%
Thrusting	22.8		15.9[a]
Swinging	—	6.5[b]	—
Sidling	18.2		9.1
Pecking	18.2		0.0
Backing	0.0		0.0
Quivering	2.8		0.0
Nibbling	0.0		0.0
Retiring	—		—
S-curving	—	7.3%[c]	—
Nipping	2.8		4.5
10 observation periods on 2 males paired for the previous 3 months with female swordtails only			
Copulation	0.0		0.0
Thrusting	0.0		30.0
Swinging	—	2.3[b]	—
Sidling	0.0		30.0
Pecking	0.0		40.0
Backing	0.0		0.0
Quivering	0.0		20.0
Nibbling	0.0		0.0
Retiring	—	0.0	—
S-curving	0.0		0.0
Nipping	0.0		0.0

[a] Four of these cases occurred when the female swordtail swam between the male and female platyfish while the male was courting the female platyfish.

[b] As swinging is independent of the female, it cannot be placed in either of the above categories. These figures represent the average number of gonopodial swings per observation.

[c] This behavior often occurred when the second female interrupted the courtship procedure. Hence it was not always possible to relate this behavior to a specific female.

No copulations took place under these circumstances either between the platyfish and their own females or with the swordtail females. Of the eight males with no previous interspecific mating experience, courtship activity with female swordtails was low. In six of the observations a male platyfish thrust his gonopodium at a female swordtail, but he displayed no other courtship activity except sidling, a behavior that precedes thrusts. During four observation periods the female swordtail swam between the courting platyfish pair. The male platy-fish then thrust at the passing female swordtail.

Concerning the two male platyfish that had lived for three months with female swordtails, one showed no sexual behavior except gonopodial swinging, and this was at the low frequency comparable to that exhibited commonly by isolated males. The other male definitely courted the female swordtail, and the frequency of appearance of the various sexual patterns was in the order of magnitude recorded for the pure platyfish. In table 43 the data represent the averages for the

TABLE 44

Behavior of a Male Swordtail Placed with Both a Female Platyfish and a Female
Swordtail in Terms of Per Cent of Observation Periods

(For comparisons, see tables 14, 20, 24, 28, 29, 33, 37, 41–43.)

	Behavior Directed Towards Platyfish Female		Behavior Directed Towards Swordtail Female
25 observation periods on 5 males previously experienced with both species of females			
Copulation	0.0%		8.0%
Thrusting	28.0		84.0
Swinging	—	1.4%[a]	—
Sidling	36.0		84.0
Pecking	0.0		0.0
Backing	4.0		40.0
Quivering	0.0		0.0
Nibbling	0.0		0.0
Retiring	—	0.0	—
S-curving	0.0		0.0
Nipping	48.0		16.0
20 observation periods on 7 males paired for the previous 3 months with female platyfish only			
Copulation	0.0		0.0
Thrusting	20.0		40.0
Swinging	—	3.0[a]	—
Sidling	20.0		40.0
Pecking	0.0		0.0
Backing	10.0		20.0
Quivering	0.0		0.0
Nibbling	0.0		0.0
Retiring	—	0.0	—
S-curving	0.0		0.0
Nipping	10.0		10.0

[a] As swinging is independent of the female, it cannot be placed in either of the above categories. These figures represent the average number of gonopodial swings per observation.

two males. Since one male showed no sexual activity, these scores when doubled represent the behavior level of the active male.

MALE SWORDTAIL, FEMALE PLATYFISH, AND FEMALE SWORDTAIL: Twelve male swordtails were tested during 45 observation periods. Five of the males had lived with females of both species, whereas the other seven swordtails had lived for the previous three months with only female platyfish.

Two intraspecific copulations were recorded. These involved males raised with members of their own species. One of the copulations resulted in insemination of the female. In this experimental situation the female platyfish did not interfere with the courtship activity of the male swordtail, and this contrasts with the reciprocal experiment described above, in which the female swordtail regularly interrupted courtship procedures of the male and female platyfish. This difference may be accounted for by the smaller size and subordinate position of the female platyfish. Swordtail males thrust at both species of females but thrust more often at their own females. Thrusts were recorded in 28 observation periods during which 125 thrusts were directed at female swordtails and 55 at female platyfish. Backing and sidling behavior of the swordtail male

was associated more frequently with the swordtail females than with the platyfish females, but, in contrast to this, swordtail males nipped more often at platyfish females than at females of their own species. In the case of the seven males that had experience with platyfish females only, the differential in the sexual reactions towards the two females was in the same direction but less pronounced as compared with the five males previously discussed. This low differential was no doubt due to the enforced association of these seven males with platyfish females. (See table 44.)

EXPERIMENTS ON SEXUAL ISOLATION

THE ISOLATION INDEX

A series of tests was conducted for the study of the mating preferences in "multiple choice" groupings over a longer period of time than observational methods would permit. A single male platyfish or swordtail was placed with three virgin female platyfish and three virgin female swordtails. Each male used was carefully examined, and each had a typical gonopodium.

The fishes were placed in 10-gallon aquaria (35.5 by 36.0 by 46.0 cm.) which contained a small amount of *Nitella*. After a varying number of days the females were checked for the presence of sperm, then they were returned to the aquarium with the male. The sperm testing procedure for the six females of the group was completed in less than 30 minutes. Most of these interspecies mating groups were kept intact for several months. The young born during the interval were closely examined for the possibility that some might be hybrids. A female that revealed a positive sperm smear for the first time was

TABLE 45

MATING SELECTION OF A MALE PLATYFISH GROUPED WITH THREE VIRGIN FEMALE PLATYFISH AND THREE VIRGIN FEMALE SWORDTAILS

(For comparisons, see tables 46 and 47.)

Group Number	Previous Experience of Male	Number of Females with Positive Sperm Smears										Total Number of Females Inseminated		Young Fish Recovered after 2 Months
		1 Day		5 Days		7 Days		12 Days		20 Days		P	X	
		P	X	P	X	P	X	P	X	P	X			
1	With P ♀ only[a]					3	0			3	0	3	0	13 pure P
2	With P ♀ only[a]	0	0	0	0			1	0			1	0	5 pure P
3	With P ♀ only[a]	0	0	0	0			0	0			0	0	—
4	With P ♀ only[a]	2	0	2	0			1	0			2	0	20 pure P
5	2 months with X ♀ only	2	0	3	0			3	0			3	0	18 pure P
6	2 months with X ♀ only	1	0	2	0			2	0			2	0	5 pure P
7[b]	2 months with X ♀ only	2	0	3	0			2	0			3	0	—
8[b]	No contact with mature ♀	3	0	3	0			0	0			3	0	4 pure P
9	No contact with mature ♀	1	0	2	0			2	0			2	0	14 pure P
10	No contact with mature ♀	0	0	0	0			0	0			0	0	

P, platyfish.
X, swordtail.

[a] Copulations resulting in insemination of females were observed in some of the males used in groups 1 to 4. The remaining males in groups 1 to 4 had been in aquaria with mature females for more than two months.
[b] One female swordtail replaced on the sixth day.

TABLE 46

MATING SELECTION OF A MALE SWORDTAIL GROUPED WITH THREE VIRGIN FEMALE
PLATYFISH AND THREE VIRGIN FEMALE SWORDTAILS

(For comparisons, see tables 45 and 47.)

Group Number	Previous Experience of Male	Number of Females with Positive Sperm Smears										Total Number of Females Inseminated		Young Fish Recovered After 2 Months
		1 Day		5 Days		7 Days		12 Days		20 Days				
		P	X	P	X	P	X	P	X	P	X	P	X	
1	With X ♀ only^a					0	1			0	3	0	3	1 pure X
2	With X ♀ only^a					0	3					0	3	b
3	With X ♀ only^a					0	1	0	1	0	1	0	1	
4	With X ♀ only^a	0	0	0	0			0	0			0	0	—
5	With X ♀ only^a	0	0	0	0			0	0			0	0	—
6	With X ♀ only^a	0	0	0	0			0	0			0	0	—
7	2 months with P ♀ only	0	1	0	1			0	1			0	1	8 pure X
8	2 months with P ♀ only	0	0	0	0			0	1			0	1	—
9	2 months with P ♀ only	0	0	0	0			0	1			0	1	12 pure X
10	No contact with mature ♀	0	1	0	1			0	0			0	1	—
11	No contact with mature ♀	0	2	0	3			0	2			0	3	—
12	No contact with mature ♀	0	1	0	1			0	1			0	1	1 pure X

P, platyfish
X, swordtail

^a Copulations resulting in insemination of females were observed in some of the males used in groups 1 to 4. The remaining males in groups 1 to 4 had been in aquaria with mature females for more than two months.

^b The male in this test died on about the seventh day, and all six females were dissected after 20 days. Three female swordtails had embryos; three female platyfish had unfertilized ova.

marked by having the first dorsal fin ray cut off. This procedure was necessary to ascertain how many females were inseminated over the entire test period, since the smear technique is not always reliable for more than seven days after a copulation (see p. 161). The dorsal fin scar was visible throughout the test period.

In the experiment, only fully mature females were used, and there was never any difficulty in obtaining sperm smears. Two of the female swordtails that died during the test period were replaced immediately with virgin females. Tables 45, 46, and 47 contain summaries of the results of 24 such mating groups in which 10 platyfish males, 12 swordtail males, and two F₁ males were tested. It can be concluded from these data that under our test conditions complete sexual isolation existed between the platy-

fish and swordtail, and hence the isolation index, according to the formula of Stalker (1942) or the combined indices of Bateman (1949),[1] both equal 1.00.

Tests (table 47) indicate that F_1 males show a slight tendency to mate with platyfish females, but owing to the scarcity of F_1 males at the time of the tests, the data are still insufficient for more definite conclusions to be drawn.

In a preliminary test, which proved to be an exception, an active male platyfish, the previous sexual experiences of which had been only with females of his own species, was used. Sperm smears of these pre-test females were positive. As in the other experiments, this male was placed with three swordtail females and three platyfish females. The swordtails were well matured (over one

[1] For description of isolation indices, see page 150.

TABLE 47

MATING SELECTION OF A FIRST-GENERATION HYBRID MALE GROUPED WITH THREE VIRGIN
FEMALE PLATYFISH AND THREE VIRGIN FEMALE SWORDTAILS

(For comparisons, see tables 45 and 46.)

Group Number	Previous Experience of Male	Number of Females with Positive Sperm Smears						Total Number of Females Inseminated		Young Fish Recovered After 2 Months
		7 Days		14 Days		21 Days				
		P	X	P	X	P	X	P	X	
1	Effective copulations with F₁ females	0	2	0	1	3	2	3	2	7 backcross P
2	Effective copulations with F₁ females	2	0	1	0	0	0	2	0	None

year old), but the three female platyfish were only about four months old. Although the latter appeared mature when selected for the experiment (i.e., the yellow ova could be seen through the translucent body wall), it was later discovered that their oviducts were not fully developed, as indicated by the force needed to insert the micropipette into the oviduct when the first smear sample was taken. Seven days from the start of the experiment, when all the females were checked, the striking effect of the apparent sexual immaturity of the female platyfish in this experiment was revealed. Smears from two of the female swordtails were positive; smears from the remaining swordtail and the three platyfish females were negative. The females were checked again 11 days later. A smear from one of the female platyfish now contained numerous sperm, and another from a swordtail had only a moderate number of sperm. The rest were negative. These last smear tests indicate that the male platyfish's last positive contacts had been made with a female platyfish and not with the swordtail females.

MATING SELECTION IN LARGE GROUPS

Table 48

All the experiments so far described gave evidence of almost complete sexual isolation in the small groupings tested. To investigate this problem further, the following experiment was designed to simulate more closely

the natural conditions under which the platyfish and swordtails live together. Four large aquaria having the following dimensions (in centimeters) were used:

	WIDTH	LENGTH	DEPTH
A	74	172	48
B	66	152	36
C	69	112	28
D	48	112	28

Gravel was placed on the bottom of all the tanks. Tanks A, B, and D were heavily planted with rooted aquatic plants: *Sagittaria*, *Vallisinaria*, hair grass, and *Nitella*, which formed an intricate maze in the body of the aquarium; duck weed and water fern covered some of the surface. Tank C was devoid of any vegetation.

Into each of these four tanks 46 fishes were introduced: 20 almost mature platyfish of both sexes, 20 almost mature swordtails of both sexes, three mature virgin platyfish females, and three mature virgin swordtail females. The mature virgin females were included in order that the first young male that matured would have available a choice of mature females of both species with which to mate.

Incidental observations of about 10- to 30-minute periods were made twice a week for the first four weeks. Copulations were never witnessed, but males were frequently observed courting and thrusting their gonopodia towards females of their own species even before their gonopodia had become fully

TABLE 48

INVENTORY OF FISHES FROM COMMUNITY TANKS CONTAINING PLATYFISH AND SWORDTAILS FOR A PERIOD OF EIGHT AND ONE-HALF MONTHS

Tanks	Platyfish			Swordtails			Hybrids
	Males	Females	Young[a]	Males	Females	Young[a]	
A	4	11	72	11	6	145	0
B	2	10	26	8	10	90	0
C	16	6	0	8	9	0	0
D	3	5	17	5	11	77	0

[a] Some of the smallest young were lost in the process of emptying these large tanks. Others were too small to be identified accurately and were set aside for two weeks after which proper identifications could be made. The numbers in this column represent the total young recovered, including those identified later.

differentiated. On one occasion a young male swordtail in tank B was observed thrusting his gonopodium at a young female platyfish.

At the end of three months, tanks A, B, and D had both platyfish and swordtail young, but no hybrids. Table 48 contains an inventory of all the fishes found at the end of eight and one-half months.

These results support the conclusions of field collectors, in that no hybrids are found in mixed populations of platyfish and swordtails (Gordon, 1947). Incidentally, although our previous experiments showed a lower copulatory frequency and a lower percentage of inseminations after copulation in the swordtail as compared to the platyfish, more swordtail young than platyfish young were found in these community tanks at the end of the test period. This could be interpreted as the results of greater prenatal and early postnatal mortality among platyfish, or simply to the fact that the larger-sized swordtail females produce larger broods than the smaller platyfish females.

DISCUSSION

SEXUAL BEHAVIOR

THREE STAGES of sexual activity may be recognized in the two xiphophorin fishes. These divisions are useful for analysis of the behavioral observations. While there are qualitative and quantitative differences in certain of the behavior patterns in the platyfish and swordtail, the stages are basically the same in the two species.

The first stage is marked by a steadily increasing frequency of gonopodial swinging and also by the characteristic and persistent manner in which the male swims after the female.

The second stage in the platyfish is characterized primarily by pecking, sidling, and thrusting. In the swordtail it is indicated by nibbling, backing, sidling, and thrusting. Also indicative of this stage is a further increase in the frequency of gonopodial swinging. Although these patterns of behavior represent an interaction of the male and female, the threshold of the second stage of sexual activity appears to depend largely on the male. For example, a highly excited male that pursues and sidles up to a female rarely fails to thrust his gonopodium towards her even though she may be non-receptive. Moreover, as seen from the tables, the male swings, sidles, pecks, quivers, nibbles, and backs up to a female more frequently at a time when his threshold for thrusting has been reached.

The third stage of sexual activity involves the act of copulation. Its threshold depends to a great extent on the receptivity of the female. The more receptive the female, the shorter the courtship period (stage 2) and the more likely that copulation will ensue. In several observation periods, some swordtail and F_1 hybrid males copulated in less than one minute after the female was presented to them, and occasionally the fish copulated almost immediately after the female was placed into the male's tank. In these instances, the only behavior recorded for the males before copulation was one or two thrusts of the gonopodia. Only thrusting increased in frequency with copulatory activity, and this was in proportion to the number of copulations. This is what might be expected, since thrusting seems to be the only indispensable act prior to copulation. On the other hand, when the female is only partially receptive or is non-receptive, the remaining items of precopulatory behavior of the male increase in frequency and duration.

It has often been suggested that courtship behavior raises the level of sexual excitability of both individuals and serves to hold the pair together through mutual stimulation to a point which may lead to a well-synchronized mating (Huxley, 1914; Marshall, 1936; Aronson, 1949). This seems to be borne out by the present data, particularly with reference to short and extended courtships. Prolonged, intense courtship (characteristic of stage 2) often results in copulatory failure. This may reflect poorly synchronized acts on the part of the female, of the male, or of both. On the other hand, short courtship periods, which are usually followed immediately by copulations, indicate that the newly introduced pair is well coordinated sexually, that is, both physiologically and psychologically. In this connection, highly receptive females and sexually excited males that have histories of multiple copulations showed practically no courtship behavior, except for a minimum of sidling and thrusting of the gonopodium.

In analyzing reproductive behavior in the oviparous mouthbreeding fish *Tilapia macrocephala*, Aronson (1949) considered it appropriate to group certain of the activities under the category of courtship, as distinct from a subsequent group of reproductive acts more closely associated with the actual spawning process. Evidences of an organic separation were considered. Also, a different adaptive significance for these two groups of behavior was postulated in that courtship is mostly concerned with pair formation and maintenance, while the latter activities have most to do with the immediate preparation for spawning, e.g., nest building, oviposition, and fertilization. Exact equivalence cannot be made, but the courtship group roughly represents stages 1 and 2 in the platyfish and swordtail which are also concerned primarily with pair formation and main-

tenance. Similarly, the second group in *Tilapia* is partially equivalent to stage 3 which is more intimately associated with the actual reproductive process. Although the observed behavior seems to form a continuum during any given testing period, it is strongly suspected that different organic processes come into play during the transition from one stage to another, and it is suggested that in physiological investigations of reproductive function, instead of considering sexual behavior as a whole, due consideration should be given to possible differences at the various stages.

Prior to the present investigation, it had been generally assumed that in poeciliid fishes the male's momentary gonopodial thrusts at the genital orifice of the female were the actual copulatory acts that facilitated insemination (Breder and Coates, 1935; Fraser-Brunner, 1947; Haskins and Haskins, 1949; Hervey and Hems, 1952). Only Stepanek (1928) suspected otherwise. Writing in a Czechoslovakian journal which did not come to our attention until long after the present experiments had been terminated, Stepanek discussed his observations on the guppy as follows: "First I maintain that the teeth at the end of the gonopodium—especially the protruding terminal tooth [terminal hook on ray three]—serve for the purpose of holding on. Although covered with a soft membrane, nevertheless it forms a protuberance on the surface of the organ so that it could hook on to the urogenital papilla.

"In support of this idea, I have observed twice in *Lebistes reticulatus*, once in *Xiphophorus helleri* and often in *Platypoecilus* [=*Xiphophorus*] *maculatus* that the end of the organ [gonopodium] is actually inserted, and contact of a few second's duration is possible. . . . At this time the spermatophores are ejaculated."

Our experiments fully confirm Stepanek's conclusions. The momentary thrusts of the gonopodium never result in the transfer of sperm to the female, whereas insemination often takes place during a single prolonged contact. This behavior, which we have designated as the copulatory act, is clearly distinguished from the momentary thrusts by duration of contact, a characteristic stance of the male and female, and the sharp break

which terminates the act.

We have observed the behavior of platyfish and swordtails in our stock tanks for many half-hour periods without ever once witnessing a copulation, although considerable courtship behavior was usually in evidence. It is likely that in home aquaria and under natural field conditions, poeciliid fishes do not copulate very frequently. It was only by the special techniques of isolation of the males, segregation of the females, bringing the females to the male's tank, and observation during the 10-minute period immediately following the introduction of the female that we were able to witness so many copulations and to standardize our observation.

In a study of the guppy, *Lebistes reticulatus* (Clark and Aronson, 1951), a similar distinction between momentary thrusts and prolonged copulations was revealed. It is well known that in stock tanks of guppies, thrusting behavior may be witnessed as an almost continuous activity. Clark and Aronson suspected that despite the high thrusting frequency in the guppy, copulations occurred very infrequently. To obtain a measure of this frequency, mature females that had been living in large stock tanks were selected at random, and genital smears were taken from them. On the basis of the results, they estimated that half of the females had not been inseminated for at least a week prior to the taking of the smears.

In our standard procedures for testing sexual behavior, the female was introduced into the male's tank. This is in accordance with the widespread practice among animal breeders of bringing the female to the residence of the male. In explanation, Beach (1947a) suggested that "The absence of sexual arousal [in the male] in a strange environment may be due to fear, but more frequently it appears that the male is merely distracted by non-sexual stimuli, a situation which occurs less commonly in the female." In the experiments conducted by Schlosberg, Duncan, and Daitch (1949) the male and female fish were placed in an aquarium that was a strange environment for both sexes. In this situation they reported an exploratory period of five to 10 minutes by the fishes before the initiation of any sexual activity.

In contrast to this, our technique induced a much more rapid onset of sexual behavior. We found that in the platyfish the median interval between the time of introduction of the female and first copulation was five minutes. In the swordtail, this median interval was only one minute (fig. 6).

In addition to the more rapid initiation of sexual behavior, our method of introducing the female to the male's tank induced a greater degree of sexual activity. Schlosberg, Duncan, and Daitch reported "lasting contacts" (copulations) in the swordtail in only 3 per cent of their 40-minute observation periods, as contrasted with 13.4 per cent in our 10-minute tests. In platyfish they report "lasting contacts" in 22 per cent to 33 per cent of their 40-minute observation series compared to 26.4 per cent to 42.0 per cent in our 10-minute tests. Finally, they report "rarely more than one lasting contact per [40 minute] session," whereas we observed as many as six copulations per 10-minute test, and Kamrin and Aronson (in preparation), using our techniques, observed up to 12 copulations per 10-minute test. Similarly, we report that gonopodial thrusts in the platyfish occur in 76 per cent of our observation periods; and in swordtails, 72 per cent. These are in contrast to 50 per cent and 55 per cent, respectively, obtained by Schlosberg and his co-workers.

Early in these studies it was noted that if a female nipped the male frequently, and if these nips were not returned by the male, the latter exhibited relatively little sex behavior. At the same period, however, the male's high incidence of gonopodial swinging indicated some degree of sexual arousal on his part. Several investigators have interpreted this nipping behavior of fishes in terms of social dominance. Thus Braddock (1949) indicated that in the platyfish, prior residence in an area confers on an individual a greater potential for dominance, especially during initial contact between individuals. A newcomer introduced into an established hierarchy was usually relegated to the omega position for a short period. Essentially the same phenomenon has been reported for the swordtail by Noble and Borne (1938), by Noble (1939), and, for immature green sunfish, Lepomis cyanellus, by Greenberg (1946,

1947). In our limited 10-minute observation periods, dominance-subordination relationships were not adequately evaluated, but the studies cited above suggest that in our experiments there was less likelihood that the newly introduced female would nip the resident male. This, too, may account in part for the relatively higher frequencies of sexual behavior observed in our tests in comparison with those of Schlosberg, Duncan, and Daitch.

In all our experiments involving homospecific pairs, young mature virgin females were used whenever possible as the older females are larger (sometimes almost one and one-third to one and one-half times the size of males). This was done because it has been shown in most studies of hierarchies, in fishes by Noble and Borne (1938), Noble (1939), Noble and Curtis (1939), and Braddock (1949), and in other animals that the larger size of an individual confers a positive advantage in establishing dominance.

Size difference appeared to be an important factor in our studies of the behavior of males given an interspecific or intraspecific choice of females (p. 196). In these studies, observations were made with three fish in the aquarium. When a male platyfish was presented with a female platyfish and a female swordtail, the female swordtail was always the largest of the three (pl. 5). The male platyfish usually started courting the female of his own species, but he was often interrupted when nipped by the female swordtail. The high frequency of nipping, an aggressive act exhibited by the swordtail female in these groups, may explain, in part, why copulation under these circumstances did not take place.

In the reciprocal situation, when a male swordtail was presented with females of the two species, the platyfish female was the smallest fish of the three. In this combination the platyfish did not interfere appreciably with the courtship between the male and female swordtails. This may account for the homospecific copulations in these latter experiments. This is particularly interesting because, in intraspecific matings, platyfish generally copulate more frequently.

In interspecific pairings of a single male and female (p. 193), the size factor did not seem

to be quite so important (although it still was evident). Copulations took place between the platyfish male and the larger swordtail female and not between the swordtail male and the smaller platyfish female. Possibly maleness (Noble and Borne, 1940) and prior residence outweighed the smaller size handicap

PLATYFISH OR SWORDTAIL	RAT[1]	GUINEA PIG[2]
Insemination	Ejaculation	Ejaculation
Copulation (no insemination)	Copulation	Copulation
Thrust	Palpation and pelvic thrust	Mounting
Sidling	Clasp without palpation	Nuzzling
Pecking and quivering, or backing and nibbling		Sniffing and nibbling
Swinging		Other behavior

of the male platyfish when paired with the female swordtail. When a platyfish male was sexually inactive and did not thrust at the swordtail female paired with him, she nipped frequently at him.

In most fishes fertilization is external, and under these circumstances comparisons with mammalian sexual behavior is limited. But in the viviparous species the general relationships and sequence of events are much closer. It is therefore of interest to make such comparisons and to note certain surprising similarities, despite the fact that viviparity in fishes and mammals must have developed quite independently; the genitalia are not homologous (e.g., gonopodium versus penis); and the peripheral and central nervous systems of teleosts differ markedly from those of mammals.

Some of these similarities are as follows:

1. LEVELS OF SEXUAL EXCITABILITY: In guinea pigs (Seward, 1940; Young and Grunt, 1951) and rats (Beach, 1944) the measure of sexual behavior forms a graded series which can be arranged from the lowest to the highest degree of sexual excitability. For example, in the rat, Beach (1944) recognizes the following stages: clasp without palpation; palpation with pelvic thrust; copulation; and ejaculation. Our previous discussion of "stages of sexual activity" (p. 201) represents a comparable interpretation. It was not possible in our studies to distinguish copulation from ejaculation by means of overt behavior, as can be done in the rat, but we do know that insemination results from only a cer-

tain percentage of copulations. This is similar to the situation reported for the golden hamster, in which Pauker (1948) was unable to distinguish copulations and ejaculations behaviorally. With this in mind, we present a graded list of behavior patterns for the platyfish and swordtail analogous to that for the rat and guinea pig.

2. RELATION OF COURTSHIP TO COPULATION: In the invertebrates, as well as the vertebrates, a series of behavioral actions generally precede copulation. These are interpreted as having stimulative value to the sexual partner and are usually termed courtship or precopulatory activity. Beach and Holz (1946) found that in rats, where penis function was interfered with by surgical removal of the os penis, copulatory frequency was markedly reduced, but precopulatory mounting with pelvic thrusts (incomplete copulation) was notably higher. The same result was obtained with rats castrated at birth and treated later with androgen. The penis of these individuals was notably underdeveloped. Copulations were infrequent, but mounting and pelvic thrusts approached the maximum that was physically possibly during a test period. Similar observations have been reported in the rabbit (Bacq, 1931) when ejaculation does not occur.

When copulation was prevented in the platyfish by surgical removal of the gonopodial tip, swinging, pecking, sidling, and thrusting behavior increased decidedly. The mean frequency of gonopodial thrusts, for example, was almost twice as high in the operated group as in the intact control fish.

3. ENVIRONMENTAL INHIBITIONS: Beach (1947a, 1951) noted that male mammals often fail to respond to receptive females encountered in new and strange environments.

[1] Data from Beach (1944).
[2] Data from Young and Grunt (1951).

He attributes the absence of sexual arousal to the disturbing effects of non-sexual stimuli. Females are much less susceptible to this form of inhibition, a difference that may be due to the relative independence of feminine behavior from cerebral functions (Beach, 1947a). As previously discussed (p. 152), male platyfish and swordtails are similarly distracted by strange situations, as contrasted with the females.

4. RELATION OF SEXUAL TO AGGRESSIVE BEHAVIOR: In mammals, as well as in other vertebrates, sexual behavior often transforms into aggressive actions, particularly when the pair is poorly matched physiologically. This same interrelationship was observed in platyfish and swordtails. Nipping, for example, was seen most frequently in tests that did not culminate in copulations. Frequently, in the swordtail, when a series of thrusts was not followed by a copulation, the male ceased thrusting and the remaining part of the test period was occupied largely by aggressive nipping. In the platyfish, sexual activity was often interspersed with nipping.

5. INTERVAL BEFORE FIRST COPULATION: In the study by Young and Grunt (1951) on sexual behavior in the male guinea pig, testing procedures were used that in a general way are similar to those used in the present study. Their data on the interval between the start of the observation and the first ejaculation in the guinea pig may therefore be compared with our data concerning time of first copulation in platyfish and swordtails, with the limitation, as noted above, that all first copulations in these fishes do not result in insemination. In the guinea pig the median interval was five minutes, as compared to five minutes in the platyfish and one minute in the swordtail. In figure 6, the distribution of these intervals in the platyfish, swordtail, and hybrids is compared with the data of Young and Grunt for the guinea pig. It may be seen that the order of magnitude and general trend are notably alike for fish and rodent.

There seems little doubt that further investigation would reveal other similarities, as suggested, for example, in the realm of bisexual behavior (Beach, 1947b; Schlosberg, Duncan, and Daitch, 1949), relation of territory to mating (Collias, 1944), sensory processes involved (Beach, 1951), and the like. Moreover, if the same testing techniques were applied to reptiles and birds, additional comparisons could surely be made, and these might even be extended to include some of the invertebrate groups as well. These findings demonstrate that certain features of sexual behavior have remained remarkably constant during vertebrate evolution despite very radical changes in structure, particularly of the nervous system. These stable elements stand in contrast to the evolving features of sexual behavior, especially in relation to progressive sensory and motor encephalization and altered hormonal relationships. These latter changes as discussed by Beach (1947a) result in "increasing variability and modifiability, both of the types of stimuli adequate to elicit sexual activity and of the overt forms of behavior by which sexual excitement may be expressed," and in freeing in some measure the "more primitive sexual mechanisms from strict control by gonadal hormones."

GENETICS OF MATING BEHAVIOR PATTERNS

A study of the mating behavior patterns of the male platyfish and swordtails and those of their F_1, F_2, and two types of backcross generation hybrids reveals some influence of heredity, but the genetic data are not of a kind that allow a precise analysis in simple Mendelian terms. In the first place, the individual behavioral rating of the two parents (P_1) was not known. Only the average ratings for the species were determined on the basis of samples of males. Similarly, the combined average rating of all available first generation platyfish-swordtail hybrid males was determined instead of the average rating for only those males that produced the particular F_2 hybrids that were tested. This was necessary because at the time the behavior studies were conducted, some of the parental individuals were no longer available.

For the usual type of genetic analysis of morphological characters the above method

of gathering data is undesirable, since the species ratings of the actual parents and their offspring are essential. Moreover, in the study of mating behavior, a single rating obtained from a particular male parent may not truly represent either the individual or the group. Therefore the analysis was based on mass groups rather than individuals. This method has been previously applied to the genetic studies of species differences in the morphology of the male genitalia. The results were interpreted by Gordon and Rosen (1951) on the assumption that the inheritance of the various structural elements were controlled by polygenes (multiple factors), the number of which could not be closely approximated. Also, in a behavioral study the techniques generally applicable to morphological analyses (pigmentation, for example) cannot be used, as a particular adult behavior pattern is likely to be based on a complex of genetic and experiential factors the precise nature of which remains to be determined.

The inherent difficulties in genetic studies of behavior are due to the extreme variability and the less clearly defined nature of the expressed characteristics. This is complicated by the difficulty of evaluating the precise rating of the male parent's behavior pattern and the even greater task of knowing anything of these patterns in the undemonstrative female parent.

These difficulties are appreciated, for example, by geneticists who have attempted to study the genetic basis for broodiness in the fowl. In birds, of course, the female's broodiness behavior may be measured to only a limited degree because it, too, is extremely variable. Hutt (1949) described the intricate interplay of intrinsic and extrinsic factors (particularly the hormonal ones) that influence broodiness. The genotype of the males for this trait, however, can be estimated only from the rating of broodiness in their sisters and daughters. Hutt (1949) in his thorough review of the genetics of the fowl pointed out that, since different breeds of fowl differ in the degree of broodiness exhibited, it is likely that the trait is hereditary. Broodiness which appeared in 91 per cent of a flock of Rhode Island Reds was reduced by selection over a period of five years

to 19 per cent. The exact genetic basis is still unknown despite the fact that careful studies on this subject were begun more than 30 years ago by Goodale, Sanborn, and White (1920).

Sexual behavior, particularly in sub-mammalian forms, is usually considered to be "instinctive" or "innate." The implications are that such behavior is gene controlled and that its characteristics are not influenced by factors of learning. However, even the most ardent supporters of this concept recognize some influences of learning or conditioning on instinctive actions (Lorenz, 1935: instinct-training interlacement; Tinbergen, 1948, 1950: conditioning). On the other hand, there is a growing trend, particularly among certain investigators in this country, to abandon as artificial the dichotomy between nature and nurture and to stress the point of view of interaction between environmental, experiential, and genetic factors in the ontogeny of the so-called instinctive or innate responses (Schneirla, 1949, 1952; Riess, 1950; Beach, 1951; Ginsberg, 1952; Lehrman, in press). Beach (1951) writes, "We have seen that sexual behavior develops in various ways in different animals and may be controlled by different external and internal correlates in males and females of the same species. . . . This kind of evidence contradicts categorization of mating and maternal behavior as unlearned or instinctive."

To evaluate the genetic influences one might eliminate all experiential factors, as Carmichael (1926) and others have attempted in studies of locomotory reflexes in larval amphibians. This, however, is often difficult if not impossible. Thus Riess (1950) notes that the commonly used procedure (e.g., Tinbergen, 1951, p. 52) of raising animals from weaning (and sometimes from before parturition) in isolated cages does not prevent the animal from having experiences with itself or with the "inanimate realia of the environment." More often the investigator strives to keep the environment as constant as possible among the individuals of the genetic strains being tested. This procedure has many pitfalls. Individuals may have very different experiences in the same environment (tank or cage), particularly when raised in groups as is so often the case.

It is with the above consideration and limitations as background that the following genetic analysis is undertaken. We believe that our evidence indicates genetic bases for some features of male sexual behavior in xiphophorin fishes, but we cannot at this time offer any specific mode of inheritance to account for the data obtained thus far. It certainly cannot be expressed in simple Mendelian terms. We must also anticipate that an array of environmental and experiential factors, of which we have only a very general and incomplete knowledge, probably influenced the genetic response.

ANALYSIS OF THE GENETIC DATA

Some of the precopulatory activities of xiphophorin fishes are characteristic of the male platyfish but are absent in the swordtail. Other traits are found in the reverse order. It might be expected that their frequency in the F_1, F_2, and the two types of backcross male hybrids would indicate the general manner of their inheritance (table 37).

Backing and nibbling, characteristic of most swordtails, were not seen in the platyfish during our test periods, although it cannot be said with certainty that they are completely absent in the platyfish. Backing was recorded for the F_1 hybrids in 9.7 per cent of the observations, which is far below the midpoint between the swordtail (32.8%) and the platyfish (0.0%). This trait was found at a slightly higher level in the F_2 hybrids (11.4%). In the platyfish backcross hybrids it fell to 4.6 per cent, and in the swordtail backcross hybrids it rose to its highest recorded level, 49.0 per cent, which is higher than in the original P_1 swordtail. We know of no morphological character in the xiphophorin fish which has this pattern of inheritance. A possible explanation is presented below after somewhat similar patterns (or lack of patterns) of inheritance are reviewed.

Nibbling is a swordtail trait that appeared in 44.3 per cent of the observations. In the F_1 hybrid male its rate was zero, which is as it was in the platyfish parent. Surprisingly, this trait reappeared in a few of the F_2 platyfish-swordtail hybrids (4.5%), was much less frequent in the platyfish backcross hybrids (0.9%), and attained about the same frequency in the swordtail backcross hybrids (4.9%) as in those of the F_2 hybrids.

Pecking and retiring were seen frequently in the platyfish but not in the swordtails. Pecking was recorded at the frequency of 58.0 per cent in the platyfish and at zero for the swordtail and zero in their F_1 hybrids. This may indicate that pecking may be attributed to the action of recessive genes. If this is so, then we should expect a number of double recessives to reappear in the F_2 hybrids, and this was found perhaps in that these hybrids had a pecking frequency of 17.8 per cent. The trait was found in some (15.7%) of the platyfish backcross hybrids (PX-P) but not at all in any of the swordtail backcross hybrids (X-PX). Retiring as a trait has approximately the same general pattern of inheritance as pecking, although the dominant suppressing influence of the swordtail behavior is not so absolute as before among their F_1 platyfish-swordtail hybrids.

In a broad comparison of the frequencies of backing and nibbling on one hand with pecking and retiring on the other, an apparent pattern of inheritance is formed, particularly in the behavior of the various backcross hybrids. Traits such as backing and nibbling, more or less restricted to the swordtail, are found more frequently in swordtail backcross hybrids. Traits such as pecking and retiring, more or less restricted to the platyfish, are found more frequently in the platyfish backcross hybrids. Some possible evidence of association of these behavior patterns to known genes for color patterns are presented and discussed at another point.

The frequencies of gonopodial swinging are approximately similar in the platyfish (92.2%) and swordtails (78.0%) and their hybrids. Except for an unexpected falling off in the frequency of this behavior among the F_1 hybrids (34.4%), all the other kinds of hybrids, the F_2 and the two backcross hybrids (PX-P and X-PX), are about the same (78.2%, 79.0%, 72.5%).

The frequencies of gonopodial thrusts, too, are approximately similar in the platyfish

(77.9%) and the swordtail (72.0%) and in their various hybrids: 46.2 per cent in the F_1 hybrids, 66.3 per cent in the F_2 hybrids, 48.1 per cent in the platyfish backcross hybrids (PX-P), and 73.5 per cent in the swordtail backcross hybrids (X-PX).

The important measurements directly associated with copulation and insemination in the platyfish, swordtails, and their various hybrids are evaluated as follows:

1. The interval between the start of the observation and the first copulation is much longer in the platyfish (median = 5 minutes) than in the swordtail (median = 1 minute). They are also short in the F_1 hybrids (median = 2 minutes), but in the F_2 hybrids they vary in duration from the shortest to the longest periods.

2. The copulatory activities (per cent of observations with copulations recorded) are higher in the platyfish (26.7% to 42.0%) than in the swordtail (13.4%). The frequency is slightly higher in the F_1 hybrids (29.0%), but lowest of all in the F_2 (8.4%) and in the two backcross generations: to platyfish (7.4%), to swordtail (4.9%). (See table 37.)

3. The maximum number of copulations in a test period is higher in the platyfish (six) than in the swordtail (two). It is intermediate in the F_1 hybrids (five). In the F_2 hybrids the maximum number is two; in the platyfish backcross hybrids, two; in swordtail backcross hybrids, three. (See table 37.)

4. The duration of copulation in the platyfish is shorter (mean = 1.36 seconds) than in the swordtail (mean = 2.39 seconds). This difference when evaluated statistically seems to be significant.

In the F_2 the average duration of copulation was very low (1.1 seconds) but here we were no longer dealing with a homogeneous group. Some males consistently copulated briefly, others coupulated for a much longer time, and still others fell into one or more intermediate groups (tables 38 and 39).

5. The percentage of females inseminated during a test period following one or more copulations (tables 8, 16, 21, 25, and 30) is higher in the playfish (86.0%) than in the swordtail (39.4%); it is intermediate in the F_1 hybrids (64.3%), low in the F_2 (23.5%), and lowest in backcross generation hybrids (9.3% in PX-P, 0.0% in X-PX). In spite of the

extremely low frequencies of successful insemination among the two groups of backcross hybrids, PX-P and X-PX, their precopulatory activities of swinging and thrusting were as high as those of the parental generation, indicating high levels of sexual arousal.

In *Drosophila*, Miller (1950) found a striking difference in the average duration of copulation in closely related species: *D. affinis*, 1.3 minutes, and *D. algonquin*, 5.7 minutes. He noted no other differences in their courtship or copulatory behavior. Apparently this and other undetected traits were sufficient to bar hybridization under natural conditions. Stalker (1942) found that *D. virilis* had an average duration of copulation of 61 seconds while its related species *D. americana* took 56 seconds on the average. Moreover, Stalker discovered that there was no correlation between the length of a copulation and a successful insemination; some of the longest copulations produced no insemination. The male *virilis* was more successful in mating with a female *americana* and in retaining its copulatory position longer than in the reciprocal heterospecific association. Differences in the duration of copulation were also noted by Spieth (1947, 1951) in the *willistoni* and *virilis* groups of *Drosophila* species. In our study it was not possible to correlate duration of individual copulations with insemination, because of the large number of test periods in which multiple copulations took place. We show (table 8) that in the platyfish the probability of insemination increases with the number of copulations per test period. However, if we consider the sum of copulatory durations per test period, no correlation with insemination could be demonstrated. For example, on one occasion, platyfish male No. 6 copulated twice with a total duration of 3.4 seconds (1.8 seconds and 1.6 seconds) without inseminating the female, although this male was perfectly fertile, as shown by previous and subsequent tests of shorter total copulatory duration. At the opposite extreme, platyfish male No. 11 successfully inseminated a female during a single copulation lasting only 0.8 second.[1]

The above data confirm preliminary indi-

[1] The mating records of these two males appear in table 5.

cations (Clark, Aronson, and Gordon, 1948) that in quantitative terms copulatory behavior in F_1 hybrids is either intermediate or more like that of the swordtail. This is contrary to the conclusion of Gerschler (1914) who reported that the behavior of the F_1 hybrid resembles more closely that of the platyfish. It may be that Gerschler puts much weight on the circumstance that he never observed in his F_1 males the exaggerated backing behavior so characteristic of the swordtail. In our studies exaggerated backing was observed occasionally in F_1 males (9.7%; see table 24). Moreover, the well-defined pecking behavior of the platyfish was completely absent in the F_1 hybrid (figs. 8, 9).

REPRODUCTIVE ISOLATING MECHANISMS

Although, as previously stated, the basic patterns of sexual behavior in the platyfish and swordtail are essentially similar, there are a number of small but notable differences between them. The frequency of copulations and the per cent of inseminations resulting from copulation are significantly higher in the platyfish. On the other hand, copulations occur sooner and last longer in swordtails under our observational methods. Thus, the median interval between the start of the test, and the first copulation was five minutes in the platyfish and only one minute in the swordtail (fig. 6). Similarly, the mean duration of copulation was 1.36 seconds for the platyfish and 2.39 seconds in the swordtail. As noted above, Stalker (1942), Spieth (1947, 1951, 1952), and Miller (1950) also recorded differences in duration of copulation in closely related *Drosophila* groups.

Our study has also revealed significant qualitative and quantitative differences in several of the courtship patterns in the two species. Pecking and retiring appear only in the platyfish, whereas nibbling and exaggerated backing were seen only in the swordtail. The appearance of sidling is rather different in the two species. Swordtails rarely quiver. S-curving is infrequent in the swordtail, and, when it is observed, the curve is much less pronounced.

The experiment in which parts of the gonopodium were amputated revealed that the tip that contains the hook and serrae (and the claw in the swordtail) is essential for copulation. Gordon and Rosen (1951) have studied the morphology of the gonopodial tip in the platyfish and swordtail and have indicated that the swordtail has a large hook on the adaxial margin of its third anal ray, while the homologous structure in the platyfish is con-siderably smaller (figs. 3, 4). This difference could conceivably have a bearing on the difference in the duration of copulation in the two species. The large hook of the swordtail may act as an important element in the holdfast mechanism during copulation, although our preliminary experiments (see p. 166) showed it is not absolutely essential. In the swordtail, the holdfast mechanism is further enhanced in effectiveness by a terminal claw-like structure on the fifth ray.

There is also a noticeable difference in the genitalia of the females (fig. 10). In the swordtail female the genital opening is proportionally .wider and farther from the anus than in the platyfish female.

It is clear from this study and from previous investigations (Gordon, 1947; Schlosberg, Duncan, and Daitch, 1949) that under special conditions of captivity none of the behavioral and morphological differences considered above are sufficient to prevent species hybridization. However, even under the optimum of these laboratory conditions, interspecies fecundity is reduced markedly. For example, the percentage of observation periods in which one or more copulations were recorded can be listed as follows:

Platyfish male with platyfish female	26.7%–42.0%
Swordtail male with swordtail female	13.4
Platyfish male with swordtail female	11.6
Swordtail male with platyfish female	0.0

The above data do not necessarily prove that swordtails do not mate successfully with platyfish females, for matings of this kind have been obtained regularly in the genetic studies by Gordon (1948). They do show that
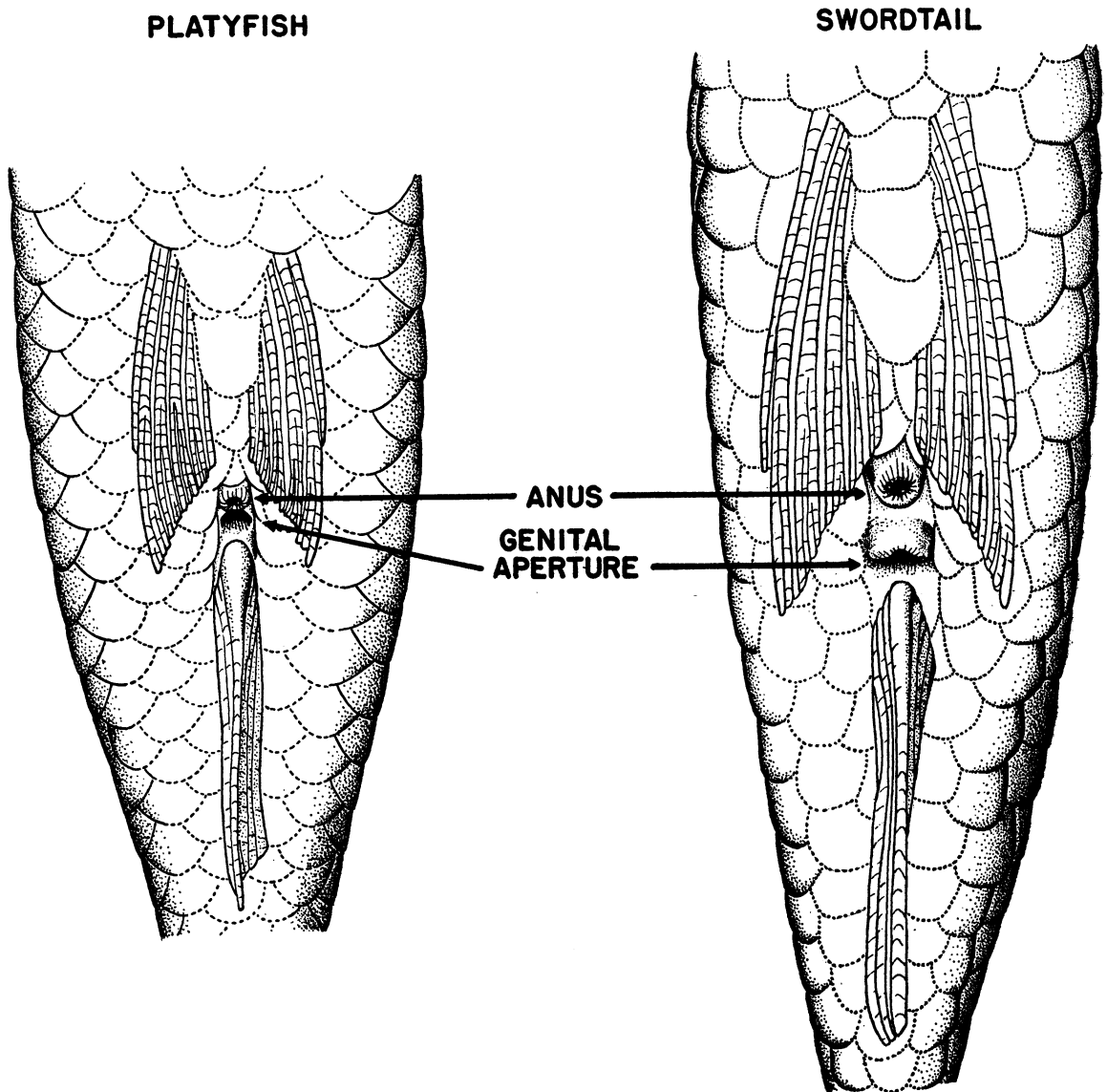
PLATYFISH   SWORDTAIL

FIG. 10. Relation of anal and genital apertures in female platyfish and swordtails. ×10.

more time is required for their association before interspecies copulations are obtained.

Our data (table 10) also demonstrate that in platyfish a succession of copulations is more likely to result in insemination than a single copulation. The same is true for the F_1 and F_2 hybrids, and a similar relationship may very well exist in heterospecific matings. This might be a further impediment to hybridization since multiple cross copulations are so infrequent even in the most appropriate situations. Rendel (1951) made the inter-

esting observation in *Drosophila melanogaster* that "double matings" (i.e., a second copulation immediately succeeding the first) are more frequent with ebony than vestigial females. The per cent of copulations resulting in insemination was 86.0 per cent in the platyfish and 39.4 per cent in the swordtail. It was only 18.2 per cent in the copulations between the platyfish male and the swordtail female; in addition, the two inseminated female swordtails did not produce embryos. Observational tests on males given a choice of fe-

males of both species show that intraspecific courtship is a frequent event, while thrusting at the heterospecific female occurs only occasionally. Insemination between interspecific fishes takes place very infrequently when a choice of females is offered.[1]

It appears that the effective barrier to hybridization between the platyfish and swordtail is not a single factor or even a few factors but is rather the summation of a considerable array of partially isolating influences. Some of these partially isolating elements have been clearly revealed in the present and previous (Gordon, 1947) studies. Others are merely suggested by our observations and results, so that their actual evaluation must await further experimentation.

Several authors have listed the principal isolating mechanisms between species, and these have been classified in a number of different ways. For our purposes the over-all arrangement presented by Schmalhausen (1949) is most suitable and is used in the synopsis and discussion that follow:

Ecological (geographic) isolation
    Partial segregation of swordtails in the swift headwaters and platyfish in the more slowly flowing lowland streams
Physiological isolation
    Differences in sensory apparatus
    Differences in thresholds for sexual responses
    Emotional differences
    Differences in sexual behavior
    Functional morphological differences
    Differing responses due to individual experiences
Gametic isolation
    Sperm less viable in oviduct of heterospecific female
    Competition between homospecific and heterospecific sperm
Genetic isolation
    Hybrid inferiority
    Partial hybrid sterility

ECOLOGICAL ISOLATION: Thorpe (1940) and Dobzhansky (1951) made a point of separating geographical (i.e., spatial) isolation from the remaining types of isolation which they group together under the heading "physiological" or "reproductive," because geographical isolation is independent of any

genetic differences between populations, while the several items of reproductive isolation are necessarily genetic. In the platyfish and swordtail, physiological (and therefore genetic) influences must be involved in segregating the two species in the lower or upper reaches of the rivers. Hence for these fishes as for many other species the distinction suggested by Thorpe and Dobzhansky cannot be made.

As previously noted, the ecological separation of platyfish and swordtails is incomplete. In about one-quarter of the collecting stations for platyfish and swordtails, both were taken, often in the same haul. In two stations they were taken in nearly equal numbers. In addition, Gordon (1947) observed that in the rainy season swordtails living in the headwaters are likely to be swept downstream into platyfish populations.

PHYSIOLOGICAL ISOLATION: Differences in the sensory apparatus of the platyfish and swordtail are indicated by the studies of Crozier, Wolf, and Zerrahn-Wolf (1937) and Crozier and Wolf (1939). They have found that the curves representing the relationship between flicker frequency (F) and mean critical illumination (I_m) for response to a moving stripe pattern exhibit marked differences in both form and proportions in the two species. This is interpreted as having a genetic basis, reflected in the physiological activity of the retinal elements and central nervous system.

Proper evaluation of threshold differences for the various sexual responses requires standardized stimulus values. This was not attempted in the present study. However, certain of our results could be explained as threshold differences, as, for example, the short interval before the first copulation in the swordtail compared with the much longer interval in the platyfish. In the swordtail, the average number of thrusts per observation period was fewer for two copulations than for one (p. 176). The reverse was true for the platyfish. This, too, might be a threshold phenomenon.

Our qualitative observations showed that swordtails are much more excitable than platyfish, and this is reflected in their general behavior. As an example, swordtails are more easily disturbed and prone to jump out of the

[1] Recent experiments of Clark (MS) give evidence that when a choice of mates is offered, cross inseminations do take place, but very infrequently

aquaria. Platyfish rarely do so. Also to be considered in this category is aggressive behavior (dominance-subordination relationships) particularly in relation to the size difference between the two species. When a male platyfish was presented with a female platyfish and a female swordtail, the latter nipped the male and courtship was continually interrupted. On the other hand, when a male swordtail was presented with a female of each species, the platyfish female being smaller and less aggressive, courtship activity proceeded more smoothly. Noble and Borne (1938) described a straight line pecking order as characteristic of the swordtail, whereas Braddock (1945) states that in the platyfish incomplete hierarchies and triangular situations are also common. Cole and Hollander (1950) noted the influence of size and aggressiveness in their studies on hybridization between the pigeon, *Columba livia*, and the domestic ring dove, *Streptopelia risoria*. According to these investigators, cross matings can be obtained when male pigeons are placed with female ring doves in "no choice" situations. However, large or pugnacious pigeons cannot be used. On the other hand, it is difficult to mate the smaller male ring dove with the larger female pigeon. Not only is size a factor, but a successful mating requires a more aggressive male.

Differences in sexual behavior between the platyfish and swordtail are discussed above. These include qualitative and quantitative differences in courtship, precopulatory activity, duration, time, and frequency of copulation.

Morphological characteristics may be considered here in so far as they reflect physiological differences between the two species. Gordon and Rosen (1951), in a genetic analysis of the differences between the platyfish and swordtail with regard to the genitalia of the males, revealed that various gonopodial structures were inherited in accordance with the multiple factors hypothesis. They summarized some of the other differences that are found between the two species, indicating that the fishes diverge significantly in the average number of dorsal fin rays, in the average number of scales in the lateral line, in the extension of the lower caudal fin rays (the platyfish has no "sword"), and in the structure of many skeletal elements associated with the gonopodial suspensoria, that is, gonapopheses and gonactinosts (Gordon and Benzer, 1945). In addition, the two species differ significantly in body size and form, coloration, pigmentation, and in the details of their embryonic development (embryonic rate of growth, rate of caudal fin development, and the time of initial pigment development; Tavolga, 1949).

Just how the physiological and correlated morphological differences considered above prevent interbreeding in mixed populations cannot be stated with certainty. It seems, however, that these items contribute to the relative effectiveness of the mating situation by influencing the interactions between the males and females and thereby building up systems of attractions and repulsions. This is the area that many writers, thinking in terms of "species recognition," refer to as the "psychological barrier." Mayr (1946, 1948) discusses the weakness of this concept, noting in particular that the term "recognition" implies the conscious ability to make judgments, for which there is no evidence in *Drosophila* and, we add, in most other animals.

In this connection, the factor of past experience must also be considered. Haskins and Haskins (1949) investigated this problem in three sympatric species of poeciliid fishes, *Lebistes reticulatus*, *Micropoecilia parae*, and *Poecilia vivipara*. Reproductive isolation between these three species is complete in natural situations, but hybrids have been obtained in the laboratory. *Lebistes* males taken directly from a *Lebistes* stock tank were placed with three females, one from each of the three species concerned. The first hundred gonopodial contacts (i.e., thrusts) were recorded. One week later a second observation was made, and 100 more gonopodial contacts were recorded. During the first observation an average of 35 per cent of the contacts were the *Lebistes* females, 59 per cent with *Micropoecilia* and 7 per cent with *Poecilia* females. One week later the average score read: *Lebistes*, 95 per cent; *Micropoecilia*, 5 per cent; and *Poecilia*, 0 per cent. Haskins and Haskins conclude that this discrimination is based essentially on the behavior of the male and that the change in behavior on the part of the males demonstrated by the above experi-

ment gives some evidence of being a "learned" reaction. Unfortunately this important study was completed before Clark and Aronson (1951) discovered that the gonopodial contacts (thrusts) counted by Haskins and Haskins never result in insemination and that actual copulations with insemination occur rather infrequently. In view of our findings in the present investigation, that interspecific thrusting occurs occasionally while interspecific insemination is an extremely rare event in multiple choice situations, it would be highly desirable to repeat the experiments reported by Haskins and Haskins, counting copulations with thrusts and checking periodically for insemination with the smear technique since developed.

Noble and Vogt (1935) studied sexual and species discrimination in the redwing blackbird (*Agelaius phoeniceus phoeniceus*). Old males, presumably sexually experienced, were distinguished by their second nuptial plumage and bright red epaulets. Young mature males, presumably inexperienced sexually, were recognized by their dappled first-year nuptial plumage and yellow epaulets. When presented simultaneously with dead mounted specimens of female redwing blackbirds and mounted specimens of several other species of birds, the older males copulated almost exclusively with the red-wing mounts, while the young males copulated with mounts of almost every species that was presented to them. Noble and Vogt attribute the rather accurate discrimination of the older birds to the influence of previous experience. Other examples concerning the relation of early experience to species discrimination in birds have been summarized by Cushing (1941).

Our own observations on the importance of previous experience are limited. When male platyfish were presented with a choice of two females, one of each species, courtship activity was directed mostly to the platyfish female. In contrast to this, a male platyfish raised in a stock tank of swordtails, when tested in the above situation, courted the swordtail female exclusively. But no copulations occurred in either case. In the reciprocal experiment of testing swordtail males with females of both species, much of the courtship was directed towards the swordtail female and two copulations occurred.

But among those swordtail males raised in stock tanks of platyfish, much less attention was directed to the swordtail female. In mixed populations in natural situations it is most probable that the previous experience of both the male and female plays an important role in determining the direction of mating activities.

GAMETIC ISOLATION: Although not actually demonstrated, this factor is strongly indicated by indirect evidence. In two cases of cross insemination (female swordtail inseminated by male platyfish) no broods or embryos were obtained after 18 days. In the general experience of Gordon, it often takes several months to obtain hybrid broods in "no choice" situations, and this is attributed in part to reduced viability of the sperm in the oviduct of the other species.

Also of importance in relation to gametic isolation is the fact that from a single insemination, or single series of inseminations, a brood may be obtained every month for six or more months. Under natural conditions females once mature are not likely to remain virgin very long. Therefore, in a chance interspecies copulation and insemination that may occasionally occur, the probability is high that the female so inseminated was already carrying an adequate complement of homospecific sperm. This then leads to the question of competition between homospecific and heterospecific sperm. Preliminary investigation of this problem has been undertaken by Clark (MS) using a newly developed technique of artificial insemination (Clark, 1950a). When a 1:2 mixture of homospecific and heterospecific sperm was introduced into virgin female platyfish, the resulting broods rarely contained any hybrids in spite of the greater abundance of potential hybrid-producing sperm. This finding can be taken only as a general indication, since the experiment should be repeated with non-virgins. Also, the relative amounts and the relative ages of the sperm may have important bearings on the problem. Mayr (1948) notes that the amount of sperm transferred may contribute to reproductive isolation, since in females of *Drosophilia pseudoobscura* and *Drosophila persimilis* the ventral sperm receptacles seem less filled after interspecific mating than after intraspecific mating. This

observation is correlated with the number of offspring produced.

GENETIC ISOLATION: The investigations of Tavolga (1949) gave no indication of lowered fecundity due to embryonic abnormalities when swordtail females were fertilized by platyfish males. The fecundity of the reciprocal cross was not examined, but there is some suggestion that this results in a slightly higher frequency of abnormal embryos. On the other hand, there is a decided increase in embryonic anomalies when F_1 females are mated to either F_1 males or to pure species males. This is particularly so when the F_1 females carry the gene Sd (spot on dorsal fin). Among five F_1 females carrying the Sd gene and mated to platyfish males, the average frequency of anomalous embryos per embryonic brood was 82 per cent (Tavolga, 1949).

When platyfish carrying the sex-linked dominant alleles (Sp, macromelanophore spotting on sides; Sd, spotting on dorsal fin; Sb, spotted belly; N, black lateral band; and Sr, stripes on sides) are mated with swordtails, the resulting F_1 hybrids develop melanosis, and some of them later show melanomas. These are most intense and destructive when the Sp, Sb, and N genes are involved (Gordon, 1948). Twenty-one per cent of the platyfish collected carry one of these genes (Gordon, 1934, 1949). However, the full effect of the melanomas is expressed later in life so that many melanotic F_1 hybrids reach reproductive maturity. When the melanotic F_1 hybrids are backcrossed to swordtails, the melanomas are intensified and are even expressed during embryonic stages (Gordon and Smith, 1938).

The genetic mechanisms for sex determination vary in the two species, for in the platyfish it is quite stable (Gordon, 1937, 1947, 1951c, 1952), whereas in the swordtail it is labile (Essenberg, 1923; Kosswig, 1939; Witschi, 1942, Regnier, 1938). With respect to their germ cell development, they are quite different, according to Wolf (1931). Male and female gonads are recognizable histologically in the postembryonic 6-mm. platyfish. In the young swordtails, however, the gonads of all appear to have ovarian elements only (Friess, 1933; Regnier, 1938); the swordtail is regarded as a protogynous species.

The chromosome numbers in platyfish and swordtails are the same (Friedman and Gordon, 1934), but there are many genic and probably chromosomal differences between them (Gordon, 1948). An outstanding example is the presence of many clear-cut, sex-linked genes in the platyfish and their absence in the swordtail. Incompatibilities of the sex chromosomes are discussed by Gordon and Rosen (1951), and they point out that in certain crosses it is rather pronounced, so that it would be incorrect to state without qualification that the F_1 hybrids are always fertile. Sterility of the F_2 generation is very pronounced, and in this connection Gordon (unpublished) has not been able as yet to maintain these hybrids by brother and sister matings beyond the F_3 generation.

Under normal conditions in the natural environment of these xiphophorin fishes, the first two isolating mechanisms, namely, the ecological and physiological, surely reduce the probability of hybridization to such an extent that isolation is almost complete. However, during the dry season the rivers become isolated pools (Gordon, 1940a), and some of these may not be much larger than our largest laboratory aquaria. In this situation, it is entirely conceivable that in exceptional circumstances a number of males of one species could be found in a pool with females, mostly of the other species. In these hypothetical situations the number of platyfish is likely to be greater than the number of swordtails, because, in the particular regions where isolated pools are formed, mostly platyfish are found. The ecological basis for this is stated above (p. 211). Although predation is high under these circumstances, and many pools dry up completely, still an occasional cross-copulation might occur, and in a small percentage of these insemination would be effected. It is here that the third factor, namely, gametic isolation, enters into the picture. The viability of sperm in the oviduct of the heterospecific female might be reduced, and, even more important, heterospecific sperm might not be able to compete favorably with homospecific sperm. It is only in that very rare event when the first three barriers are overcome that the fourth barrier, the genetic, would be effective by chromosomal incompatibility and

hybrid inviability. Hybrid sterility, the final barrier to gene interchange, is a reserve that is rarely or never invoked, since the data obtained from collections indicate that hybrids do not actually exist in nature. To summarize, there is no single item concerning these two fishes, ecological, physiological, morphological, or genetic, which prevents hybridization. The barrier to gene interchange is the sum of a number of partial barriers, each of which reduces to some extent the probability of hybridization to a point where for practical purposes we may say that it is non-existent in the Mexican and Central American rivers where these sympatric species are found.

As noted in the Introduction, Crew (1940), Dobzhansky (1941a), Hogben (1940), Huxley (1942), and Muller (1942) state that the destructive melanomas appearing in the platyfish-swordtail hybrids may serve as an isolating mechanism. It is obvious from the above discussion that these genetically induced tumors actually play an insignificant role in the prevention of gene interchange. At best they may be regarded as a potential barrier.

One of the early ideas concerning reproductive isolation was the incompatibility of the external genitalia of related species, that is, mechanical isolation. The striking complexity and variability of the copulatory apparatus in spiders have often been cited as an example of this "lock and key" type of isolation (e.g., Petrunkevitch, 1925). This view has been questioned by Berland (1932, p. 280), Petrunkevitch (1942, p. 177), Kaston (1948, p. 16), and recently by Gering (1953) who find that in interspecific pairings the male either avoids the female, or courtship behavior (which in intraspecific pairs precedes copulation in many species) is interrupted at an early stage (Kaston, 1936; Crane, 1949). These latter investigators then postulate the existence of physiological and psychological barriers to intraspecific copulation. Gering (1953) also demonstrated that among several species of agelenid spiders of the genus *Agelenopsis* morphological differences in the genitalia are not sufficient to prevent insemination. Crane (1949) found essentially the same situation in salticid spiders. The above findings are of particular interest here, since

the complex and variable gonopodium of poeciliid fishes has also been discussed in the light of the "lock and key" hypothesis (Sengün, 1949; Hubbs, 1950; Gordon and Rosen, 1951). It is apparent from the discussion of Dobzhansky (1951) that the importance of incompatibility of the genitalia as an effective barrier has been greatly overestimated.

Within recent times a new "lock and key" concept has been formulated. This is a fundamental part of the instinct theory developed by Lorenz (1935, 1950), Tinbergen (1951), and their followers. In brief, these investigators postulate that certain features of an individual which are usually innate and species specific (e.g., shapes, colors, sequence of movements, sounds, scents, or combinations of these) are specially differentiated to the function of eliciting a response in a fellow member of a species. These are called "releasers," "sign stimuli," or "key stimuli." To every releaser as an organ for sending out sign stimuli, there corresponds a "perceptual correlate," an "organ" to receive sign stimuli. This receiver is called the "innate releasing mechanism." Every releasing mechanism, according to Baerends (1950), has its own sign stimulus which in the course of evolution will develop towards a more perfect enclosure of the adequate object. The innate releasing mechanism activates an instinctive act, usually by removal of neuro-motor inhibitions, and, once released, the act proceeds independent of further stimulation.

Because of fundamental theoretical arguments concerning innateness, this approach to the study and understanding of animal behavior has not received widespread attention in this country, except perhaps among ornithologists. Detailed critiques of Lorenzian theory are presented by Ginsberg (1952) and Lehrman (in press), and further consideration of these problems is not warranted here. For the present discussion it is important to note that Lorenz (1940), Baerends (1950), and Tinbergen (1951) emphasize the species specific feature of releasers and releasing mechanisms and their biological value in the prevention of interspecies mating behavior. It is also apparent that this theory has influenced to some extent discussions of psychological isolating mechanisms, for there seems to be an

attitude among certain investigators of looking for a single critical factor or perhaps a few factors in the sense of locks and keys. Thus Seitz (1948) discusses the isolating barrier between *Tilapia heudeloti* and *Tilapia atalensis* in terms of species specific differences in nuptual coloration (key stimuli or releasers) correlated with differences in the releasing mechanism. Huxley (1942) spends several pages discussing recognition marks (auditory, visual, or olfactory) as "distinctive characters serving as barriers against intercrossing." Platyfish and swordtails certainly have an adequate supply of distinctive "recognition characters," yet they seem to play at best a minor and indirect role in the isolating mechanism. Similarly, Haskins and Haskins (1949) note that among the three sympatric poeciliid species, *Lebistes reticulatus*, *Micropoecilia parae*, and *Poecilia vivipara*, mature males of all three species differ strikingly in appearance both from females of their own species and from one another. Yet these distinctive characters seem to have little direct relation to reproductive isolation. In this connection Amadon (1950) notes that biological differences are often found in populations not even subspecifically distinct. It seems unsafe to assume *a priori* that because two sympatric species differ in a particularly striking characteristic, this feature is the isolating factor.

Although Dobzhansky (1951) postulates that two complementary genic mutations are the hypothetical minimum required for the development of an isolating mechanism in obligatorily cross-fertilizing organisms, both he and others (Muller, 1940; Mayr, 1942) indicate that in general isolation arises through the accumulation and integration of a large array of small genetic differences. This appears to be the case with the platyfish-swordtail isolating mechanism, with its series of interdependent phenotypic differences which undoubtedly depend on a large number of gene changes. Our results are therefore in agreement with the conclusion of Mayr (1947) who writes, " . . . there is great diversity in the factors that prevent hybridization between individuals of different species. . . . All the recent evidence shows that in the separation of a given pair of species many, if not

most, of these mechanisms are somewhat involved. . . . Reproductive isolation is thus not a simple lock and key mechanism that is controlled by a few genes." A similar conclusion was reached by Blair (1951).

Somewhat in contrast to our conclusions, Spieth (1947, 1949), in a painstaking investigation of the isolating mechanisms between several species of the *willistoni* group of *Drosophila*, while recognizing the existence of many isolating factors, placed great emphasis on a single item in courtship behavior, namely, the response of the female to the tapping behavior of the male, as responsible for much of the observed reproductive isolation among these species. In the *willistoni* group, a male approaches another individual and taps with his forelegs. If the individual tapped is a female of his own species, the male receives a stimulus that incites him to tap vigorously and he then proceeds to the next stage of the courtship, namely, posturing. If the individual tapped is the female of another species of the *willistoni* group, the tapping male receives a different type of stimulus. In most cases the males receiving this altered stimulus fail to posture, and courtship procedure is terminated. In other cases the males posture, but courtship procedure breaks down at a later stage. If we accept the hypothesis proposed by Dobzhansky (1940, 1941b, 1951) that reproductive isolating mechanisms are a product of natural selection (see also Koopman, 1949), it is logical to assume that the isolating factors in the *willistoni* group were once more diffuse—perhaps not unlike the situation found in our xiphophorin fishes. We may assume that in *Drosophila* species an early step in the courtship procedure was gradually emphasized by natural selection as the most economical stage to break off unprofitable reproductive activity. Similarly, it may be assumed that the isolation between the platyfish and the swordtail has not as yet reached the stage of effectiveness and completeness that is exemplified by the *willistoni* group, although it has reached a point where under natural conditions the uneconomical production of hybrid offspring must be an extremely infrequent phenomenon, However, Mayr (1947) notes that a multiplicity of isolating mechanisms is of selective advantage

and that it is genetically easier to build up a multiple, mutually reënforcing mechanism than a single-factor, all-or-nothing barrier. As an alternative hypothesis, one may postulate that the type of isolating mechanism described in this study is not only effective and complete, as the field data demonstrate, but also that it is of sufficient economy to the species to the extent that at present it is not undergoing reduction by the forces of natural selection to a more rigid and unitary type.

SUMMARY AND CONCLUSIONS

QUALITATIVE AND QUANTITATIVE RECORDS of sexual behavior patterns in males and females of the platyfish, Xiphophorus (Platypoecilus) maculatus, and the swordtail, Xiphophorus helleri, and in the males of their F_1, F_2, and backcross hybrids have been taken from a series of over a thousand 10-minute observation periods. The accuracy and significance of these observations were greatly facilitated by the development of two techniques:

1. An electrically controlled polygraphic recording apparatus was used to record the duration and sequence of each behavior during most of the 10-minute observation periods. The machine was operated by a "touch-type" system enabling the operator to record each item of behavior rapidly and without loss of observation.

2. Gonaductal smears from living females were taken with a micropipette after all critical observations. This proved to be a reliable method for determining whether insemination had taken place during the observation.

A series of experiments were designed to elucidate the mechanism of insemination in these fishes. These led to the following conclusions:

1. Gonopodial thrusting may be distinguished behaviorally from copulation. As determined by the gonaductal smear technique, thrusts alone never result in the insemination of the female. Rather, inseminations result from some but not all copulations.

2. The gonopodium is a holdfast organ in which the serrae, hook, and claw on the tip form an effective device for attachment. In the absence of this holdfast mechanism, copulations do not occur and males so deprived do not inseminate females.

3. The pelvic fin on the side to which the gonopodium is swung also moves forward, and this is an integral part of the copulatory mechanism. In the absence of both pelvic fins, the ability to transfer sperm to the female is greatly reduced.

Although courtship patterns are basically similar in the platyfish and swordtail, a number of qualitative and quantitative differences have been revealed. Several items of behavior, namely, copulation, thrusting, swinging, sidling, quivering, and nipping, were observed during courtship in both platyfish and swordtails. Two behavior patterns typically shown during courtship by male platyfish, namely, pecking and retiring, were not observed during swordtail courtship. Male swordtails, on the other hand, showed types of courtship behavior referred to as exaggerated backing and nibbling which were not seen in platyfish.

The most striking quantitative differences in the sexual behavior among platyfish, swordtails, and their various hybrid combinations were associated with copulation. The mean duration of copulation was longer for swordtails (2.39 seconds) than for platyfish (1.36 seconds), and swordtail pairs copulated sooner (average one minute) during the 10-minute observation period than did platyfish (average five minutes). However, platyfish copulated more frequently (in 26.7% of observations as compared to 13.4% in swordtails), and the number of inseminations resulting from copulations was higher in platyfish (86.0%) than in swordtails (39.4%). In the F_1, the frequency of copulation was slightly higher than in platyfish (29.0%), and the number inseminated after copulation was intermediate between the parent types (64.3%). In the F_2 and backcross hybrids these values were much lower. In general, copulatory behavior in F_1 hybrids was either intermediate or more like that of the swordtail. Some features of male sexual behavior in these xiphophorin fishes apparently are influenced by genetic factors, but we cannot offer any specific mode of inheritance to account for the data obtained. The inheritance is likely to be based on many genes, each of small effect.

Studies on interspecies groups revealed almost complete reproductive isolation between platyfish and swordtails when a choice of mates is offered, even though some heterogamic courtship activities may be observed. When no choice of mates is offered, heterogamic pairings occasionally result in copulations with a relatively low percentage of inseminations (18.2%).

The isolating mechanism between the

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swordtail and the platyfish appears to depend on an array of partially isolating factors. Each alone is not sufficient to insure isolation, but acting together these factors so reduce the probability of hybridization that under natural conditions the isolation seems to be complete, as indicated by extensive field data.

The gamut of partially isolating factors is summarized on page 211.

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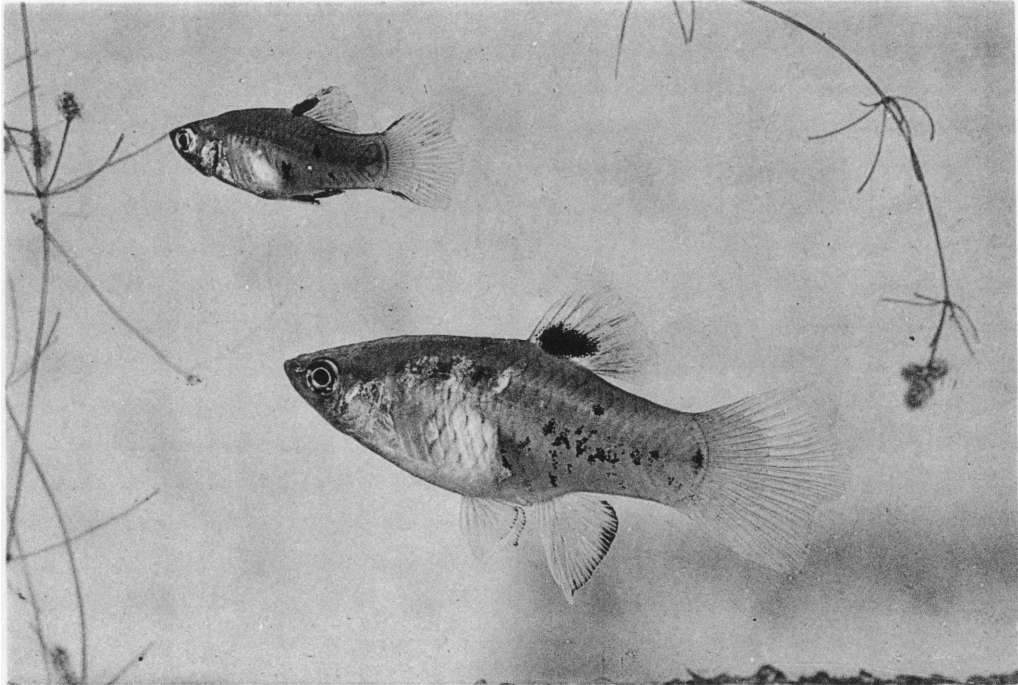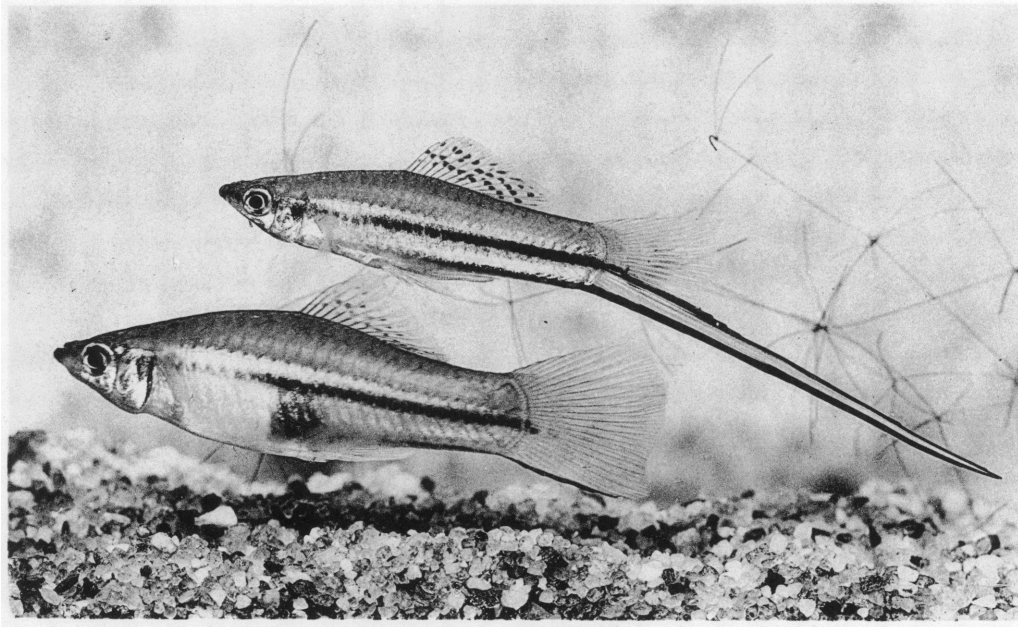
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1. Typical swordtail habitat in the headwaters
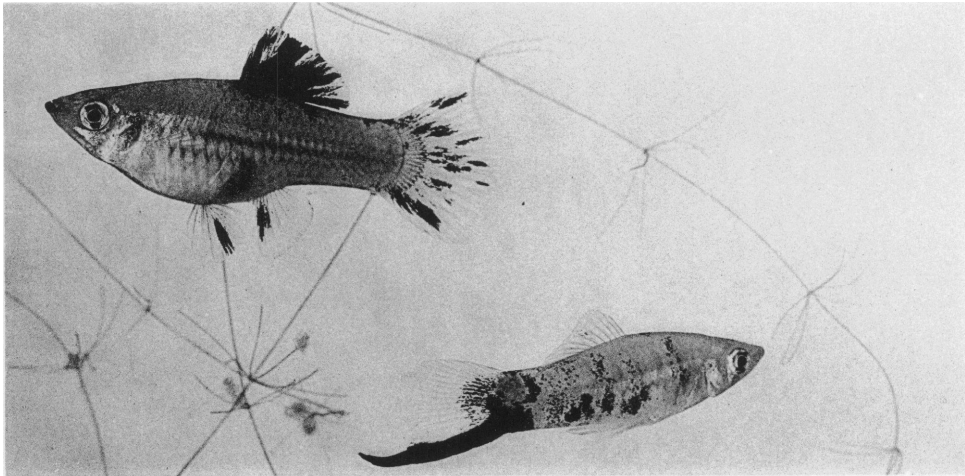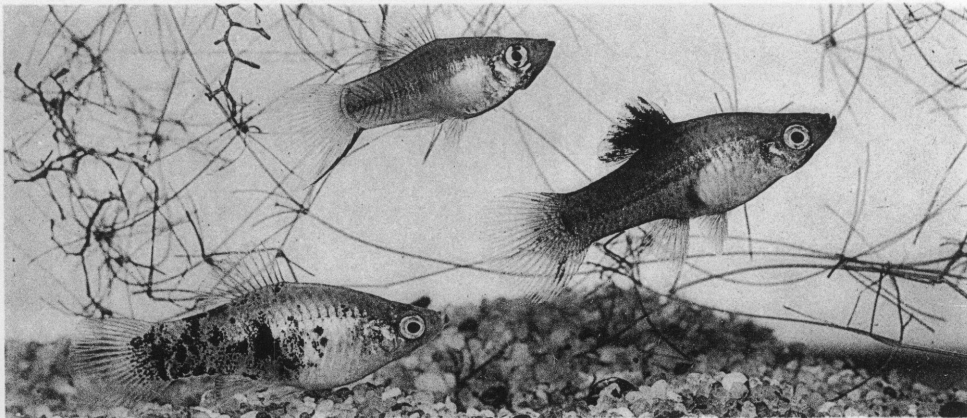2. Typical platyfish habitat in the lowlands; a few swordtails may be swept down into these areas
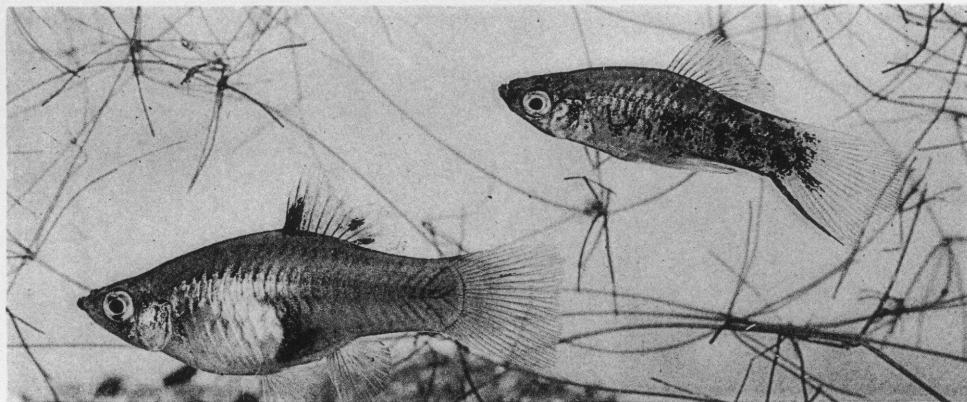
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1. Male (above) and female (below) platyfish
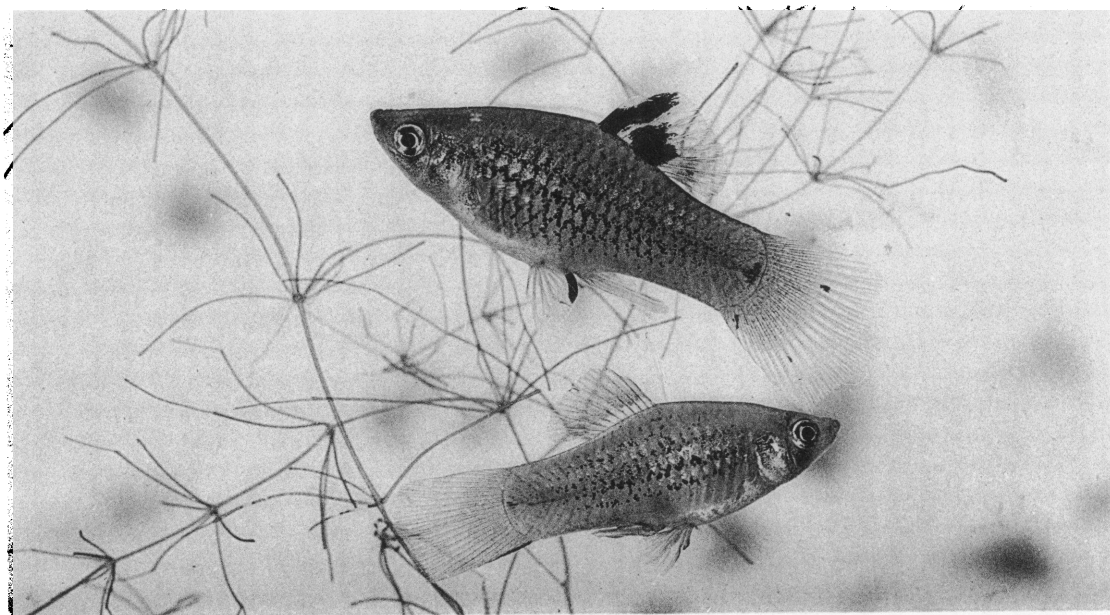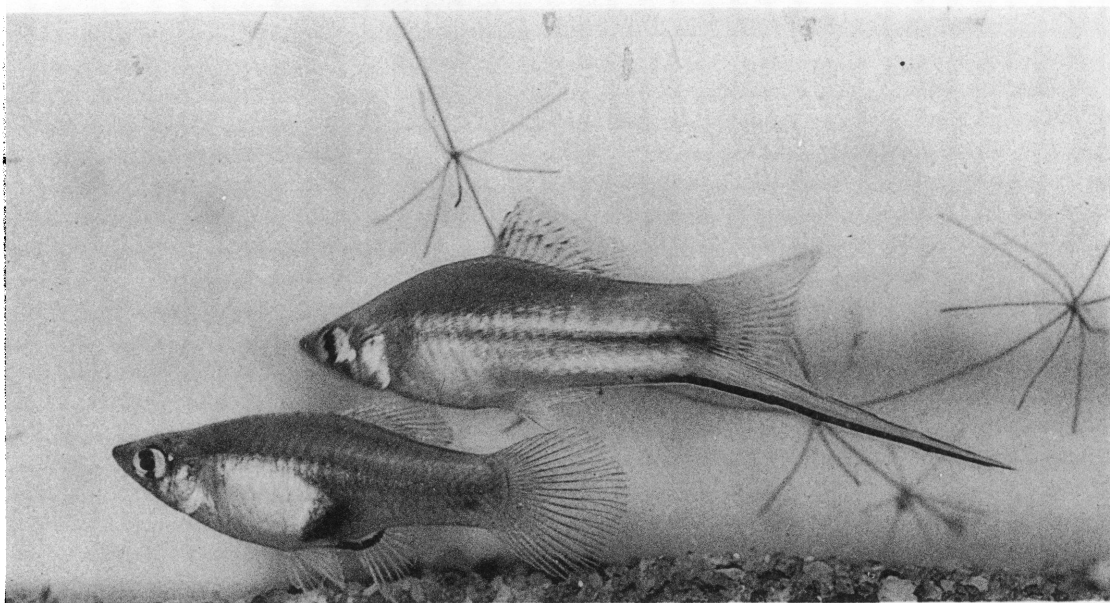2. Male (above) and female (below) swordtails

1. Male (below) and female (above) first-generation hybrids of platyfish female and swordtail male

2. Male and two females of second-generation hybrids. The male (uppermost of the three fish) is double recessive

3. Male (above) and female (below) second-generation hybrids. The male carries the dominant gene for spotting

1

2

1. Male backcross hybrids produced by mating an F_1 hybrid female to a platyfish male
2. Male (above) and female (below) backcross hybrids produced by mating a swordtail female with an F_1 hybrid male
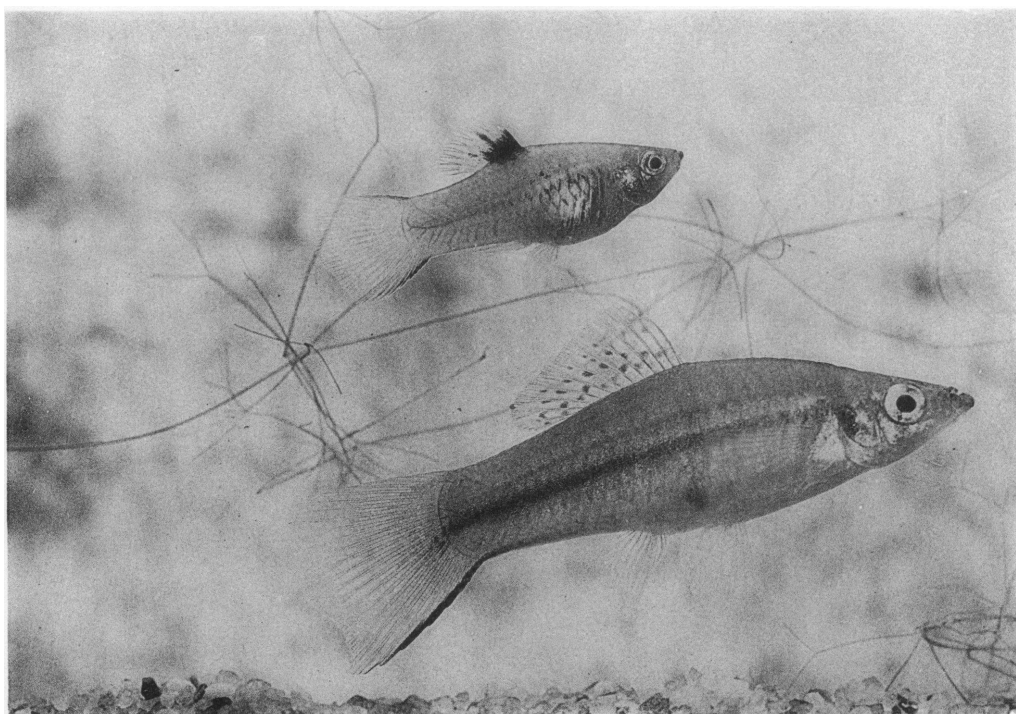
1
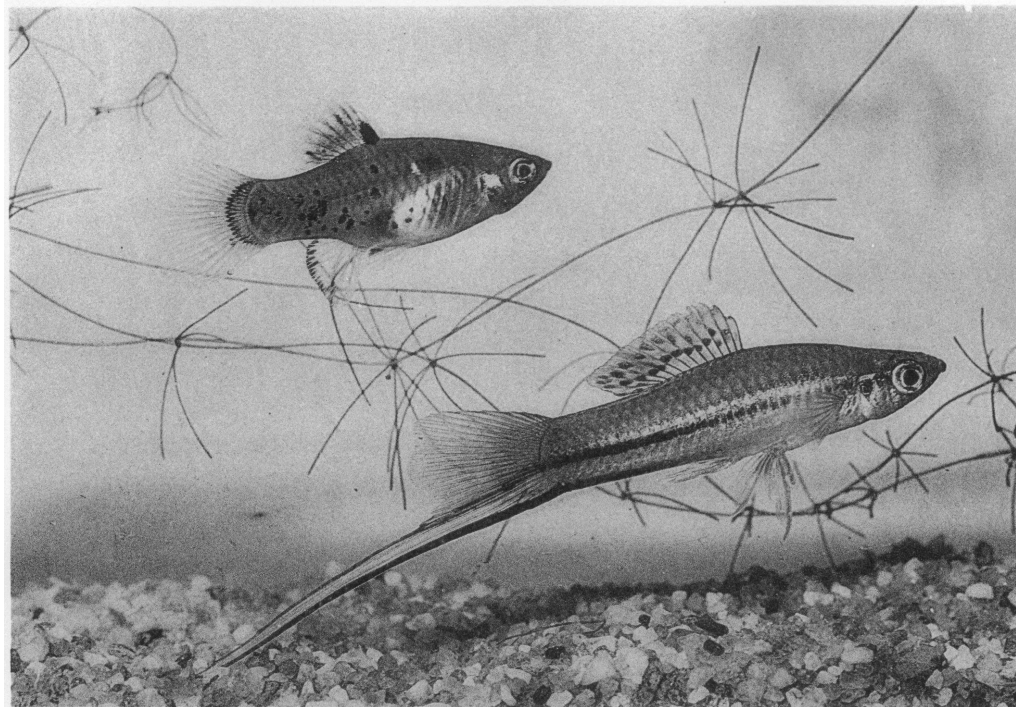
2

1. Male platyfish (above) and female swordtail (below) to show relative size difference
2. Male swordtail (below) and female platyfish (above) to show relative size difference
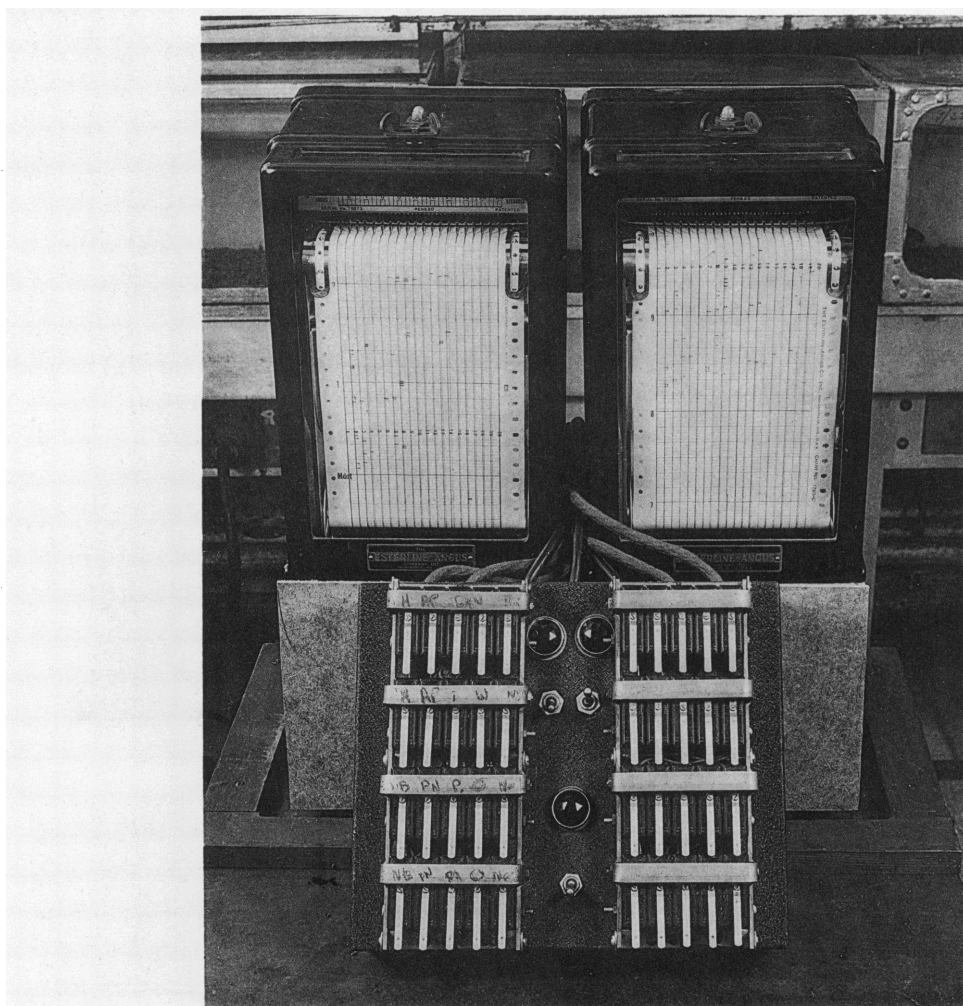
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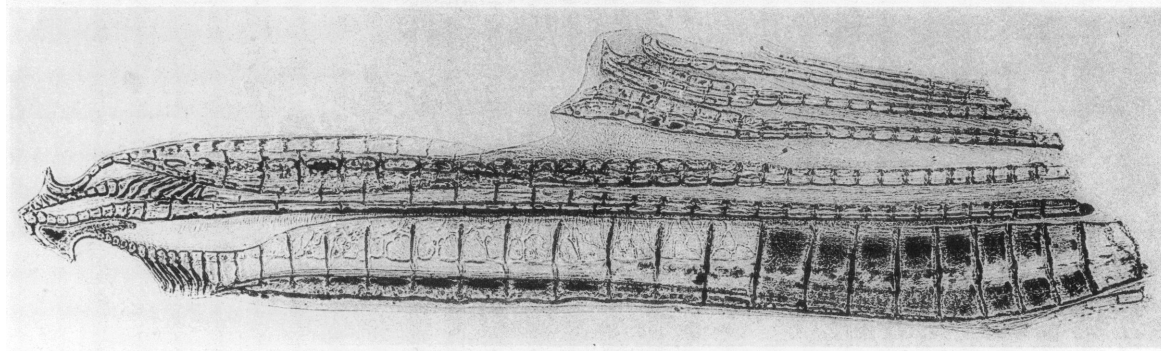
2

1. The electrically controlled polygraph and portable keyboard used for recording behavioral observations
2. Gonopodium of swordtail
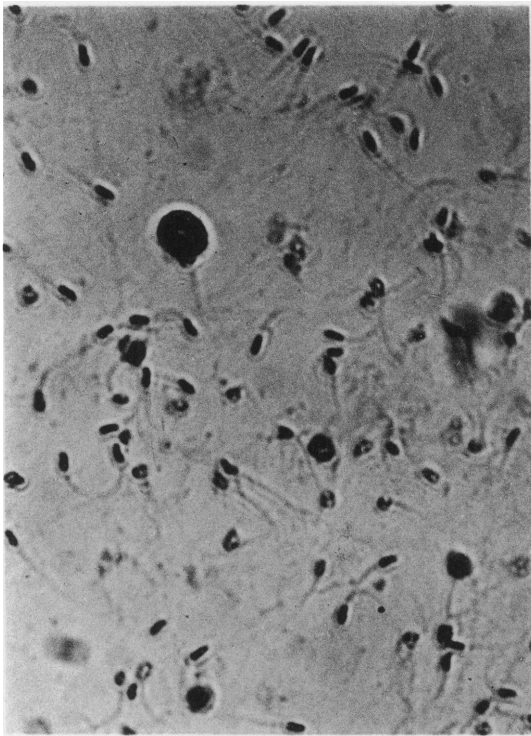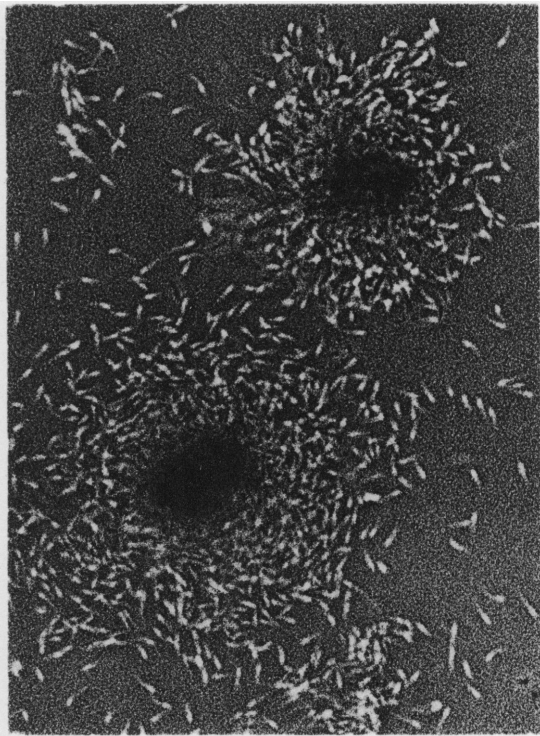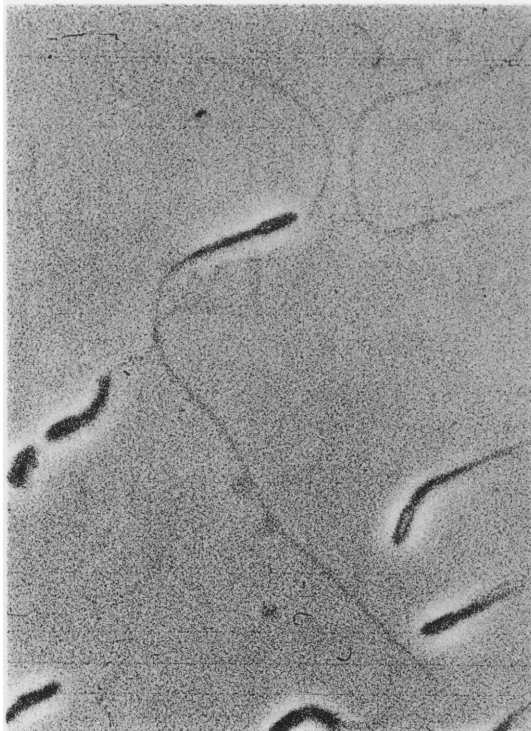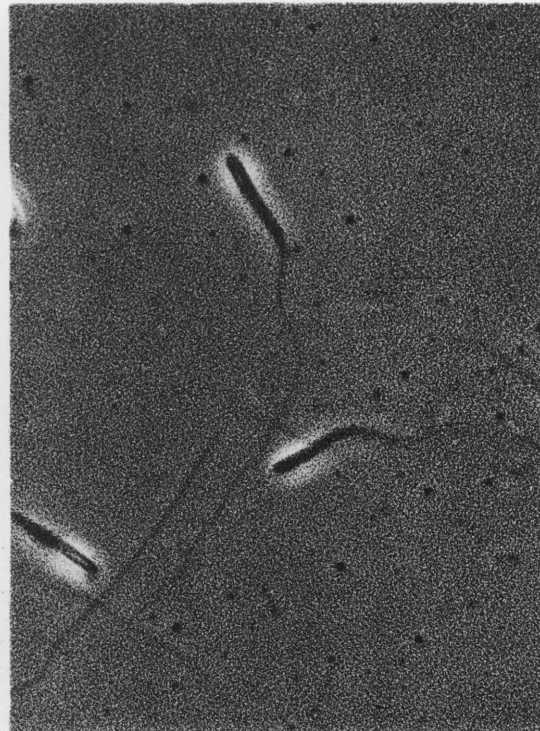
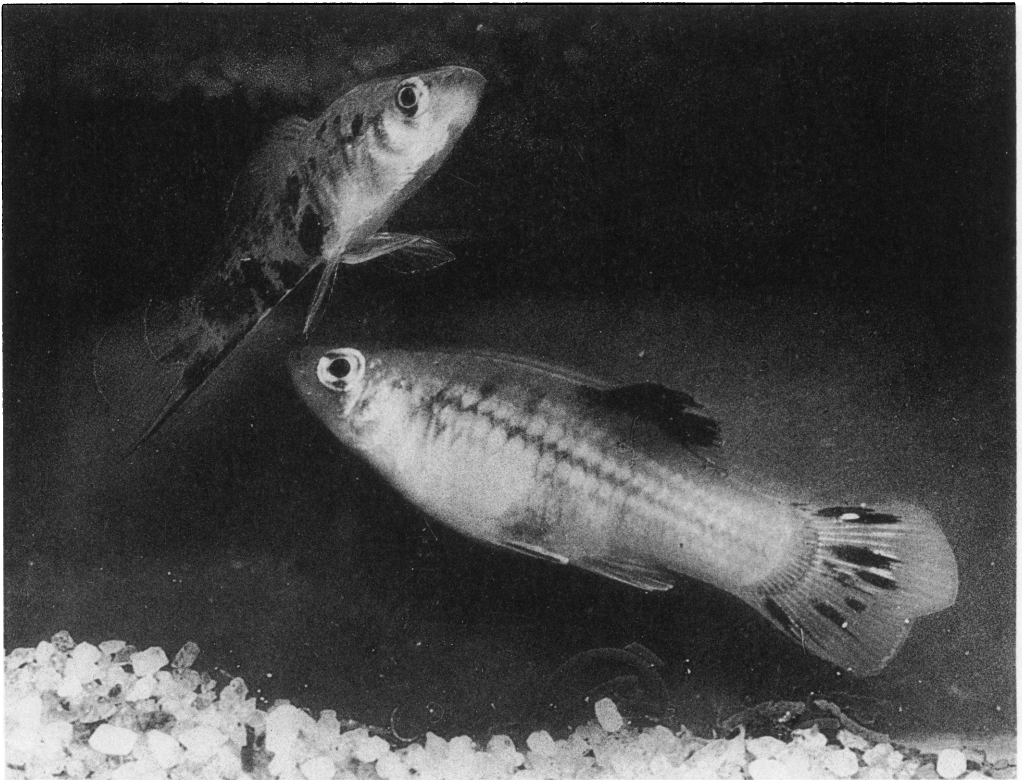1. Microphotograph of a sperm smear taken from a female platyfish 20 minutes after copulation. Stained with hemotoxylin and eosin. × 750
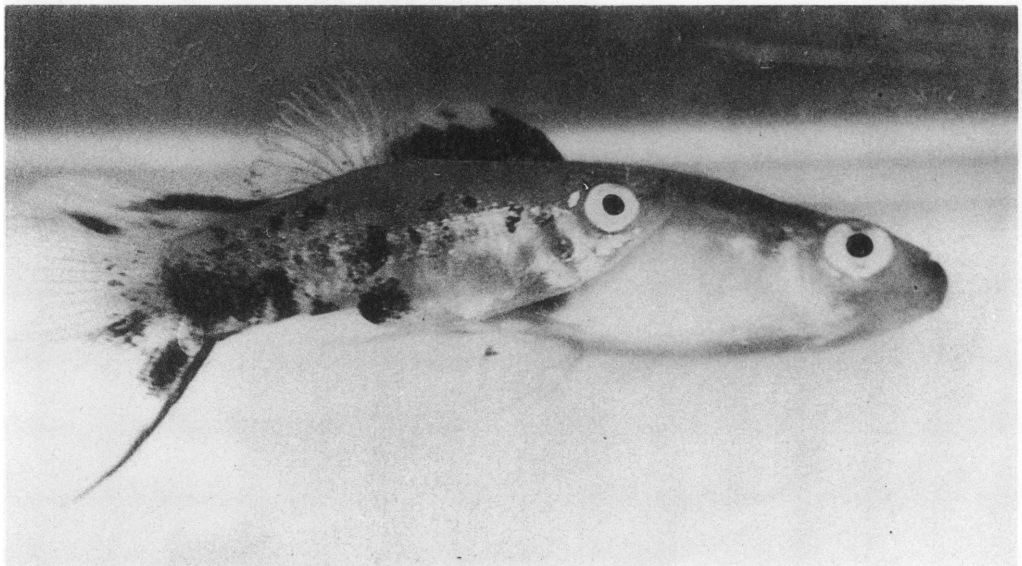2. Phase microphotograph of two swordtail spermatophores breaking up. Bright phase. × 343
3. Phase microphotograph of platyfish sperm. Dark phase. × 1500
4. Phase microphotograph of swordtail sperm. Dark phase. × 1500

1

2

1. Male (above) and female (below) first-generation hybrids of a platyfish and swordtail. The male is at the peak of a gonopodial swing, showing the gonopodium and pelvic fin in the forward position on his left side

2. Male and female first generation hybrids during copulation. The male is on the right side of the female. Note the forward position of the gonopodium
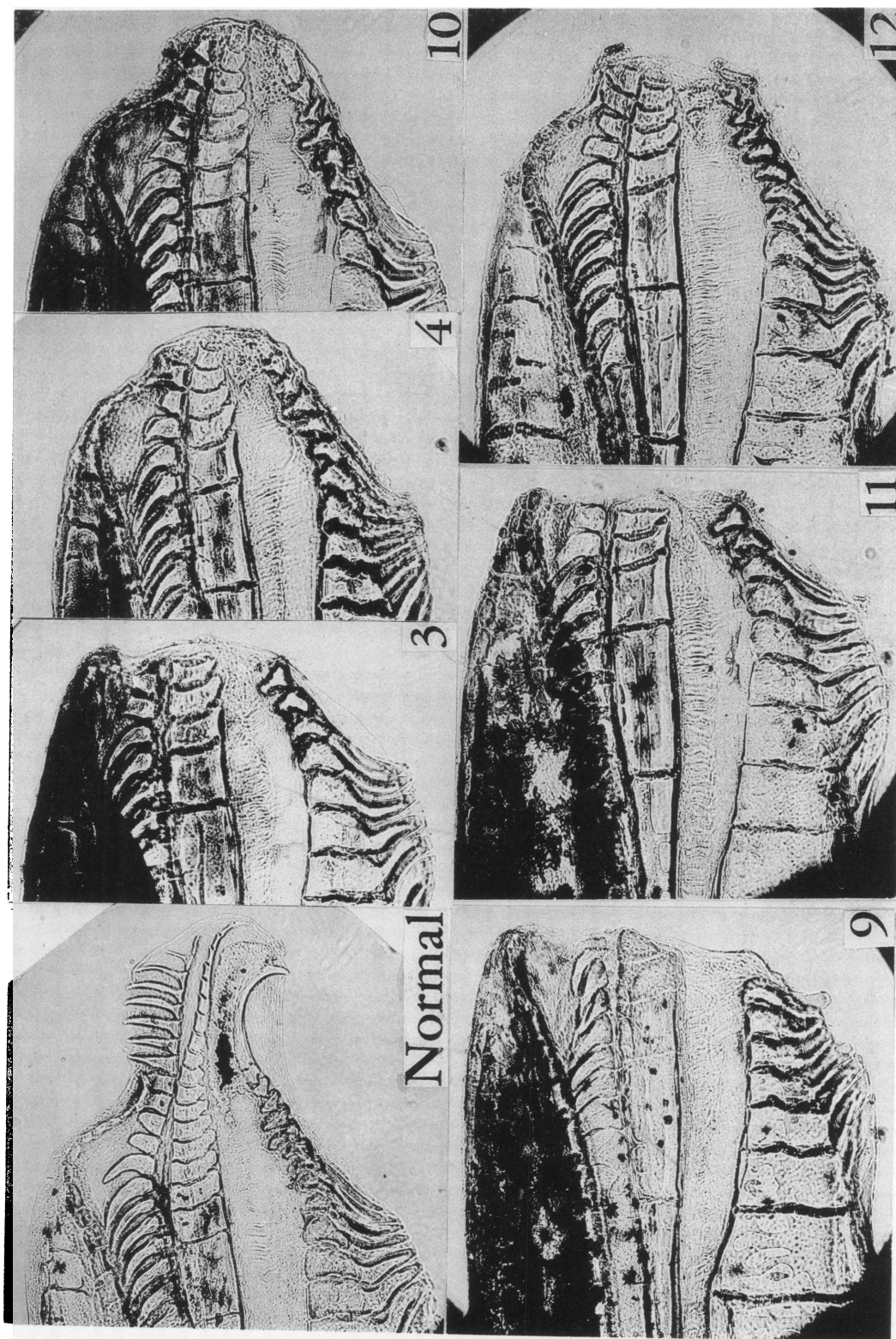
Distal end of gonopodium of a normal platyfish male and of six other males in which the tip had been amputated. These males thrust vigorously and at a high frequency but never copulated or inseminated the females.