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Biochemical and cytogenetic studies of Poecilia from eastern Mexico. II. Frequency, perpetuation, and probable origin of triploid genomes in females associated with Poecilia formosa*

by

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ABSTRACT: Triploid female fishes that closely resemble the gynogenetic, diploid, unisexual species *Poecilia formosa* Girard and its sympatric, bisexual congener *Poecilia mexicana* Steindachner constitute a significant, but not static, component of naturally occurring populations of *Poecilia* in headwater localities of the Río Soto la Marina drainage in northeastern Mexico. The frequency of triploid females fluctuates markedly from year to year, from season to season, and from one locality to another. In laboratory breeding studies to assess the reproductive competence of triploid females as a factor influencing structure of wild populations of *Poecilia* spp., we have used electrophoresis of blood plasmas and DNA-Feulgen cytophotometry of the nuclei from blood cells or scale epithelium from live fishes to monitor the persistence of triploid genomes in four laboratory-reared generations of female progeny in stocks initially derived from gravid, triploid females collected from the Río Purificación at Barretal or from Vado el Moro, near Cd. Victoria, México. We conclude that many of the triploid females are reproductively competent and regularly transmit triploid genomes to their unisexual offspring. They presumably reproduce by gynogenesis, since breeding stocks of triploids can be maintained in the laboratory by matings with sympatric males of *P. mexicana*.

Unisexual triploid fishes of the genus *Poecilia* occur in headwater tributaries of the Soto la Marina drainage in northeastern Mexico in the context of

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a breeding complex involving competitive yet dependent relationships between an all-female, gynogenetic, diploid species, *Poecilia formosa* Girard, and its sexually parasitized, sympatric congener, *Poecilia mexicana* Steindachner (2, 4, 16, 17, 26, 30, 31, 32). In this and in several other systems (1, 8, 9, 12, 23, 34, 37), considerable evidence has been reported during the past five years in support of the suggestion by SCHULTZ (33) that hybridization, unisexuality, gynogenesis, and polyploidy are related evolutionary phenomena involved in the speciation of at least certain groups of lower vertebrates (34, 35, 38). An essentially similar model to account for the occurrence of bisexual polyploid species among several groups of invertebrates, particularly in the case of the silkworm, *Bombyx mori,* was independently proposed by ASTAUROV (3) in formulating his hypothesis of the indirect origin of polyploid animals via parthenogenesis and hybridization.

In view of the implications of these ideas pertaining to problems of speciation in the subgenus *Poecilia,* and to gain insight as to functional significance of the diploid unisexual, *P. formosa,* in the repatterning and evolution of its parental genomes (2, 17, 18, 27), we began a series of studies several years ago to monitor different facets of the interactions of diploid and triploid unisexuals of *Poecilia,* with each other and with their progenitor species, particularly in the dynamic context of naturally occurring populations of these forms (4, 30, 31). In addition to intensive analysis of field collections, however, laboratory breeding experiments were also required to answer important questions about (a) the reproductive capacity of triploid hybrid females, (b) the genetic constitution of their offspring, and (c) the long range consequences of a sustained program of breeding triploid unisexuals with males from related, gonochoristic species.

The present report summarizes our findings to date on marked seasonal fluctuations and site to site differences in the incidence of diploid and triploid unisexuals of the subgenus *Poecilia* from several different natural populations in northeastern Mexico. We have also found that wild-caught, triploid females are reproductively competent, giving rise in the laboratory to stocks of all-female offspring which consistently transmit triploid genomes to their progeny when mated with males of sympatric *P. mexicana.* Evidence is also presented to show that triploid hybrid females of *Poecilia* spp. may arise spontaneously in the laboratory from matings of male *P. mexicana* with the diploid unisexual, *P. formosa.*

For reasons summarized by MASLIN (22) and discussed by others (33, 34, 37), the International Code of Zoological Nomenclature does not include adequate provisions for devising a system of appropriate designations for uniparental forms where sperm from related, bisexual (gonochoristic) species are required to stimulate embryonic development but do not contribute hereditary material to the resultant offspring. Problems of precise nomenclature are even more complex in cases like the naturally occurring triploid unisexuals resembling *P. formosa* and either *P. mexicana* or *P. latipinna,* depending on which of the

latter species is utilized as the source of sperm in particular localities within the range of the gynogen *P. formosa* (13, 16, 31). As stressed by SCHULTZ (33) in considering a similar type of taxonomic problem posed by triploid females of the genus *Poeciliopsis*: "The fact remains that these all-female forms are hybrids and should be treated as such. To do otherwise not only befuddles the nomenclatural system and species concept but detracts from the significance of hybridization to evolution." We have therefore elected to use the name *Poecilia formosa* for both the diploid and triploid unisexual fishes described in the present report and to identify the latter class of females as a variant, triploid form for purposes of designating particular fishes as the source of certain data in the charts and tables that follow. Relevant issues are discussed in a later section, after presentation of the evidence upon which our interpretations are based. A preliminary note reporting persistence of triploid genomes in the progeny of triploid females has appeared (29).

MATERIAL AND METHODS

FIELD COLLECTIONS: The present report is based on our analysis of more than 3,600 specimens of the genus *Poecilia* in population samples from 12 different, inland localities of the Soto la Marina drainage in Tamaulipas, Mexico. Sites of these collecting stations are shown in the map of Fig. 1, which also includes the field designations used to identify populations from particular localities. In subsequent charts or tables, the numbered subscripts following a given field site designation indicate year of collection. Preserved specimens and live stocks were obtained during five expeditions: June, 1968; June, 1970; April, 1971; April, 1972; and July, 1972. Restriction of the distribution of *P. formosa* to northeastern Mexico and southern Texas and a listing of its sympatric congeners within this range have been previously summarized by DARNELL and ABRAMOFF (13).

All fish collections were made with seines and transported to local motels where fishes were bled to make preparations for cytophotometry and electrophoresis, and then fixed in 10 per cent formaldehyde and subsequently preserved in 70 per cent ethanol for morphometric and gravida determinations. Counts of fin rays for each specimen were recorded by the method of HUBBS and LAGLER (19), in which the last two rays of the dorsal fin are regarded as split to the base and thus counted as a single ray. Other criteria for morphological comparsisons between inland populations of *P. formosa, P. mexicana* and the triploid hybrid females associated with them are considered in detail elsewhere (27). For further discussion of the distribution and systematics of species of the *P. sphenops* complex in Mexico, particularly *P. mexicana,* see the recent reviews by SCHULTZ and MILLER (36) and MENZEL and DARNELL (26).

ELECTROPHORESIS: Plasma samples obtained in the field from wild-caught specimens were stored in dry ice for shipment by air to the laboratory for electrophoresis, using procedures and an apparatus for horizontal polyacrylamide gel sheets reported elsewhere (5).

Blood plasmas from laboratory-reared fishes, prepared as specimens became available, were stored at -10 C for periods of up to two years, so that samples from as many as 20 different, individual fishes could be applied and analyzed simultaneously in a vertical microsystem that employs 8% polyacrylamide gel sheets with tris-glycine buffers at pH 6.7 and 8.3 for high resolution, discontinuous electrophoresis of 0.1 to 0.5 μl aliquots of fish plasma (7). Observed differences in albumin mobilities (= albumin phenotypes) were verified by mixing isoaliquots of test plasmas with plasma samples containing albumins of known electrophoretic mobilities (2, 4). Methodological details for sample preparation, apparatus design, gel castings, and microspectrophotometric evaluation of stained sample tracks after electrophoresis were as previously described (5, 7).

CYTOPHOTOMETRY: *Slide preparation and staining.* Samples of fish blood obtained by cardiac puncture were smeared on chemically cleaned slides, quickly air-dried, and post-fixed for 30 minutes in 17:5:1 (v/v) methanol: formalin: glacial acetic acid (MFA), as recommended by BEAMISH *et al.* (6) and JAMES (20). Other preparations were made by lightly touching the freshly cut surfaces of small pieces of fish liver onto glass slides, the upper thirds of which were coated with a thin film of chicken blood cells. After staining for DNA with a Feulgen procedure for quantitative purposes as described elsewhere (31), blood smears and liver prints were mounted for cytophotometry in refractive index liquids (n_D 1.548-1.556, Cargille Laboratories, Inc., Cedar Grove, New Jersey) carefully matched to minimize non-specific light loss due to unstained cytoplasmic proteins.

To survey broods of putative triploid offspring without sacrificing such high-value animals, samples of epithelial cells were obtained by removing one or two scales from just behind the dorsal fin of young fishes lightly anesthetized by a one minute exposure to MS-222 or Finquel (1:25,000, w/v). The injured region was gently swabbed with 0.02 per cent mercurochrome before returning specimens to well aerated aquaria containing 0.6 per cent saline to which was added 15 cc per 20 L of "Holdex" (Jungle Laboratories Corp., P.O. Box 2018, Sanford, Florida). All but two of the 90 specimens handled in this way survived the operation and none developed fungal infections at the site of scale removal. Each set of scales was fixed for one hour in MFA, washed for at least one hour in 10 changes of distilled water, stored overnight in 70 per cent ethanol, and rehydrated in graded ethanols the following morning. After three rinses in distilled water, sets of scales from different fishes were hydrolyzed in 5 N HCl at room temperature (14) for 30 minutes, briefly rinsed in 0.1 N HCl, and then stained for two hours in Schiff's leucofuchsin sulfurous acid reagent. After three 10-minute changes in SO_2 water, and at least a dozen changes in distilled water to remove all traces of sulfite, small pieces of stained epithelial tissue were stripped from each scale and dispersed into individual cells by persistent flattening of the tissue fragments in a small drop of 45 per cent acetic

acid under a coverslip of linear polystyrene (1 mil gauge, Rohm and Haas, Philadelphia, Pa.). The latter was subsequently removed with a razor blade after freezing the squashed preparation betwen blocks of dry ice (11). As internal reference standards assumed to contain 2.5 $\times$ 10^{-12} g DNA per cell (28, 31) and 1.6-1.7 $\times$ 10^{-12} g DNA per cell, respectively, smears of chicken blood cells and dorsal scale epithelium from a male *P. mexicana* were routinely processed through the above steps with each series of scale samples from *P. formosa* or from the offspring of putative triploid females.

Measurement of DNA amounts. The average amount of DNA per fish blood cell, taken as an index of the size of the genome for wild-caught specimens of *Poecilia* spp., was estimated by determining the Feulgen dye content of 20 or more individual erythrocyte nuclei from each fish preparation and at least 30 nuclei from the chicken cell reference standard processed with each set of fish slides. Measurements at 560 nm were made in triplicate using an image-scanning, integrating microdensitometer (model GN 2, Barr and Stroud, Ltd., Glasgow, Scotland) with a 100 $\times$ oil immersion objective (N.A. 1.32), a 10 $\times$ projector lens, and a condensor aperture of 0.4 to 0.6. Full condensor aperture (1.3) was used to measure the extremely flattened nuclei of scale epithelial cells (15). A Wang 520-14 electronic programmable calculator (Tewksbury, Mass.) was used for statistical treatment of relative Feulgen absorbancies and for translation of the latter values into estimates of actual DNA content per cell, based on determinations of Feulgen staining intensity in simultaneously processed and similarly measured chicken erythrocyte nuclei. Under the conditions for measuring described here, the coefficient of variation for repeated measurements on a single chicken nucleus was 1.8 per cent, and for a population of 30 different nuclei from the same preparation, 4.7 per cent.

A number of the specific tests recommended by MAYALL (24) were carried out to evaluate possible sources of instrumental error and other types of systematic bias in measuring, such as the extinction effects in Feulgen-DNA scanning photometry recently discussed by JAMES (20). Several series of measurements were made on preparations of blood cells, scale epithelium, and hepatocytes from particular fishes. Each slide was measured at 560, 600, and 620 nm to compare the intensity of Feulgen staining by small erythrocyte nuclei containing densely compacted chromatin with the absorbance found for nuclei from hepatocytes, lymphocytes, or scale epithelial cells which characteristically show a more open or looser meshwork or chromatin dispersed within nuclei that are several times the diameter of erythrocyte nuclei from the same animal. In most of the cases tested, highly condensed red cell nuclei showed considerably lower values (10 to 25 per cent) for integrated Feulgen-DNA adsorbances than found for nuclei with presumably the same karyotype and in an equivalent phase of the cell cycle, but which had more finely dispersed chromatin. BEAMISH (6) has also noted an apparent depression of Feulgen-DNA values in fish erythrocyte nuclei. If not evaluated, these perturbations in the proportionality of measured stain content to the amount of DNA per nucleus can introduce

an appreciable source of error when very different types of cells are used to estimate actual DNA content as an index of genome size. However, as shown by inspection of the values in Table 1 for three kinds of nuclei from different animals whose karyotypes had been independently determined in preparations of gill epithelium made according to the method of McPHAIL and JONES (25), diploid and triploid unisexuals of *Poecilia* spp. can be readily distinguished from one another on the basis of DNA-Feulgen measurements. Comparisons of this

TABLE 1

Correlations between apparent differences in the DNA-Feulgen content of various types of fish cells and the degree of chromatin condensation shown by the nuclei of these cells *

Specimen Designation	Cell Type	Degree of chromatin condensation	DNA-Feulgen per nucleus mean	±	S.E.	n	Ratio (erythrocyte = 1.00)
P. formosa (2n=46)	erythrocyte	+++	59.12	±	1.21	20	1.00
	lymphocyte	++	66.39	±	1.79	20	1.12
	hepatocyte	+	88.98	±	1.22	20	1.52
P. formosa (n=46)	erythrocyte	+++	59.62	±	0.79	20	1.00
	lymphocyte	++	64.87	±	1.65	20	1.09
	hepatocyte	+	90.65	±	0.86	20	1.52
triploid variant (3n=69)	erythrocyte	+++	96.44	±	1.00	20	1.00
	lymphocyte	++	106.46	±	1.04	20	1.11
	hepatocyte	+	134.55	±	1.30	20	1.40

* Measurements of total relative absorbance for individual, Feulgen-stained nuclei were obtained by triplicate scans at 600 nm with a Barr and Stroud integrating microdensitometer. For each set of 60 nuclei from a particular specimen, the DNA-Feulgen value found for its erythrocytes was set equal to 1.00 to obtain the ratios given in the last column.

sort, however, should be restricted to nuclei of a given cell type. Hepatocyte nuclei from the triploid specimen, for example, contained, as expected, 50 per cent more Feulgen stain than found for hepatocyte nuclei from either of the diploid specimens of *P. formosa* (Table 1). A similar relationship holds true in comparing the levels of Feulgen staining found for erythrocyte nuclei from the same triploid and diploid specimens. Within any one specimen, however, DNA-Feulgen values for erythrocytes are significantly different from those found for hepatocytes (Table 1). An extreme example of such disparities in measured Feulgen-DNA amounts has been shown here to emphasize the potential difficulties sometimes encountered when cell samples from different tissues are to be compared. For this reason, samples of scale epithelium from

a male *P. mexicana* were processed and measured with all test samples of scales from the offspring of triploid mothers, in addition to our usual reference standard of chicken blood cells.

TABLE 2

Estimates of genome size for male and female specimens of Poecilia *spp. collected in* 1970 *from different localities in northeastern Mexico**

Collecting Location	Statistic	*Poecilia mexicana* (Diploid; 2n=46) ♂	*Poecilia mexicana* (Diploid; 2n=46) ♀	*Poecilia formosa* (Diploid; 2n=46) ♀	Variant form (Triploid; 3n=69) ♀
Río Purificación at Barretal	Mean	1.592	1.574	1.598	2.394
	± S.E.	0.0116	0.0190	0.024	0.023
	N	(5)	(3)	(5)	(7)
	95 % confidence interval	1.56-1.62	1.49-1.66	1.53-1.66	2.34-2.44
Río Soto La Marina at Vado el Moro	Mean	1.568	1.557	1.580	2.394
	± S.E.	0.0122	0.0225	0.0161	0.019
	N	(16)	(9)	(5)	(11)
	95 % confidence interval	1.54-1.59	1.50-1.61	1.54-1.62	2.31-2.39

Analysis of variance for diploid specimens

SOURCE OF VARIATION	SUM SQUARED	degrees of freedom
within groups	0.00253	37
between groups	0.00157	5

Variance ratio: 0.620
$P < 0.001$

* Numbers of different fish specimens examined at each locality for these comparisons are shown in parentheses. The average relative absorbance for 30-50 individual, Feulgen-stained erythrocyte nuclei was determined with a Barr and Stroud integrating microdensitometer for each animal. The relative absorbance for at least 50 chicken erythrocyte nuclei, simultaneously stained and similarly measured, was used as a reference standard equivalent to 2.5×10^{-12} grams DNA per cell to compute estimates of actual DNA content of nuclei from particular fish specimens. Thus, the DNA values given here summarize triplicated cytophotometric measurements of more than 2175 blood cell nuclei from a total of 61 different fishes.

RESULTS AND OBSERVATIONS

CRITERIA FOR SPECIMEN IDENTIFICATION: Triploid unisexuals of the genus *Poecilia* are identified in natural populations of fishes in terms of three criteria: (i) DNA-Feulgen cytophotometry to determine genome size for individual specimens, (ii) polyacrylamide gel electrophoresis to reveal distinctive albumin phenotypes for the same animals, and (iii) assessment of morphological characteristics in body form, color, and fin ray counts that are intermediate between those found for typical diploid specimens of *P. formosa* and females of similar age from sympatric *P. mexicana.* Representative data of genome size estimates and albumin phenotypes for specimens from two different collecting sites are shown in Tables 2 and 3, which illustrate the kinds of detailed analyses that are involved in our use of these operational definitions to establish taxonomic designations for individual specimens in populations of *Poecilia* spp. In general, triploid females contain approximately 2.3 to 2.4 $\times$ 10^{-12} g DNA per blood cell nucleus and evidence a two-component serum albumin pattern in which the faster fraction (albumin 3) stains more intensely with Coomassie brilliant blue than does the slower fraction (albumin 4). As described in detail elsewhere (5), the albumin phenotype of these triploid specimens is designated *3-dark, 4-light* to distinguish it from the single albumin fraction 3 shown in electropherograms of plasma from *P. mexicana*, the single albumin fraction 4 shown by *P. latipinna,* or the two, equally stained albumin components (fractions 3 + 4) found in electropherograms of plasma from *P. formosa.* Finally, triploid females identified by the above criteria characteristically have a body morphology intermediate between *P. formosa* and *P. mexicana* (Fig. 2) and show dorsal ray counts of 10 or 11 and anal ray counts of 8 or, rarely, 9.

Estimates of genome size for somatic cells of *P. mexicana, P. formosa,* and their proponed triploid hybrid are shown in Table 2, which includes values for 61 representative specimens in populations collected at two different localities, Barretal and Vado el Moro, in the Soto la Marina drainage. Confidence limits for the mean DNA content of cells from *P. formosa* are indistinguishable from those of either male or female *P. mexicana,* as might be predicted from the overall similarity in the karyotypes of these species (31). The confidence limits for the genome size estimated for triploid females in these same two populations, on the other hand, provide a useful diagnostic parameter by which such specimens can be unequivocally identified, and in comparative Feulgen-DNA measurements, readily distinguished from their congeneric forms (Table 1). It is also worth noting that the DNA levels found for naturally ocurring triploid unisexuals are consonant with expectations based on the probable hybrid origin of such fishes, since a haploid genome from *P. mexicana* (about 0.8 $\times$ 10^{-12} g DNA), plus the diploid somatic genome of *P. formosa* (about 1.6 $\times$ 10^{-12} g DNA), generates the DNA amount actually determined for wild-caught triploid females (2.3 to 2.4 $\times$ 10^{-12} g DNA).

FREQUENCY OF TRIPLOIDS IN NATURAL POPULATIONS OF *Poecilia*: Triploid females associated with *P. formosa* constitute a significant, but not static, component of wild fish populations in northeastern Mexico, as shown by the

TABLE 3

Identification by DNA cytophotometry of triploid specimens of Poecilia *in a population collected from the Río Purificación near Barretal, Mexico in June,* 1970. *Taxonomic designations for* P. mexicana *and* P. formosa *are based on the characteristic albumin phenotypes shown in electropherograms of their blood plasmas. Estimates of DNA amounts for fish blood cells were computed by setting the Feulgen values determined for chicken blood cell reference standards equal to* 2.5 x 10^{-12} *g per nucleus*

Specimen	Taxonomic designation	Albumin phenotype 4	3	DNA-Feulgen per nucleus mean	±	S.E.	n	Estimated DNA per cell (x 10^{-12}g)
1	*P. mexicana*			91.2	±	0.71	30	1.67 ± .015
2	*P. mexicana*			87.4	±	0.81	30	1.60 ± .017
3	triploid			130.0	±	0.90	30	2.35 ± .021
4	triploid			133.3	±	0.71	30	2.40 ± .019
5	triploid			122.8	±	0.53	30	2.22 ± .016
6	triploid			130.9	±	1.27	30	2.36 ± .027
7	triploid			126.3	±	0.74	30	2.28 ± .019
8	*P. formosa*			86.4	±	0.70	30	1.58 ± .015
9	triploid			127.4	±	1.05	30	2.31 ± 023
10	triploid			131.1	±	0.56	30	2.38 ± .017
11	*P. formosa*			86.1	±	0.42	30	1.58 ± .012
14	triploid			126.6	±	1.34	30	2.32 ± .028
19	triploid			130.6	±	0.56	30	2.36 ± 0.17
20	*P. formosa*			89.2	±	1.06	30	1.63 ± .021
22	*P. formosa*			91.2	±	0.80	30	1.67 ± .017
26	*P. mexicana*			88.6	±	0.55	30	1.62 ± .013
27	triploid			127.7	±	0.75	30	2.34 ± .019
28	*P. mexicana*			86.8	±	0.69	30	1.59 ± .015
29	*P. mexicana*			87.1	±	0.58	30	1.60 ± .014
30	triploid			129.0	±	0.62	30	2.32 ± .011
31	triploid			128.5	±	0.79	30	2.31 ± 014
32	triploid			127.1	±	0.90	30	2.29 ± .016
33	*P. mexicana*			85.8	±	0.71	30	1.55 ± .013
34	triploid			122.6	±	0.80	30	2.21 ± .014
35	triploid			127.0	±	.082	30	2.29 ± .015
36	triploid			126.7	±	1.13	30	2.28 ± .020
37	*P. formosa*			85.1	±	0.63	30	1.53 ± .012
38	triploid			131.8	±	0.69	30	2.37 ± .013
Chicken RBC standard I				138.8	±	0.512	120	2.5
Chicken RBC standard II				136.5	±	0.748	80	2.5

TABLE 4

Frequency of triploids associated with natural populations of Poecilia formosa *from various localities in the Soto la Marina drainage*

Locality and date	Spring collections						Summer collections					
	Poecilia spp.					Percent triploids (% ± S.E.)	*Poecilia* spp.					Percent triploids (% ± S.E.)
	N	*P. mexicana* ♂	*P. mexicana* ♀	*P. formosa*	triploids		N	*P. mexicana* ♂	*P. mexicana* ♀	*P. formosa*	triploids	
Arroyo la Presa												
6-25-70							54	11	12	4	27	50.0 ± 6.8
4-12-71	60	12	48	0	0	0						
4-6 & 7-14-72	125	31	94	0	0	0	67	16	43	0	8	11.9 ± 4.0
Río Casas												
4-14-71	14	0	1	6	7	50.0 ± 13.4						
4-13 & 7-15-72	35	3	3	14	15	42.9 ± 8.4	115	7	8	73	27	23.5 ± 4.0
Río Cobe												
6-25-70							18	4	13	0	1	5.6 ± 5.4
4-12-71	36	8	27	0	1	2.8 ± 2.7						
4-11 & 7-14-72	36	7	25	0	4	11.1 ± 5.2	48	3	40	0	5	10.4 ± 4.4
Río Corona												
6-24-70							36	4	15	0	17	47.2 ± 8.3
4-12-71	44	6	23	0	15	34.1 ± 7.1						
4-11 & 7-14-72	48	2	37	1	8	16.7 ± 5.4	100	3	84	0	13	13.0 ± 3.4
Río Pilón												
7-1-70							21	1	20	0	0	0
4-7-71	50	8	42	0	0	0						
4-14-72	30	1	28	0	1	3.3 ± 3.3						

Río Piriuli												
7-19-72							30	7	19	0	4	13.3 ± 6.2
Río Purificación												
at Barretal												
6-20-70							125	7	56	14	48	38.4 ± 4.4
4-9-71	75	2	61	1	11	14.7 ± 4.1						
4-5 & 7-11-72	194	12	122	17	43	22.2 ± 3.0	208	21	111	25	51	24.5 ± 3.0
Río Purificación												
at Padilla												
6-17-70							45	5	20	10	10	22.2 ± 6.2
4-10-71	3	1	1	0	1	33.3 ± 27.2						
4-4 & 7-18-72	26	3	17	4	2	7.7 ± 5.2	84	14	63	5	2	2.4 ± 1.7
Río San Antonio												
6-30-70							14	6	7	0	1	7.1 ± 6.9
4-10-71	67	14	50	1	2	3.0 ± 2.1						
4-4 & 7-17-72	60	4	52	0	2	3.3 ± 2.3	96	20	71	1	4	4.2 ± 2.1
Río Soto la Marina												
7-19-72							4	0	0	0	4	100.0
Vado el Moro												
6-19, 24, 26, 28-70							228	38	97	3	90	39.5 ± 3.2
4-10-71	234	67	153	2	12	5.1 ± 1.4						
4-10 & 7-20, 21-72	343	31	282	4	26	7.6 ± 1.4	182	23	67	16	76	41.8 ± 3.7
Vado el Sarnoso												
6-25-70							35	2	29	0	4	11.4 ± 5.4
4-14-71	27	6	17	0	4	14.8 ± 6.8						
TOTALS:	1505	218	1083	50	154	10.2 ± 0.8	1510	192	775	151	392	26.0 ± 1.1

TABLE 5

Frequency of triploids associated with natural populations of Poecilia formosa *from different sites within two localities in the Soto la Marina drainage*

Locality / Date / Site	Spring collections						Summer collections					
	Poecilia spp.					Percent triploids (% ± S.E.)	*Poecilia* spp.					Percent triploids (% ± S.E.)
	N	*P. mexicana* ♂	*P. mexicana* ♀	*P. formosa*	triploids		N	*P. mexicana* ♂	*P. mexicana* ♀	*P. formosa*	triploids	
Río Purificación at Barretal												
June, 1970:												
Site 1							14	1	9	0	4	28.6 ± 12.1
Site 2							51	1	8	10	32	62.8 ± 6.8
Site 3							60	5	39	4	12	18.3 ± 5.0
April, 1971:												
Site 1	75	2	61	2	10	13.3 ± 3.9						
April & July, 1972												
Site 1	90	1	60	7	22	24.4 ± 4.5	16	1	8	4	3	18.8 ± 9.8
Site 2	36	6	21	2	7	19.4 ± 6.6	84	5	27	10	42	50.0 ± 5.5
Site 2A	68	5	41	8	14	22.1 ± 5.0	108	15	76	11	6	5.6 ± 2.2

Vado el Moro June, 1970:												
Site 1							18	0	2	0	16	88.9 ± 7.4
Site 2							16	4	3	0	9	56.3 ± 12.4
Site 4							39	7	17	0	15	38.5 ± 7.8
Site 5							95	11	53	3	28	31.6 ± 4.7
Site 6							60	16	22	0	22	36.7 ± 6.2
April, 1971:												
Site 2	66	20	42	0	4	7.6 ± 3.3						
Site 4	62	13	48	0	1	1.6 ± 1.6						
Site 5	106	34	63	2	7	6.6 ± 2.4						
April & July, 1972												
Site 5	343	31	282	4	26	9.0 ± 1.5	68	13	44	0	11	16.2 ± 4.5
Site 6							114	10	23	16	65	57.0 ± 4.6
TOTALS:	846	112	618	25	91	10.8 ± 1.1	743	89	331	58	265	35.7 ± 1.8

data in Tables 4 and 5, which are based on our analysis of field collections from twelve different inland localities in the Río Soto la Marina drainage. A total of 546, or roughly 20 per cent, of the more than 3000 specimens of *Poecilia spp.* preserved during the 1970-1972 collecting expeditions were identified as triploid unisexuals by application of the criteria described above.

As seen by inspection of the values given in Table 4, the frequency of triploid females fluctuates markedly from year to year, from season to season, and from one locality to another. Also, there are variations in the incidence of triploids within different habitats of the same locality during particular seasons of the year (Table 5). These data suggest that there is a higher frequency of triploid females in intermediate areas of tributaries in contrast to coastal areas or headwaters. Very few triploids have been found in the more headwater habitats of Río Cobe, Río El Pilón, Río Piriuli, or at Río San Antonio (Table 4), although some 30 to 40 per cent, or more, of the specimens in summer collections from intermediate regions of the Río Purificación at Padilla or Barretal and from Vado el Moro have been identified as triploid unisexuals (Table 4). Even in the latter localities, however, where we have consistently found triploid specimens since 1966 (31), there are highly significant, seasonal fluctuations in the frequencies of triploid females (Table 5). Finally, within a single locality for a given season of any one collecting year, there are marked variations in the frequencies of triploids from different habitats, as shown by the summary of data in Table 5 for the site to site and season to season differences observed at several specific collecting stations sampled repeatedly during 1970 and 1972 at Barretal and Vado el Moro. Although the basis for these fluctuations in population structure is unclear at present and obviously requires more study, the extensive data summarized here clearly demonstrate the fact that triploid unisexuals of the genus *Poecilia* comprise a significant and persistent, if variable, feature of naturally occurring populations of *P. formosa* and *P. mexicana* in northeastern Mexico.

PERPETUATION OF TRIPLOID GENOMES: The occurrence in nature of triploid unisexuals resembling the all-female species of *P. formosa* quite logically leads to several questions about the reproduction of these forms. How do the triploid females arise in wild populations? Are they reproductively competent? What is the probable genetic constitution of their offspring? Do they consistently transmit triploid genomes to their progeny or does a reversion to the diploid status regularly occur? Are the offspring of wild-caught triploids invariably female? Or, as recently reported for a stock of triploids in the related genus *Poeciliopsis* (10), do the triploids associated with *P. formosa* occasionally give rise to male offspring? Results from laboratory breeding experiments with stocks of triploid unisexuals maintained by matings with sympatric males of *P. mexicana* provide partial answers to some of these questions and help to establish a basis for assessing the reproductive capacity of triploid females as a factor influencing the structure of natural populations of *Poecilia* spp.

As summarized by the lineages of triploid fishes depicted in Fig. 3, wild-caught triploid females and their descendents are reproductively competent and consistently transmit triploid genomes to their unisexual offspring. These results were obtained by the use of both cytophotometric and electrophoretic criteria to monitor up to four laboratory-reared generations of triploid fishes derived from the F_1 offspring of gravid, hybrid females initially collected in 1966 from the Río Purificación at Barretal (SM Pub 66) or in 1970 from Vado el Moro (Sm VM_{70}). These stocks represent the two localities in the Soto la Marina drainage where we have previously encountered the highest frequencies of triploid unisexuals in field collections made during summer months (Tables 4 and 5). To date, 12 such "founding" mothers and their descendants have given rise to a total of 269 female offspring, more than 50 of which have been confirmed as triploids, either by electrophoresis of their plasma proteins as reported elsewhere (29) or by DNA-Feulgen cytophotometry of samples of their blood cells (Table 6) or scale epithelium (Table 7).

Three of the original hybrid mothers collected in 1966 at Barretal, and identified as A, B, or C in Table 6, produced broods in the laboratory containing 10 to 18, or more, daughters. Two of these original females and ten of their F_1 offspring were identified as triploids on the basis of albumin phenotypes and by the size of the genome found in their erythrocyte nuclei (Table 6). Similar determinations of DNA content as an index of genome size were made for laboratory reared specimens of *P. formosa* and *P. mexicana.* These values are also shown in Table 6 for comparison with the DNA levels found for the offspring of triploid mothers.

A number of the F_1 laboratory-reared triploid offspring from Barretal females A and C were subsequently mated with males of *P. mexicana* from the same or a near-by locality (at Padilla). These matings gave rise to several small broods, comprising a total of 23 putative triploid females of the F_2 laboratory-reared generation. Of the nine F_2 females reaching sexual maturity and examined by Feulgen cytophotometry of scale epithelium samples, all have been found to contain triploid genomes (STROMMEN and RASCH, unpublished). Matings of these F_2 females with sympatric males of *P. mexicana* have now produced 11 small broods in all, constituting a total of 48 females of the F_3 laboratory-reared generation. In six of these cases, DNA levels consonant with the possession of triploid genomes have been found both for a particular mother and one or more of her F_3 offspring. Two of the latter have recently produced small broods totaling 11 putative triploid hybrid females of the F_4 laboratory-reared generation from the Barretal C-line stocks (Fig. 3). Two of these very young, F_4 specimens and their F_3 mothers have been assessed for triploidy by determining the DNA content of their scale epithelial cells. Both the F_3 mothers and their F_4 daughters possess triploid genomes (Table 7). For comparison, values are also shown in Table 7 for the amounts of Feulgen-DNA staining and estimated genome size found for scale epithelium cells from

TABLE 6

Estimates of DNA levels as an index of genome size in erythrocytes from laboratory-reared, diploid and triploid specimens of the genus Poecilia. *The all-female F_1 and F_2 offspring tested were derived from three different, wild-caught hybrid females originally collected in* 1966 *from the Río Purificación at Barretal, near Cd. Victoria, Mexico**

Specimen designation	DNA-Feulgen per nucleus mean	±	S.E.	n	Estimated DNA per cell ($\times$ 10-12 g) mean	±	S.E.
P. formosa	92.02	±	0.660	25	1.60	±	0.017
P. formosa	91.44	±	0.434	30	1.67	±	0.014
P. mexicana	93.02	±	0.384	30	1.71	±	0.014
P. mexicana	90.94	±	0.832	30	1.67	±	0.019
SM Pub mother "A"	134.06	±	0.558	50	2.46	±	0.020
F_1 female $8A_1$	131.82	±	0.760	50	2.32	±	0.022
F_1 female $9A_1$	130.74	±	0.624	50	2.40	±	0.021
SM Pub mother "B"	—		—	—	—		—
F_1 female $1B_1$	130.88	±	1.240	50	2.41	±	0.028
F_1 female $2B_1$	138.36	±	0.598	30	2.54	±	0.021
SM Pub mother "C"	133.94	±	1.256	30	2.46	±	0.029
F_1 female $1C_1$	139.52	±	0.616	50	2.54	±	0.022
F_1 female $3C_1$	133.74	±	0.910	60	2.45	±	0.024
F_2 female $3C_2$	128.26	±	1.006	60	2.36	±	0.025
F_2 female $7C_2$	134.96	±	1.042	30	2.48	±	0.026
Chicken Blood Cell Standard	136.12	±	1.042	150	2.50	±	0.025

* Measurements of individual, Feulgen-stained nuclei were obtained by triplicate scans at 560 mn with a Barr and Stroud integrating microdensitometer. A total of 725 values are shown. Estimates of actual DNA content for fish erythrocytes presume a value of 2.5 $\times$ 10^{-12} g DNA per cell for the chicken erythrocyte nuclei used as a reference standard here.

a male *P. mexicana* whose tissue samples were prepared and processed with those from the presumptive triploid females to serve as a separate reference standard in this experiment.

The breeding studies described here are obviously still in progress. For example, we do not as yet have experimental data to confirm the putative triploid status of the 8 or more F_4 offspring born on June 9, 1973 to specimen 9 C$_3$ (Fig. 3), a female in the Barretal line whose scale epithelium sample has already identified her as carrying a triploid genome (STROMMEN and RASCH, unpublished).

ORIGEN OF TRIPLOID FEMALES FROM *P. formosa*: During the past year we have obtained positive confirmation on both electrophoretic and cytological grounds to document a case in which an F_1 brood of four triploid females was

TABLE 7

Estimates of genome size in somatic cells from laboratory-reared specimens of Poecilia *spp. The all-female F_3 and F_4 offspring tested were derived from the progeny of a triploid female initially collected in* 1966 *from the Rio Purificación at Barretal, near Cd. Victoria Mexico* *

Specimen designation	Cell type	DNA-Feulgen per cell mean ± S.E.	n	Estimated DNA per cell ($\times 10^{-12}$ g)
116 *P. mexicana* ♂	erythrocytes	111.6 ± 2.36	20	1.60 ± 0.035
	scale epithelium	109.5 ± 1.96	40	1.57 ± 0.030
117 F_3 ♀	erythrocytes	168.3 ± 2.84	20	2.41 ± 0.044
	scale epithelium-1	159.4 ± 0.60	40	2.33 ± 0.013
	scale epithelium-2	164.0 ± 4.20	20	2.39 ± 0.063
118 F_3 ♀	scale epithelium-1	177.0 ± 1.54	40	2.58 ± 0.025
	scale epithelium-2	166.4 ± 1.19	53	2.38 ± 0.023
120 F_4 ♀	scale epithelium	165.1 ± 5.20	13	2.41 ± 0.076
121 F_4 ♀	scale epithelium	169.9 ± 1.60	40	2.43 ± 0.028
Chicken standard AJ 1	erythrocytes (from liver print)	171.2 ± 0.70	30	2.45 ± 0.019
Chicken standard AJ 2	erythrocytes (from blood smear)	174.7 ± 1.18	30	2.50 ± 0.024

* Measurements of individual, Feulgen-stained nuclei were obtained by triplicate scans at 560 nm with a Barr and Stroud integrating microdensitometer (model GN2). A total of 346 values are shown. DNA estimates for fish specimens presume a value of 2.5 $\times$ 10^{-12} g DNA per cell for the chicken erythrocyte nuclei used as a reference here.

produced from a mating that involved a diploid *P. formosa* from the Río Purificación and a sympatric male *P. mexicana* (Table 8 and Fig. 5). All four offspring showed dorsal and anal fin ray counts, plasma albumin phenotypes, and DNA levels that were indistinguishable from those found for naturally occurring triploids of the genus *Poecilia* collected from the same or near-by localities (cf. Table 3). In addition to the DNA values presented in Table 8 for the diploid mother (specimen 71) of the triploid brood (specimens 71-1, 71-2, 71-3, and 71-4), data are shown for two wild-caught triploid females (specimens 91 and 205). Also, for comparison with the data obtained for the triploid offspring of mother No. 71, plasma albumin phenotypes (Fig. 5) and estimates of genome size (Table 8) were determined in this experiment for another sexually mature *P. formosa* (specimen 72) and her small brood of laboratory-born offspring (specimens 72-1 and 72-2). All of the latter fishes were identified as *P. formosa* by their 3 + 4 albumin phenotype, their possession of about 1.6×10^{-12} g DNA per somatic cell, and by the presence of 46 chromosomes in metaphase configurations of gill epithelium preparations made from mother No. 72 according to the technique of McPHAIL and JONES (25).

DISCUSSION

Findings of the present study add support to the already considerable body of circumstantial evidence suggesting that hybridization, unisexuality, and polyploidy are important factors in the speciation of certain groups of lower vertebrates (2, 3, 4, 8, 12, 23, 33, 37, 38). From detailed analyses of several thousand specimens of the genus *Poecilia* from a dozen inland collecting localities in the Soto la Marina drainage in Tamaulipas, México, it is clear that triploid unisexuals, intermediate in form between *P. formosa* and *P. mexicana,* constitute a significant, but quite variable, feature of certain populations of wild fishes, particularly in the vicinity of Cd. Victoria, Mexico (Table 5). Many of these triploid females are reproductively competent. Their laboratory-reared descendants can be bred successfully for at least four consecutive generations by appropriate matings with sympatric males of *P. mexicana* (Fig. 3). In all of the cases examined to date, triploid females transmit triploid genomes to their all-female progeny (Tables 6 and 7), which show a common, characteristic phenotype when judged by electrophoresis of blood plasma samples or by morphological criteria such as fin ray counts (30). On these grounds, triploid females of the genus *Poecilia* can be readily and reliably distinguished from sympatric females of *P. mexicana* or the diploid unisexual, *P. formosa.*

Determinations of the DNA content of nuclei from several types of somatic cells provide an estimate of roughly 2.3 to 2.4×10^{-12} g DNA per cell as the size of the genome for triploids of *Poecilia* spp. Similar types of determinations with cells from diploid congeners yield estimates of genome size

of about 1.6×10^{-12} g DNA (Table 2). From these relationships, we infer that the elevated DNA content of the triploid genome is derived from the addition of a haploid chromosome complement, presumably the gamete nucleus from *P. mexicana.*

Postulated interactions between *P. formosa* and *P. mexicana* that result in the formation of triploid hybrids are summarized in Fig. 4. Normally, sperm from *P. mexicana* function only to stimulate embryonic development of functionally diploid eggs of *P. formosa.* The male genome itself is regularly excluded by an as yet unknown mechanism from contributing hereditary material to the resultant offspring, which exhibit strictly matroclinous inheritance. In a certain number of cases, however, failure or absence of the usual mechanism for sperm exclusion results in the fortuitous inclusion of a male gamete nucleus and the production of a triploid hybrid. The latter can be thought of as carrying one "new" or contemporary genome from *P. mexicana,* in addition to an ancestral *mexicana* genome plus an ancestral *latipinna* genome contributed by the diploid egg of *P. formosa,* itself a hybrid species derived and stabilized in times past (2, 17), supposedly by reproductive isolation from its bisexual progenitor species, *P. mexicana* and *P. latipinna.*

As depicted in Fig. 4, production of the first generation of triploid hybrids from a diploid *P. formosa* would be considered a rare event. As previously reported for cases of exceptional triploid hybrids produced in the laboratory by matings of *P. formosa* with allopatric males of *P. sphenops* or *P. vittata,* only two offspring showing some paternal characteristics were identified from among several thousand fishes (31). Recently, however, we have documented the occurrence under laboratory conditions of an F_1 brood of four triploid daughters from a wild-caught, diploid *P. formosa* (Table 8). This particular case provides direct experimental evidence in support of the general scheme shown in Fig. 4 to account for the spontaneous and probably contemporary origin of triploid unisexuals in nature. The diploid mother of this triploid brood had been isolated for several months in the laboratory and subsequently mated with a sympatric male *P. mexicana.* We do not know of any special past history effect, such as an extreme environmental stress, that can account for the simultaneous production of four triploid offspring from diploid parents. At any rate, the requisite coincidence of events during fertilization and/or syngamy in this particular case suggests that there could be several separate instances involving the origin of triploid unisexuals in populations at various localities within the range of *P. formosa* and *P. mexicana* (13, 26, 36).

In view of the marked seasonal fluctuations observed in the frequencies of triploid females from populations at Barretal or from the Vado el Moro, it is tempting to suggest that misadventures during March and April in the gynogenetic reproduction of the diploid unisexual *P. formosa* may account for the observed shift to a predominance of triploid unisexuals in field collections taken during June or July. Alternatively, triploid forms may be better

TABLE 8

Summary of evidence for the occurrence of triploid genomes in the all-female, F_1 laboratory-bred offspring from a diploid Poecilia formosa*

Specimen designation		Specimen size SBL (mm)	Morphometrics Dorsal ray count	Morphometrics Anal ray count	Plasma Albumin Phenotype	Cell type	Estimated Genome size** DNA content per cell ($\times 10^{-12}$ g) mean	± S.E.
71	*P. formosa* (2n=46)	49	10	8	3+4	erythrocytes	1.680	0.0155
						hepatocytes	1.780	0.0410
	71 F_1-1	24	10	8	3D+4L	erythrocytes	2.198	0.0294
						hepatocytes	2.339	0.0283
	71 F_1-2	29	10	8	3D+4L	erythrocytes	2.320	0.0460
	71 F_1-3	24	10	8	3D+4L	erythrocytes	2.324	0.0162
	71 F_1-4	25	10	8	3D+4L	erythrocytes	2.291	0.0119
72	*P. formosa* (2n=46)	50	11	8	3+4	erythrocytes	1.597	0.0090
						lymphocytes	1.603	0.0204
	72 F_1-1	12	11	8	3+4	erythrocytes	1.543	0.0183
	72 F_1-2	14	11	8	3+4	erythrocytes	1.510	0.0201
91	Triploid Variant (3n=69)	68	10	8	3D+4L	erythrocytes	2.481	0.0240
						hepatocytes	2.566	0.0180
205	Triploid Variant (3n=69)	35	10	8	3D+4L	erythrocytes	2.322	0.0157
						hepatocytes	2.410	0.0867
						scale epithelium	2.315	0.0188

* Mother (specimen 71) of the presumptive triploid brood (71-F_1) and other sympatric *P. formosa* (for example, specimen 72) were originally collected in 1970 from the Río Purificación at Padilla, near Cd. Victoria, Mexico. They were subsequently mated in the laboratory during 1972 with males of *P. mexicana* collected from the same locality. For comparison with data obtained from the immature offspring of mothers 71 and 72, values are also shown for two mature triploid specimens of *Poecilia* spp. collected near Barretal, Mexico in 1970. Measurements of standard body length (SBL) and counts of fin rays were made using the method of HUBBS and LAGLER (19).

Albumin phenotype 3+4 denotes the pattern for a typical *P. formosa,* whereas the phenotype 3D+4L is characteristic of variant triploid forms associated with *P. formosa* in headwater tributaries of the Soto la Marina.

** The DNA values shown summarize the results from cytophotometric measurement of the Feulgen dye content of more than 600 individual nuclei from fish blood smears or liver prints, and were computed using the pooled mean and its variance from 200 similar determinations of levels of Feulgen staining in nuclei from the chicken blood cells carried with each fish preparation as a reference standard of approximately 2.5 $\times$ 10^{-12} g per cell.

suited to withstand the stressful conditions imposed by the environment in northeastern Mexico during late spring months. Differences in the reproductive schedules of both the diploid and triploid unisexuals could also be involved, of course, since they both presumably compete for sperm with each other and with females of *P. mexicana.*

Although details of the mechanism of reproduction by unisexual triploids of the genus *Poecilia* are not known, the evidence available to date on their consistent perpetuation of triploid genomes suggests that they reproduce by gynogenesis, probably in a manner similar to that operating in their diploid relative *P. formosa.* This would involve the production of triploid eggs that only require stimulation by sperm from a related bisexual species in order to develop. A comparable situation has been described for "species" of triploid salamanders of the genus *Ambystoma* (37), in which meiosis is preceded by an endonuclear replication of chromosomes that is not followed by cytokinesis so that the resultant oöcyte contains a hexaploid chromosome complement (21). Two subsequent meiotic divisions, then, lead to the production of triploid eggs (= automixis). As recently discussed by UZZELL (37) and WHITE (38), alternative mechanisms of meiosis in unisexual systems have distinctive genetic consequences. Given appropriate genetic markers, one should be able to infer the kind of perturbation in meiosis operating for a particular system. In *Poecilia,* however, there is no compelling evidence to exclude the possibility that meiotic divisions are suppressed altogether to prevent normal reduction (= apomixis), so that the functional ova of a triploid female would convey an unreduced set of maternal chromosomes to the next generation (37). Additional information on the course of meiosis in both the diploid and triploid unisexuals of the genus *Poecilia* is needed to clarify questions posed by previous reports on the appearance of a single diploid offspring of a triploid mother (31) or the spontaneous occurrence of a diploid male among the otherwise all-female triploid offspring of a triploid mother in the related genus *Poeciliopsis* (10). The consistent perpetuation of triploid genomes in the invariably female progeny of the Barretal "C" line described earlier suggests that reversion to the diploid status is an extremely rare event in these populations, once triploidy has been established. In comparison with the large brood sizes often observed for cultures of *P. formosa* and *P. mexicana* reared in captivity (16, 18) the generally small broods of 1 to 8 offspring produced by triploid females indicate some reduction of fecundity associated with possession of the triploid genome.

By using Feulgen-stained cell samples from scale epithelium of live fish, it is now feasible to monitor the probable levels of polyploidy in entire broods of young fishes and their mothers without killing them and thereby precluding their use in future breeding studies or tissue transplantation experiments. Because scale epithelial nuclei in squash preparations are appreciably larger than erythrocyte nuclei and also contain finely dispersed chromatin, the diploid hepatocyte nuclei in liver prints of chickens provide a more appropriate reference standard than chicken erythrocyte nuclei, when estimating actual DNA content of fish epithelial nuclei.

Consideration of data on the frequency of triploid females in nature and the demonstrated capacity of these fishes to perpetuate triploid genomes immediately poses several additional questions relating to the origin and persistence of reproductively competent triploids of the genus *Poecilia*. Are all of the triploid females now found in a given collecting site derivatives from one or more triploid hybrid sibs produced by an initial, one-time-only rare event there? Is there any evidence for a spread of triploid hybrids into adjacent populations? Since triploid females have been found in quite separate and remote inland localities, these populations may very well indicate the past occurrence of different "rare events" in each of these several localities.

Under environmental conditions subject to dramatic seasonal shifts, there may be an inherent positive value in the genetic variability resulting from hybridization. Formation of polyploid hybrids, such as the triploid unisexuals of *Poecilia* spp., could represent an extreme example of bringing together all of the available genetic material needed for making accomodations to a changing environment. In this sense, unisexuals may function in natural populations as a type of "genetic bridge" between differentiated bisexual species. Even though there might be little selective pressure for long-term survival of the triploid form itself, there may be an appreciable selective advantage for the sporadic occurrence of triploid hybrids in terms of the success of the breeding complex as a whole.

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RESUMEN

Parte importante pero no estática de las poblaciones de *Poecilia* de la cuenca alta del Río Soto la Marina del noreste de México son las hembras triploides que se asemejan mucho a la especie ginogenética unisexual *P. formosa* Girard y a su congénere simpátrica bisexual *P. mexicana* Steindachner. La abundancia de estas hembras triploides fluctúa marcadamente de año en año, de estación en estación y de una localidad a otra. En estudios de reproducción experimental para determinar la competencia reproductiva de las

hembras triploides como factor influyente en la estructura de las poblaciones naturales de *Poecilia* usamos electroforesis de plasma sanguíneo. Para comprobar la persistencia de los genomas triploides en cuatro generaciones sucesivas, invariablemente de hembras, derivadas inicialmente de hembras triploides grávidas colectadas entre 1960 y 1970 en Barretal, en el Río Purificación o en Vado el Moro cerca de Ciudad Victoria, México, usamos citofotometría de ADN-Feulgen de núcleos de células sanguíneas o de epitelio de escampas. De estos datos, y del análisis de las albúminas fenotípicas de los mismos peces, se concluye que muchas de las hembras triploides son reproductivamente competentes y transmiten con regularidad genomas triploides a su descendencia unisexual. Se puede suponer que se reproducen por ginogénesis, ya que se puede mantener en el laboratorio las estirpes fértiles triploides cruzándolas con machos simpátricos de *P. mexicana*.

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Fig. 1 Map. showing 12 localities in northeastern Mexico from which populations of *Poecilia* spp. were collected during 1970-1972. Coded designations for these sites are as follows:

AP: Arroyo la Presa, 14 km N Cd. Victoria
C: Río Corona, at Corona
Co: Río Cobe, at Subida Alta
Cs: Río Casas, at Casas
Pi: Río El Pilón, at Maguayes
Pl: Río Piriuli, 110 km. E Cd. Victoria
Pu: Río Purificación, at Padilla
Pub: Río Purificación, at Barretal
SA: Río San Antonio, at Hidalgo
SM: Río Soto la Marina, at Soto la Marina
VM: Vado el Moro, 68 km E Cd. Victoria
VS: Vado el Sarnoso, 32 km E Cd. Victoria

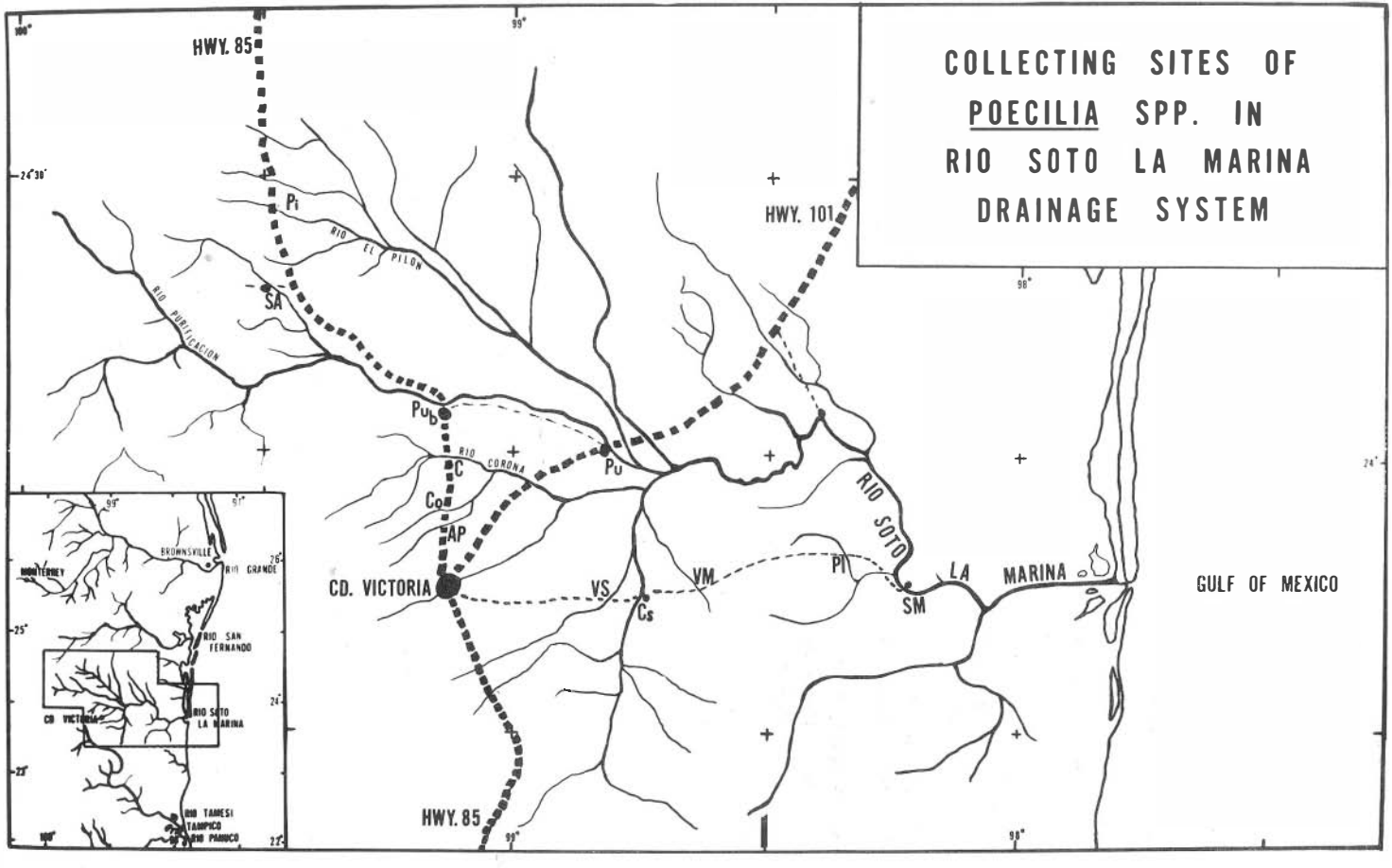
COLLECTING SITES OF
POECILIA SPP. IN
RIO SOTO LA MARINA
DRAINAGE SYSTEM
HWY. 85
HWY. 101
HWY. 85
Pi
RIO EL PILON
SA
RIO PURIFICACION
Pub
RIO CORONA
C
Co
AP
Pu
CD. VICTORIA
VS
VM
Cs
PI
SM
LA MARINA
RIO SOTO
GULF OF MEXICO
BROWNSVILLE
RIO GRANDE
MONTERREY
RIO SAN FERNANDO
CD VICTORIA
RIO SOTO LA MARINA
RIO TAMESI
TAMPICO
RIO PANUCO

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Fig. 2 Females of the genus *Poecilia* collected during 1972 from inland localities of the Soto la Marina drainage in Tamaulipas, Mexico.
A. *P. mexicana* from the Río El Pilón at Maguayes (SBL, 60 mm).
B. *P. formosa* from the Río Purificación at Padilla (SBL, 62 mm).
C. Triploid variant associated in nature with *P. formosa*, collected from the Río Purificación at Padilla (SBL, 59 mm).
D. Triploid variant associated with *P. formosa* in the Vado el Moro, 68 km. east of Cd. Victoria (SBL, 58 mm).

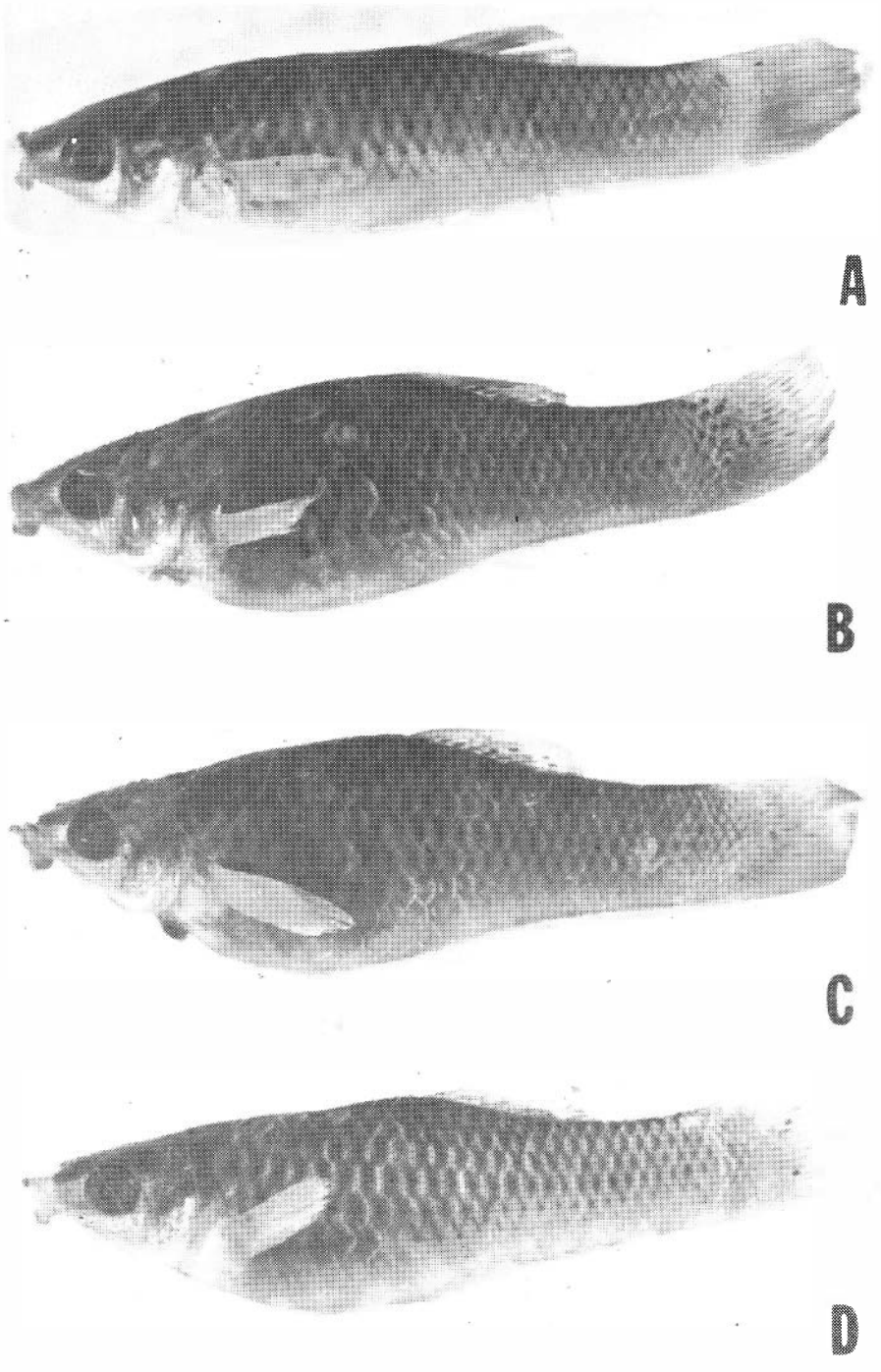

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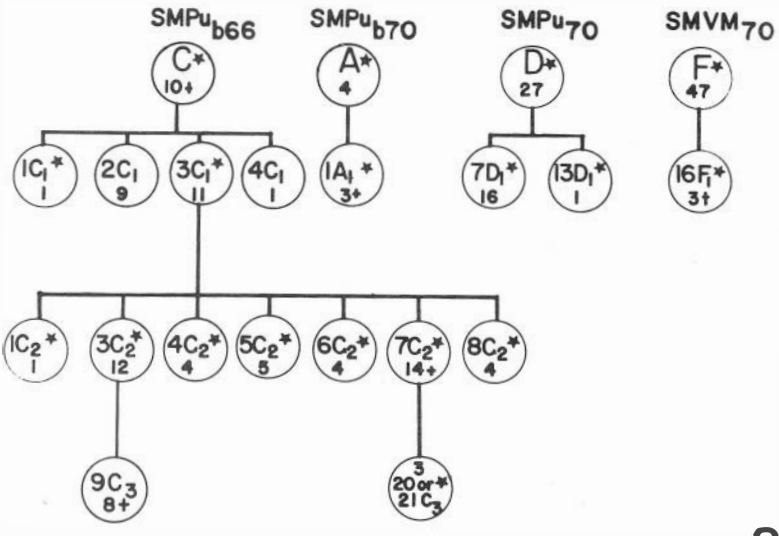
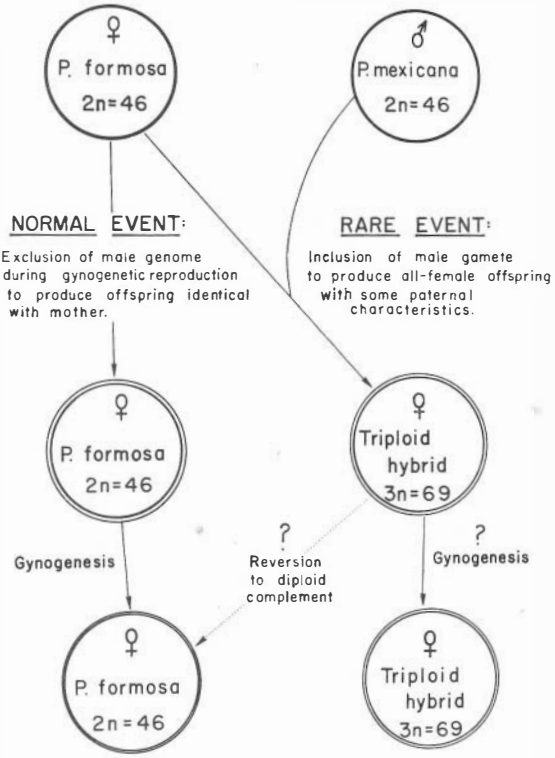
Fig. 3 Lineages of four stocks of laboratory-reared, unisexual offspring derived from wild-caught, triploid females of the genus *Poecilia*, originally collected from inland localities of the Soto la Marina drainage in Tamaulipas, México. Designations for collection sites, as in Fig. 1. Each circle represents a female, the specimen number-letter designation identifies descendants from a particular "founding" mother. An asterisk (*) denotes positive confirmation of triploid genomes in mother and one or more offspring by cytophotometric determinations of DNA levels in Feulgen-stained nuclei from erythrocytes or scale epithelium cells. The unlettered number within a circle indicates the number of young produced by each female that were reared to sexual maturity. From the four, original, wild females and their descendants shown here, more than 260 young were born, 180 of which reached maturity. Eleven of these, the offspring from specimens $9C_3$ and $20C_3$ or $21C_3$, constitute an F_4 laboratory-bred generation of triploid females produced by matings with males of sympatric *P. mexicana*.

Fig. 4. Diagram of postulated relationships between the all-female, diploid gynogen, *Poecilia formosa*, its sexually parasitized relative, *Poecilia mexicana*, and the triploid unisexuals found in nature associated with these two species.


SMPu b66
SMPu b70
SMPu 70
SMVM 70
C* 10+
A* 4
D* 27
F* 47
1C1* 1
2C1 9
3C1* 11
4C1 1
1A1* 3+
7D1* 16
13D1* 1
16F1* 3+
1C2* 1
3C2* 12
4C2* 4
5C2* 5
6C2* 4
7C2* 14+
8C2* 4
9C3 8+
3 20 or* 21C3

3


♀ P. formosa 2n=46
♂ P. mexicana 2n=46
NORMAL EVENT:
Exclusion of male genome during gynogenetic reproduction to produce offspring identical with mother.
RARE EVENT:
Inclusion of male gamete to produce all-female offspring with some paternal characteristics.
♀ P. formosa 2n=46
♀ Triploid hybrid 3n=69
Gynogenesis
?
Reversion to diploid complement
?
Gynogenesis
♀ P. formosa 2n=46
♀ Triploid hybrid 3n=69

4

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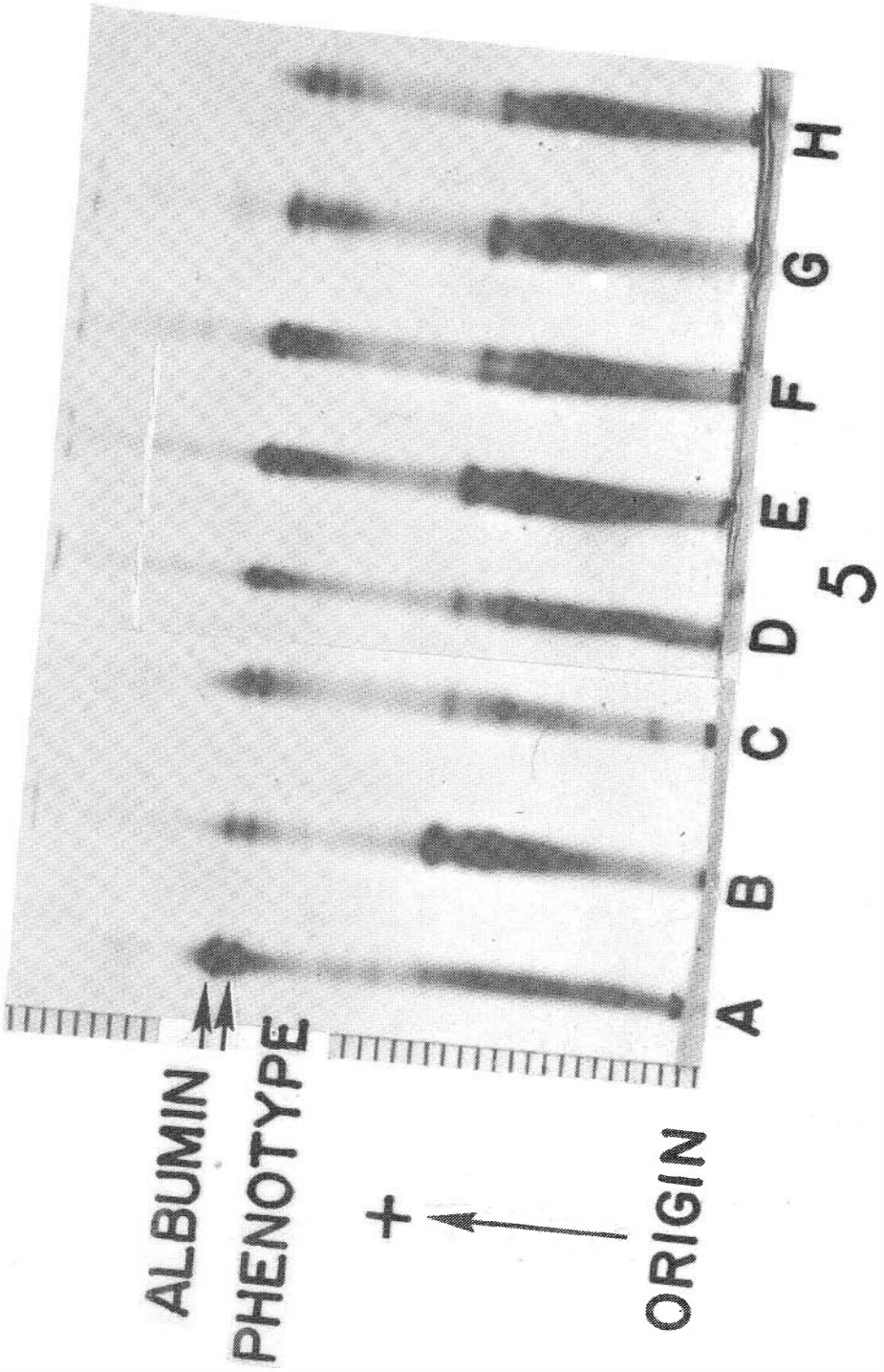
Fig. 5 Electrophoretograms of plasma proteins from females of *Poecilia* spp. for which DNA values and estimates of genome size are given in Table 7. A: Specimen 91, a triploid hybrid female collected in 1970 from the Río Purificación near Barretal, Mexico; B: Specimen 72-1, laboratory-reared F_1 diploid offspring from mother 72; C: Specimen 72, a typical, diploid *P. formosa* from the Río Purificación at Padilla; D, E, F, and G: Specimens 71-1, 71-2, 71-3, and 71-4, respectively, the laboratory-reared brood of F_1 triploid females derived from Specimen 71, a diploid *P. formosa*, whose plasma protein pattern in shown in track H.
Separations of 0.2 μl samples of plasmas were carried out in thin sheets of 8 per cent polyacrylamide gel, using tris-glycine buffer at pH 8.3 in an apparatus especially designed for vertical, discontinuous electrophoresis of microsamples of tissues (7). Gel stained for 48 hours in 0.1 per cent Coomassie brilliant blue and then destained in 7 per cent acetic acid. Each interval of the scale shown at the left of the figure represents 1 mm.


A
B
C
D
E
F
G
H
5
ALBUMIN
PHENOTYPE
+
ORIGIN