time is consumed in picking the moist seeds out of the peat used in stratification, recounting them and sowing them in the flat. Use of the peat mat saves this time, since the mat is a suitable medium for stratification as well as for germination. The seeds are counted while still dry and easily handled, set up on the mat in position for the germination test, refrigerated for the desired period and removed to the germination room without further manipulation.

The peat mat used by the station is 19 by 19 cm square by 2 cm thick, and fits loosely into a square glass baking dish which is covered by a pane of ordinary window glass during the test. The mat is molded on a form consisting of ten triangular wooden strips, 17.7 cm long, 6 mm wide at the base and 4 mm from base to apex, tacked on a board parallel to each other and 1.6 cm apart from apex to apex. A square frame or collar of galvanized screen wire, 2 cm deep and 19 cm on a side, is held in position around the block of strips by four headless nails driven vertically into the board. Around this wire frame a snugly fitting square wooden frame is slipped to keep the wire from bulging. Moistened peat is packed into the frame and down upon the triangular strips, and is compressed with the hands into a firm mat 2 cm deep. The apparatus is inverted, the board with the strips is gently lifted off, and the completed peat mat and its wire border are carefully pushed out of the square frame into the glass dish. The triangular strips leave what is now the upper surface of the mat marked with ten equally-spaced grooves or drills, each suitable for 25 to 50 seeds, depending on size.

By keeping a trace of free water in the bottom of the dish at all times, it is possible to maintain nearly ideal moisture conditions at the top of the mat throughout the tests.

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SPECIAL ARTICLES

APPARENT PARTHENOGENESIS IN NATURE, IN A FORM OF FISH OF HYBRID ORIGIN

WE are now clinching with experimental proof a number of ideas developed out of a study in systematic ichthyology. As these ideas express phenomena which are new for the vertebrates, this preliminary announcement is being published.

Through northeastern Mexico and the southern tip of Texas there occurs in abundance a form of viviparous cyprinodont, of the family Poeciliidae, which has been thought to be a distinct species, *Mollienisia formosa*. From circumstantial evidence we concluded that this form was probably the hybrid between *Mollienisia latipinna* and *Mollienisia sphenops*, species so distinct that they were long placed in different genera. This form was found to be exactly intermediate between those species in all distinctive features—depth of body, strength of rows of spots, position and size of dorsal fin and number of dorsal fin rays. These characters are all closely correlated and may be briefly illustrated by the usual number of dorsal rays: 9 in *sphenops*, 11 in *"formosa"* and 13 in the local race of *latipinna*.

Mollienisia "formosa" varies somewhat geographically, but in each region it is intermediate between the particular local forms of *sphenops* and *latipinna* which occur there. Recently we have received an apparent hybrid from the Yucatan Peninsula, exactly intermediate between the local race of *M. sphenops* and *M. velifera*. The dorsal rays are 13, midway between the approximate average for the local *sphenops* (9.5) and for *velifera* (17.5).

In the laboratory, a culture of males and females of this supposed hybrid stock, obtained by Dr. Myron Gordon in Rio Papaloapan, Vera Cruz, Mexico, has shown various reproductive abnormalities. Although several of the females apparently have become pregnant, only one of them in our aquaria has delivered young, two in one brood and one in the next brood (a female *Mollienisia* of this size should produce 10 to 60 young in a brood). No such lack of fecundity is apparent in the initial cross producing the hybrids, nor in the back crosses with the parent species. Most of the apparently pregnant females reverted to a thin condition, as though resorbing young. A rather high percentage of the young are abnormal, and most or all of the abnormal ones are developing into males. An unusually high percentage of the adult males develop irregular black blotches.

The hypothesis that *Mollienisia "formosa"* is the hybrid of *M. latipinna* by *M. sphenops* has just been verified by an aquarium mating of a male *sphenops* and a virgin female *latipinna*. The 22 young produced are clearly hybrids. We also have apparently pregnant females from the reciprocal species-cross.

This verified hybrid exists in nature in great abundance. Many hundreds of specimens have been collected. It has almost every characteristic of a species: a definite, homogeneous range; clear consistency of characters, and, as shown below, the ability to repro-

duce in approximate genetic constancy. This constitutes the nearest approach known to us of a demonstration that hybridization plays a rôle in the process of speciation of animals.

The outstanding peculiarity in the natural relations of this hybrid form is that it exists over much of its range only as females. Not a single male has been found, among about two thousand specimens examined, from Tamaulipas and Texas. Such a condition obtains among invertebrates, but has not previously been encountered among the vertebrates.

The only male hybrids we have seen from nature are those from the Rio Papaloapan, where or near where both parent species occur together. Where this hybrid form exists in nature solely as females, it occurs with only one of the parent species; never with neither. In Tamaulipas it lives with *M. sphenops,* well inland from the coastwise range of *latipinna.* In Texas it abounds in the resacas of the Brownsville region, in company with *M. latipinna,* but considerably farther north than *sphenops* occurs in the coastal area. Yet the female hybrids of these two regions appear virtually identical, in all their characters. For example, they both usually have 11 dorsal rays.

Our natural supposition was that these female hybrids can mate only with *sphenops* males in the Tamaulipas streams, and only with *latipinna* males in Texas. Since the males thus assumed to be utilized are very different, while the offspring are entirely alike (and all females) in the two areas, some genetic process very peculiar for a vertebrate must be involved.

This supposition has been completely verified in our aquaria. A number of females of *M. "formosa,"* from Forlon in Tamaulipas and from near Brownsville in Texas have produced young after being received from nature. Although the females from Tamaulipas had almost certainly mated with males of *M. sphenops,* while those from Texas had mated with males of the very different *M. latipinna,* they have to date produced several hundred young, showing throughout a marked uniformity in characters. Neither lot of young (many already adult) shows any apparent approach toward the characters of the male involved. The characters of the female parent have been inherited as a block. Although the broods have been large and many, not a single male has appeared among them. This result is wholly consistent with and explanatory of the occurrence of females only in nature, in the regions from which the two stocks came.

That we are dealing with entirely matroclinous inheritance has been proved by controlled matings. Virgin female hybrids, which have never been with any males, mate readily with males of either parent species, and soon become pregnant. At the time of writing, two such hybrid females of the Texas stock, mated with males of *M. latipinna,* have already given birth to purely matroclinous young.

The consistent and abundant production of purely matroclinous and constantly female offspring by this hybrid form of fish finds its most plausible explanation as parthenogenesis. It is apparently not a spontaneous parthenogenesis, since many controls, unmated, have shown no indication of becoming pregnant. We provisionally assume that we are probably dealing with a case of gynogenesis (parthenogenetic development initiated by sperm which for some reason is prevented from taking part in heredity)[1]—a condition recorded as naturally occurring among certain invertebrates, but not among vertebrates. This hypothesis does not exclude alternatives, and requires cytological verification. Plans have been made for this cytological investigation.

The breeding experiments with these fishes are being rapidly expanded, and further results are anticipated in the near future (as many as three or four generations in a year are possible). The matings already productive are being repeated, and many other matings have been and will be made, between the numerous stocks we have from localities between Mississippi and Panama. Inbreeding and back-crossing of the young hybrids we have produced in wholly controlled matings will of course be carried out. Attempts will be made by both individual and mass matings, starting with the assumed original material, to fully recreate the uniquely characterized and peculiarly distributed hybrid form under discussion. The anticipated results of breeding both natural and aquarium-reared male hybrids to female hybrids known to be virgin, and to females of both the parent species, will be vital to the analysis of the origin and distribution of this form with such surprising reproductive behavior.

These viviparous poeciliid fishes, not only of *Mollienisia* but also of other genera, furnish prime material for studies in experimental evolution. The researches by Winge, Gordon and others have already shown how valuable this material is in the interpretation of the gene and chromosome basis for the development of characters and for the production of sex. A genetic explanation for the production of wholly female and purely matroclinous young by the hybrids in *Mollienisia* may contribute critical data for the solution of these problems.

In conclusion, the conditions demonstrated by this study, so far as we know novel in the biology of the

[1] Wilson excludes gynogenesis from the limits of parthenogenesis, but his classification of reproductive methods on the basis of differences in the manner of egg activation seems less logical and less significant than one based on the genetic constitution of the offspring. Parthenogenesis we regard as unisexual reproduction, as the result of which the offspring are genetically like the mother.

vertebrates, are: (1) The abundant occurrence in nature of a form of demonstrated hybrid origin, having nearly all of the characteristics of a natural species; (2) the occurrence of a form as females only, over a wide portion of its range; (3) the consistent and abundant production of wholly female and purely matroclinous young; (4) apparent parthenogenesis in nature.

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THE VITAMIN C ACTIVITY OF HEXURONIC ACID FROM SUPRARENAL GLANDS[1]

THE isolation and identification of vitamin C (from lemons), first reported by the writers,[2, 3] has been followed by independent and in part concurrent evidence from other laboratories,[4, 5, 6, 7, 8] which adds support to our conclusion that vitamin C is identical with the hexuronic acid previously studied as a reducing agent by Szent-Gyorgyi[9] and Kendall.[10] The earlier papers from other laboratories did not include quantitative assays of the acid prepared from suprarenal glands. Only one feeding level (1 mg per day) was recorded, so that the minimum protective dosage was not evident, and from this point of view there was need of further evidence to answer Zilva's criticism.[11] This point was further emphasized by an indication that the original crystals from animal glands could be further purified,[12] even though they were of reasonable purity when tested. Reasoning from analogy between the vitamin C content of lemon juice and its hexuronic acid content is conditioned by a variation of over 100 per cent. in the antiscorbutic activity of the juice and by very little data regarding its hexuronic acid content. The paper by Harris and others[13] included an assay using a different technique than ours, but their product was evidently about the same as our own in activity. It is clearly evident from his later publications[14] that Szent-Gyorgyi had no knowledge of the vitamin function of the acid previous to the experimental work published in 1932.

To provide further evidence that the crystalline vitamin was not a case of inactive crystals accompanied by active material, we have made an assay of the hexuronic acid prepared by Dr. E. C. Kendall[15] from suprarenal glands. This product was prepared according to a distinctively different procedure from that used in our laboratory and from a different type of starting material. Hence a quantitative study of its activity provided strong evidence regarding the identity of the vitamin. This product corresponded with our own in crystalline form, melting point, reducing value, titration equivalent and rotatory power. The essay showed strikingly that the two preparations were identical in activity within the limits of measurement. A dosage of 0.5 mg daily provided a minimum protective level with slightly suppressed growth (133 g. gain in 55 days) and with two of five animals showing slight scurvy symptoms (av. score 2). The 0.75 mg level was above the amount required for protection, and the 0.25 mg level was distinctly too low.

The procedure for isolating the hexuronic acid from suprarenal glands involved an ether extraction of the solution when neutralized by sodium bicarbonate. This would have removed such a substance as the o-diphenol derivatives of narcotine, which has been considered by Rygh[16] to be identical with vitamin C.

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