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Biochemical and cytogenetic studies of Poecilia from eastern México.
I. Comparative microelectrophoresis of plasma proteins of seven species*

by

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ABSTRACT: Over 2000 fisch plasmas from six species of *Poecilia* were collected from 33 populations in eastern Mexico and one from western Mexico. These plasmas were electrophoretically separated in 7.5% polyacrylamide gel which was stained for specific enzymes or total protein. Identifications of albumin band mobilities were verified by mixing isoaliquots of test plasmas with plasmas of known standards and by comparing test plasmas with plasmas from F_1 hybrid progreny of known parentage.

In the *latipinna* complex *P. latipinna* is polymorphic in albumin phenotype whereas *P. velifera* has an albumin mobility that is intermediate between two of those of *P. latipinna;* an unidentified *petenensis*-like species exhibits the slowest migrating albumin whose mobility is identical to that found in the distantly related *P. latipunctata.* In the *sphenops* complex *P. mexicana* is polymorphic, containing five electrophoretically different albumins whereas *P. sphenops* tentatively appears to be monomorphic. *P. formosa* is characterized by two albumin bands in its plasma that are electrophoretically identical to those of northern *P. mexicana* and western *P. latipinna.*

The poeciliid fishes of Middle America have proven particularly refractory to classical taxonomic analysis. Insight into the complex evolutionary processes of the various groups can be achieved only through multidisciplinary analysis.

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For the past several years species of the genus *Poecilia* (commonly called mollies) inhabiting eastern Mexico have been under investigation in our laboratories through an extensive breeding program coupled with techniques of morphology, plasma electrophoresis, cytology, and ecology. This report is concerned with variation in plasma proteins found in natural populations.

General distribution patterns of *Poecilia* species inhabiting eastern Mexico were summarized by DARNELL and ABRAMOFF (8), MILLER (16), and ROSEN and BAILEY (18). *P. latipinna* and *P. velifera* are morphologically quite similar and have been considered to be closely related, if not conspecific. Both are euryhaline coastal forms which are seldom found far inland. The former species occurs along the south Atlantic and Gulf coasts from South Carolina to the vicinity of Tuxpan, Mexico. The latter is limited to the Yucatán peninsula. Populations occurring at the base of the Yucatán peninsula have characteristics of both species, and their identity is, at present, questionable. An apparently isolated population occurring well inland in the Río Usumacinta system is clearly related but has some distinct morphological features.

P. sphenops and *P. mexicana* constitute another species pair of close morphological similarity. Although often confused in the past, these species are clearly distinguishable on the basis of dentition and other characters (SCHULTZ and MILLER, 19). Both occur far inland as well as in coastal habitats. *P. mexicana* occurs in tributaries of the Río Grande and in various drainages down into South America. *P. sphenops* reaches its northern distributional limit a short distance north of Veracruz and occurs well down into Central America. Both species are also known from the Mexican west coast where they are sympatric with *P. butleri*.

An all-female species, *P. formosa,* is morphologically intermediate between *P. latipinna* and *P. mexicana* (HUBBS and HUBBS, 12, 13). Its plasma contains two albumins: one is identical in electrophoretic mobility to the albumin found in western populations of *P. latipinna* and the other is identical to the albumin found in northern populations of *P. mexicana* (ABRAMOFF et al., 1). *P. formosa* occurs naturally from the coastal waters of southern Texas down to Tuxpan and well inland in tributaries of three intervenning streams, the Ríos San Fernando, Soto la Marina, and Tamesí.

P. latipunctata, which is morphologically quite distinct, is limited to certain tributaries of the Rio Tamesí (DARNELL, 7). Although there are other mollies found in eastern Mexico, they were not collected by us and, hence will not be considered here.

MATERIAL AND METHODS

Specimens for the present study were taken from 33 collecting sites representing every major drainage and many minor waters of the western Gulf of Mexico. Additional samples were taken from Baton Rouge, Louisiana, and the Río Grijalva and Yucatán in southeastern Mexico. Several of the localities were sampled repeatedly. Details are summarized in Table 1 and Fig. 1. Sam-

TABLE 1

Field sampling data. Stations are listed from north to south

Code	Drainage	Locality	Dates	Species
M-BR	Mississippi River	Baton Rouge, La.; drainage ditch below lake on Louisiana State University campus; 30°23'N, 91°11'W.	4-1-63, 8-26-63	*P. latipinna*
RG-RRV	Río Grande	Resaca del Rancho Viejo; coastal tributary near Lulu Sam's state fish hatchery; Hwy 77, 8 km. N Brownsville, Texas; 25°56'N, 97°29'W.	3-17-64, 4-17-65 7-28-65, 7-17-66 6-25-68	*P. formosa* *P. latipinna*
RG-C	Río Grande	Río Cadereyta (or Río Monterrey); Hwy 40 at Cadereyta, 34 km E Monterrey; 25°35'N, 99°58'W.	7-14-65, 6-24-68	*P. mexicana*
SF-C	Río San Fernando	Río Comacho; Hwy 85 west of Linares, 132 km SE Monterrey; 24°53'N, 99°37'W.	7-15-65, 6-23-68	*P. formosa* *P. mexicana*
SM-Pu	Río Soto la Marina	Río Purificación; Hwy 101 at Padilla, 52 km NE Victoria; 24°1'N, 98°47'W.	3-24-64, 4-15-65 7-29-65	*P. formosa* *P. mexicana*
SM-Pub	Río Soto la Marina	Río Purificación; Hwy 85 at Barretal, 40 km N Victoria; 24°5'N, 99°8'W.	7-16-65, 7-17-66 6-22-68	*P. formosa* *P. mexicana*
SM-VM	Río Soto la Marina	Vado el Moro; about 60 km E Victoria; 23°45'N, 98°36'W.	6-21-68	*P. formosa* *P. mexicana*
T-G	Río Tamesí	Río Guayalejo; Hwy 85 at Llera, 59 km S Victoria; 23°16'N, 99°1'W.	4-9-63, 3-17-64 4-9 65, 4-15-65 7-17-65, 7-28-65 6-20-68	*P. formosa* *P. latipunctata* *P. mexicana*
T-Gx	Río Tamesí	Río Guayalejo; at Xicotencatl, 39 km N Mante; 23°1'N, 98°40'W.	7-17-65, 7-16-66 6-18-68	*P. formosa* *P. latipunctata* *P. mexicana*

TABLE 1 (cont.)

Code	Drainage	Locality	Dates	Species
T-S	Río Tamesí	Río Sabinas; two sites near Encino, **50 km N Mante;** nacimiento of Río Sabinas (T-Snac), 23°8'N, **99°9'W;** Arroyo Sarco (T-Ssar), 23°7'N, 99°7'S.	3-21-64, 4-11-65	*P. mexicana*
T-Bo	Río Tamesí	Río Boquilla; east of Chamal, 25 km NW **Mante;** 22°43'N, 99°9'W.	3-20-64, 4-10-65 6-18-68	*P. mexicana*
T-M	Río Tamesí	Río Mante; at dam 6 km W Mante; 22°38'N, **99°1'W.**	3-19-64, 4-10-65 7-18-65, 7-16-66 3-20-67, 6-19-68	*P. formosa* *P. latipunctata* *P. mexicana*
T-LCh	Río Tamesí	Laguna de Champoyan; coastal lagoon, Hwy 80 at Altamira, 23 km N Tampico; 22°23'N, 97°56'W.	3-23-64, 4-13-65 7-21-65	*P. formosa* *P. latipinna* *P. mexicana*
T-LPt	Río Tamesí	Laguna de la Puerta; coastal lagoon, Hwy 80, S Altamira, 22 km N Tampico; 22°22'N, 97°56'W.	7-21-65, 7-15-66 3-20-67, 6-17-68	*P. formosa* *P. latipinna* *P. mexicana*
P-AG	Río Pánuco	Arroyo de los Gatos; Hwy 80 near Nuevo **Morelos,** 46 km SW Mante; 22°32'N, 99°14'W.	3-22-64, 4-14-65	*P. mexicana*
P-Pi	Río Pánuco	Tributary at Puente Pimienta; Hwy 105, **91 km NW** Tuxpan; 21°12'N, 98°4'W.	7-27-65	*P. mexicana*
Tn-Az	Río Tuxpan	Tributary at Alazán; Hwy 105, 45 km **NW Tuxpan;** 21°9'N, 97°46'W.	7-14-66, 6-16-68	*P. mexicana*
Tn-LT	Río Tuxpan	Laguna de Tampamachoco; coastal lagoon at **La Barra,** 11 km E Tuxpan; 20°58'N, 97°20'W.	7-14 66	*P. formosa* *P. latipinna* *P. mexicana*
Tn-AF	Río Tuxpan	Río Tuxpan at Alamo ferry crossing; 38 km **W Tuxpan;** 20°10'N, 97°56'W.	6-15-68	*P. mexicana*

Tn-A	Río Tuxpan	Tributary south of Alamo; 14 km N Tihuatlan which is 33 km SW Tuxpan; 20°49'N, 97°34'W.	7-13-66	*P. mexicana*
Tn-T	Río Tuxpan	Tributary 9 km N Tihuatlan which is 33 km SW Tuxpan; 20°47'N, 97°33'W.	7-23-65, 7-13-66	*P. mexicana*
C-C	Río Cazones	Río Cazones; at bridge NW of Poza Rica, 57 km S Tuxpan; 20°33'N, 97°29'W.	7-23, 26-65	*P. mexicana*
C-Ca	Río Cazones	Río Cazones; at Cazones, 39 km S Tuxpan; 20°43'N 97°19'W.	7-13-66	*P. mexicana*
GM-V	Gulf of Mexico	Río de Vega (or Río Colipa); coastal river, Hwy 180 at Vega de Alatorre, 184 km S Tuxpan and 164 km N Veracruz; 20°1'N, 96°37'W.	7-24-65, 7-12-66 3-18-67, 6-15-68	*P. mexicana*
SC-SC	Río San Carlos	Río San Carlos; Hwy 180 at Ursulo Galvan, 66 km SE Jalapa; 19°24'N, 96°21'W.	7-25-65, 7-12-66 6-13-68	*P. mexicana* *P. sphenops*
SC-PM	Río San Carlos	Río Paso de la Milpa; Hwy 140, 43 km SE Jalapa; 19°24'N, 96°38'W.	7-11-66, 3-19-67 6-14-68	*P. mexicana* *P. sphenops*
J-M	Río Jampa	Coastal tributary near Medillín; 12 km S Veracruz; 19°5'N, 96°9'W.	7-25-65, 7-11-66	*P. mexicana* *P. sphenops*
GM-AP	Gulf of Mexico	Arroyo Piedra; Hwy 180 between La Piedra and Salinas, 44 km SE Veracruz; 18°54'N, 96°0'W.	7-9-66, 3-18-67	*P. sphenops*
B-Belt	Río Blanco	Río Blanco; at bridge S Entronque la Tinaja on Hwy 150, 60 km S Veracruz; 18°45'N, 96°24'W.	7-8-66	*P. mexicana*
Mo-Mo	Río Moreno	Río Moreno; at road to Tierra Blanca, 86 km S Veracruz; 18°37'N, 96°23'W.	7-8-66	*P. mexicana* *P. sphenops*
E-E	Río Estanzuela	Río Estanzuela; at road to Tierra Blanca 94 km S Veracruz; 18°33'N, 96°22'W.	7-8-66	*P. mexicana* *P. sphenops*
Pn-LC	Río Papaloapan	Laguna de Conejo; coastal lagoon, Hwy 180, S Buena Vista, 84 km SE Veracruz; 18°42'N, 95°37'W.	7-9 66, 6-12-68	*P. mexicana* *P. sphenops*
Pn-SJ	Río Papaloapan	Tributary of Río San Juan; Hwy 180, 27 km NW Acayucan; 18°6'N, 95°4'W.	6-12-68	*P. mexicana*
Gr-CP	Río Grijalva	Tributary near Ciudad Pemex which is 65 km SE Villa Hermosa, Tabasco; 17°53'N, 92°30'W.	6-10, 11-68	*P. mexicana* *P.* spp.
GM-P	Gulf of Mexico	Coastal cienega 2 km S Progreso, Yucatan; 21°16'N, 89°39'W.	3-16-67	*P. mexicana* ***P. velifera***

ples of *P. sphenops* from the Río Papagayo and of *P. butleri* from the Río Tepic on the Mexican west coast were also included through the courtesy of R. R. Miller of the University of Michigan Museum of Zoology.

All fish collections were made with seines and transported to local motels where fish were bled. Blood was collected in heparinized capillary tubes through cardiac puncture of anesthetized fish. The plasma was separated from the blood cells by centrifugation at 1200 rpm for 8 to 10 minutes. All samples were initially kept in dry ice and then stored at -20 C. Plasma samples were obtained from 2137 adult fish, predominantly females.

Plasma proteins were separated electrophoretically in 7.5% polyacrylamide gels using tris-glycine buffer at pH 8.3 as described by DAVIS (9) and modified by BALSANO and RASCH (4) for horizontally-run gel sheets. This modification incorporates the following features: (i) only one to three microliters of plasma are required for each sample; (ii) from 10 to 15 plasmas are simultaneously separated within a single, thin sheet (100 × 90 × 0.8 mm) of polyacrylamide gel; (iii) a 25 ma current is applied for 35 to 45 minutes which results in minimal heating of the gel, which can be eliminated for enzyme studies by placing a tray of crushed ice on the glass cover of the gel; (iv) the mobilities of particular protein fractions can be compared directly, thus eliminating the need to calculate relative mobilities; (v) replicate samples of a single plasma in a given gel sheet can be processed through separate visualization procedures to identify different classes of enzymes such as lactate dehydrogenase isomers or various esterases (SHAW, 20; DESSAUER, 10). Mobilities of bands showing the same enzymatic activity can then be compared directly for samples from different fish or can be compared with other migration tracks of the same plasma samples stained for total protein; (vi) resultant thin gel sheets of uniform thickness are easily photographed on a translucent glass light box or they can be mounted on glass slides for densitometric evaluation. Further details for handling fish and for preparation and analysis of plasmas are described elsewhere (2, 4).

RESULTS

Electropherograms with generalized results are shown in Fig. 2. Although plasmas were analyzed for ten different enzymes, consistently reproducible results were only obtained for general esterases, lactate dehydrogenases, and peroxidases. Most of our biochemical data, however, are concerned with plasma albumin because this protein exhibited considerable phenotypic variation in different species of *Poecilia* and it was stable over long periods of storage and after repeated freezing and thawing. The fastest moving, darkly stained band was verified as albumin in an immunoelectrophoretic set-up: electropherograms of fish plasmas and purified bovine albumin were cut into lengthwise strips; one strip was stained to localize the various protein bands, and the other was tested with an absorbed anti-albumin reagent in an agar matrix; arcs of precipitation formed only in the region of the protein band that was designated albumin in the stained half of the electropherograms of both the fish plasmas and the purified bovine albumin

control (BALSANO, 2). The phenotypic variation included differences in the electrophoretic mobilities of albumin bands (Fig. 3) and the number and staining intensities of albumin bands within individual plasmas (Figs. 2 and 3). The numbers of specimens that exhibited these various phenotypes are summarized in Table 2: about 60% (1307) had single albumin band phenotypes whereas about 40% (830) had two to four albumin bands of varying staining intensities. At this time no attempt was made to specifically identify the specimens with the multiple banded phenotypes within the *P. latipinna-velifera* complex and within the *P. mexicana-sphenops* complex because the majority of these specimens were collected in southeastern Mexico where the systematics of these fishes is under current study.

TABLE 2

Summary of albumin phenotypes observed in Poecilia *species**

	Total Number	Albumin Mobility Values 1	2	2½	3	3½	4	5
Single banded phenotypes:								
P. latipinna (eastern population)	39				x			
" " (western populations)	52						x	
P. velifera	6					x		
Poecilia sp. (Ciudad Pemex population)	6							x
P. mexicana limantouri (northern subspecies)	824				x			
" " (coastal populations)	172		x					
" " (southern inland populations)	11	x						
" " (southern coastal populations)	10						x	
" " (southern coastal populations)	4					x		
P. sphenops (east coast)	97			x				
" " (west coast)	7			x				
P. butleri (west coast)	11					x		
P. latipunctata	68							x
Multiple banded phenotypes:								
P. formosa	314				x		x	
triploids associated with *P. formosa*	177				x		x	(x)
P. latipinna-velifera complex	106		x		x	x	x	x
P. mexicana-sphenops complex	233	x	x	x	x	x	x	x
Grand Total	2137							

* The mobility values indicate proximity of albumin band to anode, with number 1 having the fastest mobility. An "x" indicates which mobility values were observed in one or more of these plasmas. Two to four bands with various staining intensities were observed in the multiple banded phenotypes.

Since a modification of the DAVIS (9) method of electrophoresis was employed, the relative mobilities of various proteins could be due to molecular size, shape, charge or a combination of these factors. Consequently, three dif-

ferent methods were used to confirm these observed differences; typical data are shown in Table 3. The plasma mixing method involved mixing isoaliquots of an unknown plasma with one or more plasmas containing albumins of known mobilities; the resultant positions of albumin bands provided the basis for the currently employed numbering system. The albumin band with the fastest anodal migration was designated number 1 whereas the slowest was designated number 5. One-half was assigned to an albumin band mobility which overlapped the mobilities of two electrophoretically distinct bands. It is important to realize that this numbering system refers to relationships in the sequence of mobilities which may not necessarily correspond to amino acid sequences and genetic relationships. The cellulose acetate results were obtained with the standard model R-100 Beckman Microzone Electrophoresis System (published by Spinco Division of Beckman Instruments, Inc.; Palo Alto, California). The fastest migrating albumin band was the standard set at 100 and the slower migrating albumins were compared to it. Using different concentrations of acrylamide gel yielded different values for specific albumin bands but the relative sequence, i.e., which band migrated slowest, fastest, etc., remained the same. Similar results were obtained when different amounts of current and/or time for electrophoresis were used. DAVIS (9) discussed the interpretation of results obtained by procedural variations in his study of human plasma and BALSANO (2) and BALSANO and RASCH (4) modified these procedures to better resolve proteins in the plasmas of poeciliid fishes. On the basis of the results from these three different methods we conclude that variations in albumin band mobilities are due to electrophoretic charge rather than to molecular size or shape.

TABLE 3

Relative mobilities of albumins of Poecilia *spp.**

Species	Values based upon various methods: Plasma Mixing	Cellulose Acetate	Polycrylamide 8.0%	Sheets 7.5%
P. mexicana	1 (fastest)	1.00		
P. mexicana	2	.92	.604	.638
P. sphenops	2½	.89	.577	.610
P. mexicana	3	.85	.569	.595
P. velifera	3½	.81		.584
P. formosa	3 + 4	.85 + .76	.571 + .545	.596 + .573
P. latipinna	4		.546	.579
P. mexicana	4			.582
P. latipunctata	5 (slowest)	.69	.519	.568

* All values given for cellulose acetate are taken from a single electropherogram; the same is true for each concentration of polyacrylamide gel.

Fig. 4 illustrates that these differences in relative mobilities as well as differences in staining intensity are readily demonstrated by densitometric analysis of the electropherograms using a Leitz microspectrophotometer. The plasma samples from *P. latipunctata* (albumin mobility 5) and *P. mexicana* (albumin 1) have about the same amount of albumin and it is less than the amount in the plasma samples from *P. latipinna* (albumin 4) and *P. mexicana* (albumin 3). These comparisons and differences in relative albumin concentration apply to the samples analyzed and should not be construed to apply to the species.

Ten different enzyme staining techniques described by DESSAUER (10) were initially used, including acid and alkaline phosphatase, ceruloplasmin, cholinesterase, general esterases, glucuronidase, lactate dehydrogenase, leucine amino peptidase, peroxidase, and transferrin. Consistently reproducible data were only obtained with staining techniques for general esterases, lactate dehydrogenases, and peroxidases, probably because most of these plasmas had been stored for as long as a year at -20 C. These data are summarized in Figs. 5 and 6. Since these data were derived from many different electropherograms, all enzyme relative mobilities were normalized in relation to the albumin band set at 85.0 for albumin band 3 of *P. formosa* and *P. mexicana* and 89.0 for albumin band of *P. sphenops*; these were the relative albumin band mobilities determined on cellulose acetate (Table 3). For these three enzymes the all-female *P. formosa* appears to be as variable as the bisexual *P. mexicana*, both of which are more variable than *P. sphenops*. These results suggested that several of these enzymes are polymorphic. Preliminary data indicated that F_1 hybrids possess unique esterase bands as well as those exhibited by both parents. Detailed breeding experiments, however, have not been carried out to determine the mode of inheritance for these proteins.

When plasmas containing albumins of different mobilities were mixed and then electrophoretically separated, the resulting electropherograms displayed albumin patterns identical with the albumin pattern of F_1 hybrids. Similarly, pre-electrophoretic mixing of plasmas from parent and F_1 hybrid offspring resulted in a dark and a light albumin band on the electropherogram, i.e., the intensity of one band was increased, indicating the identity of that band and the lack of interference by the second band (Fig. 7). The same is true for comparisons of 123 plasmas from intraspecific and interspecific F_1 hybrids with plasma from their corresponding parental types. Consequently, any question regarding the relative mobility of an albumin band was resolved by mixing samples of the plasma in question with one or more plasmas containing albumins of known mobilities. A summary of such mixings is given in Table 4. No differences were observed in reciprocal matings or in plasma mixings of male versus female plasmas. The albumin phenotypes that resulted from the electrophoresis of these mixed plasmas were always in complete agreement with predicted results.

Since the protein concentrations of these plasmas were quite variable (i.e., 214 $\pm$ 77.5 μg protein/10 μl plasma), it was important to demonstrate

TABLE 4

Summary of results of isoaliquot mixtures of plasmas to confirm albumin mobility phenotypes in Poecilia *spp**

Species (source of sample population or albumin types)	Sample Size	Albumin mobility values 1	2	2½	3	3½	4	5
P. latipinna (albumin 4) plus:								
P. latipinna (eastern)	2				x		x	
P. mexicana (southern coastal)	1						xx	
P. sphenops (east coast)	5			x			x	
P. mexicana (albumin 3) plus:								
P. latipinna (eastern)	1				xx			
" " (western)	7				x		x	
" " (3 + 4 albumins)	1				xx		x	
" " (2 + 3 + 4 albumins)	3		x		xx		x	
" " (3 + 3½ + 4 albumins)	1				xx	x	x	
P. velifera	1				x	x		
P. mexicana (northern inland)	447				xx			
" " (coastal)	102		x		x			
" " (southern inland)	10	x			x			
" " (southern coastal)	2				x		x	
" " (2 + 3 albumins)	54		x		xx			
" " (1 + 3 albumins)	3	x			xx			
" " (3 + 4 albumins)	5				xx		x	
" " (2 + 3 + 4 albumins)	1		x		xx		x	
P. sphenops (east coast)	23			x	x			
P. formosa	75				xx		x	
P. latipunctata	17				x			x
P. mexicana (albumin 2) plus:								
P. latipinna (western)	1		x				x	
P. mexicana (coastal)	51		xx					
" " (southern inland)	8	x	x					
" " (2 + 3 albumins)	12		xx		x			
" " (3 + 4 albumins)	1		xx		x		x	
" " (1 + 3 albumins)	1	x	xx		x			
P. sphenops (east coast)	20		x	x				
P. formosa	11		x		x		x	
P. mexicana (albumin 1) plus:								
P. mexicana (southern inland)	2	xx						
P. sphenops (albumin 2½) plus:								
P. velifera	1			x		x		
P. mexicana (southern inland)	4	x		x				
P. sphenops (east coast)	26			xx				
P. formosa (albumins 3 + 4) plus:								
P. latipinna (eastern)	3				xx		x	
" " (western)	8				x		xx	
P. velifera	2				x	x	x	
P. mexicana (southern inland)	1	x			x		x	
P. butleri (west coast)	4				x	x	x	
P. sphenops (east coast)	10			x	x		x	
" " (west coast)	5			x	x		x	
P. formosa	13				x		x	
P. latipunctata	6				x		x	x
P. latipunctata (albumin 5) plus:								
P. velifera	1					x		x
P. mexicana (southern inland)	1	x						x
Total:	953							

* Darker bands are designated by "xx".

that an albumin band from a plasma of low protein concentration would still be visible when mixed with a plasma sample containing a high protein concentration. Fig. 8 illustrates that two different albumin bands could be identified from mixed plasmas whose protein concentrations varied as much as 54 μg to 442 μg/10 μl of plasma, which extends over the range of observed protein concentrations of all our samples. Furthermore, Fig. 9 demonstrates that Coomassie Brilliant Blue which was selected as the most sensitive protein stain available for quantitative densitometry (FISHBEIN, 11) follows Beer's law for the amounts of plasma that were employed, i.e., usually 1 to 2 μl volumes and rarely 3 μl volumes. Hence, relative approximations could be made of the albumin concentrations within a single sample or among samples within a single electropherogram (Figs. 10 and 11).

The identity of the two albumin mobilities of *P. formosa* with those of the F_1 hybrid and the synthetic mixture of plasmas from *P. latipinna* and *P. mexicana* is clearly evident in Figs. 10 and 11. Such data were presented by ABRAMOFF *et al.* (1) to substantiate the postulated origin of *P. formosa*. Fig. 11 also illustrates the relative concentration differences in the albumin bands of *P. formosa*, a diploid, as compared with its associated triploid hybrid: the two bands in the diploid comprise .472 $\pm$ .036 and .527 $\pm$ .036 of total albumin in contrast to the triploid variant which has .638 $\pm$ .022 and .356 $\pm$ .023 for bands 3 and 4, respectively, which suggested that the triploids contained an additional "dosage" of albumin 3 when compared with *P. formosa* (3, 17).

DISCUSSION

The current evidence indicates that *P. latipinna* is at least dimorphic in albumin type. The Lousiana population (eastern) is characterized by albumin mobility 3 which is faster than albumin mobility 4 characteristic of the Brownsville, Texas population (western). The two Tampico populations of *P. latipinna* contain these two albumins plus a third one, albumin mobility 2. This albumin, however, was observed only in multiple banded phenotypes and never as a single band. Albumin mobility 2 is one of the two most common bands observed in *P. mexicana* populations. Since these two species are sympatric in the Tampico area and they are known to hybridize, we postulate that albumin 2 of *P. latipinna* was derived from *P. mexicana* via introgression. *P. velifera* is characterized by an albumin whose mobility is intermediate, i.e., 3½, between the two types commonly found in *P. latipinna* populations. These data agree with the morphology-based conclusion that *P. latipinna* and *P. velifera* belong to the same complex. In the Río Grijalva population there were some *P. petenensis*-like specimens that had the ventral portion of the caudal fin elongated and melanistic. These specimens were characterized by an albumin mobility 5. Initially one might postulate that there is a third species in this complex. Such an hypothesis must, however, be evaluated in light of the fact that most of the long-finned specimens from the Papaloapan-Grijalva region of Mexico had multiple banded albumin phenotypes containing various combinations of albumins 3, 3½, 4 and 5.

P. mexicana exhibited the greatest amount of albumin phenotype variation among the seven species examined. Five single banded phenotypes were found: albumin mobilities 1, 2, 3, 3½, and 4. Types 2 and 3 are the most abundant ones in eastern Mexico; they are characteristic of the recently described (MENZEL and DARNELL, 14) southern and northern subspecies, respectively. Multiple banded phenotypes contained various combinations of all seven observed albumin mobilities; four albumin bands were observed in three plasma electropherograms. Both the east coast and west coast populations of *P. sphenops* exhibited albumin mobility 2½ which is intermediate between the albumin mobilities of the two most abundant forms of *P. mexicana.* All except one fish that possessed tricuspid dentition, which is regarded as diagnostic for *P. sphenops*, had an albumin mobility of 2½; one had a multiple banded phenotype containing albumins 1 + 2½. There were, however, five plasmas with an albumin mobility 2½ that came from specimens of *P. mexicana,* in addition to the many multiple banded phenotypes containing albumin mobility 2½. These data suggest that either there may be more differentiation within *P. mexicana* than what is currently recognized or that there is considerable polymorphism and/or hybridization occurring in this species.

P. formosa invariably possesses a double albumin pattern: albumin 4 is identical in mobility to the western *P. latipinna* and to some *P. mexicana* from southern coastal populations; albumin 3 is identical in mobility to that of northern *P. mexicana* and eastern *P. latipinna.* Theoretically this 3 + 4 albumin pattern of *P. formosa* could have been derived as a hybrid from four possible combinations, three of which are excluded on the basis of other information. *P. formosa* is morphologically intermediate between *P. latipinna* and the northern subspecies of *P. mexicana.* Its distributional range is sympatric with *P. latipinna* and/or *P. mexicana* north of Tuxpan, Mexico (DARNELL and ABRAMOFF, 8). Such data led ABRAMOFF *et al.* (1) to conclude that *P. formosa* originated as a hybrid between western *P. latipinna* and northern *P. mexicana.* Recent studies have shown that triploids frequently occur in populations of *P. formosa* (3, 15, 17). These triploids show one albumin band stained darker than the other, suggesting that *P. mexicana* males or more rarely *P. latipinna* males contribute a haploid genome to a presumably diploid egg of *P. formosa.* Three triploids exhibited triple albumin bands: the typical 3 + 4 plus a slower migrating band, presumably albumin 5 of a *P. latipunctata* male (3). These triploids may be evolutionarily significant to *P. formosa* populations if they can provide new genetic material into a relatively closed genetic system. The enzyme data suggest that the all-female, gynogenetic *P. formosa* may be as variable as its sympatric, bisexual congeners. It would appear that more than the accumulation of mutations along essentially asexual lines is needed to account for such variability.

All specimens of *P. latipunctata* from three different populations exhibited a single banded phenotype of albumin 5. These data suggest that this species is monomorphic and rarely, if ever, hybridizes with its sympatric congeners.

It is informative to consider the albumin mobility data in light of other available information regarding the various species of *Poecilia* in eastern Mexico. The most parsimonious explanation to account for all these albumin mobilities would assume that the ancestral poeciliid had an albumin with an intermediate mobility, i.e., about 3. Albumins of the *P. latipinna-velifera* complex are all identical in mobility to those found in the *P. mexicana-sphenops* complex which possesses, however, two additional albumins, i.e., 1 and 2½. Thus, it would appear that divergence between these two major complexes has been expressed in terms of their morphologies, habitats and distributions rather than in their albumins. This implies that morphology represents the better parameter for distinguishing poeciliid species whereas albumin phenotypes represent the better means for distinguishing intraspecific taxa, at least as they are currently recognized.

Genetic variability enables a species to evolve in response to changes. This same variability may also allow a population to exploit a constant or changing environment more efficiently than a single genotype could do (CAIN & SHEPPARD, 6; BAND, 5). For major evolutionary advances to occur, a highly variable population must be placed in a rapidly changing environment. Such an environment offers the population new ecological niches to which it can adapt. The evolutionary lines most likely to take advantage of a changing environment are those in which variation due to genetic recombination is raised to a maximum. The widespread distribution of *P. mexicana* in diverse, changing environments in eastern Mexico may be, in part, the result of the various albumin types that this species possesses.

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RESUMEN

El plasma de más de 2000 ejemplares de *Poecilia* de seis especies colectadas en 33 localidades del este y una del oeste de México se analizó elec-

troforeticamente con geles de poliacrilamida (7.5 %). Estos plasmas fueron teñidos específicamente para detectar enzimas o proteínas totales. La identificación de las bandas móviles de albúminas se realizó mezclando alícuotas de estos plasmas con plasmas patrón y comparándolos con el plasma de la progenie F_1 de individuos conocidos.

El fenotipo de albúminas en *P. latipinna* es polimórfico, mientras que la especie cercana *P. velifera* tiene una movilidad intermedia entre las dos de *P. latipinna*. Una especie no identificada, similar a *P. petenensis*, exhibe la albúmina migratoria más lenta, idéntica a la que se encuentra en su pariente lejano *P. latipunctata*. *P. mexicana* es polimórfica, con cinco albúminas electroforéticamente diferentes, mientras que *P. sphenops* parece ser monomórfica. *P. formosa* se caracteriza por tener dos albúminas en su plasma que son electroforéticamente idénticas a las de *P. mexicana* del norte de México y *P. latipinna* del oeste.

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Fig. 1. Collecting sites of *Poecilia* spp. from eastern Mexico showing drainage patterns, major highways (dashed lines), major cities, and collecting localities (code abbreviations are explained in Table 1).

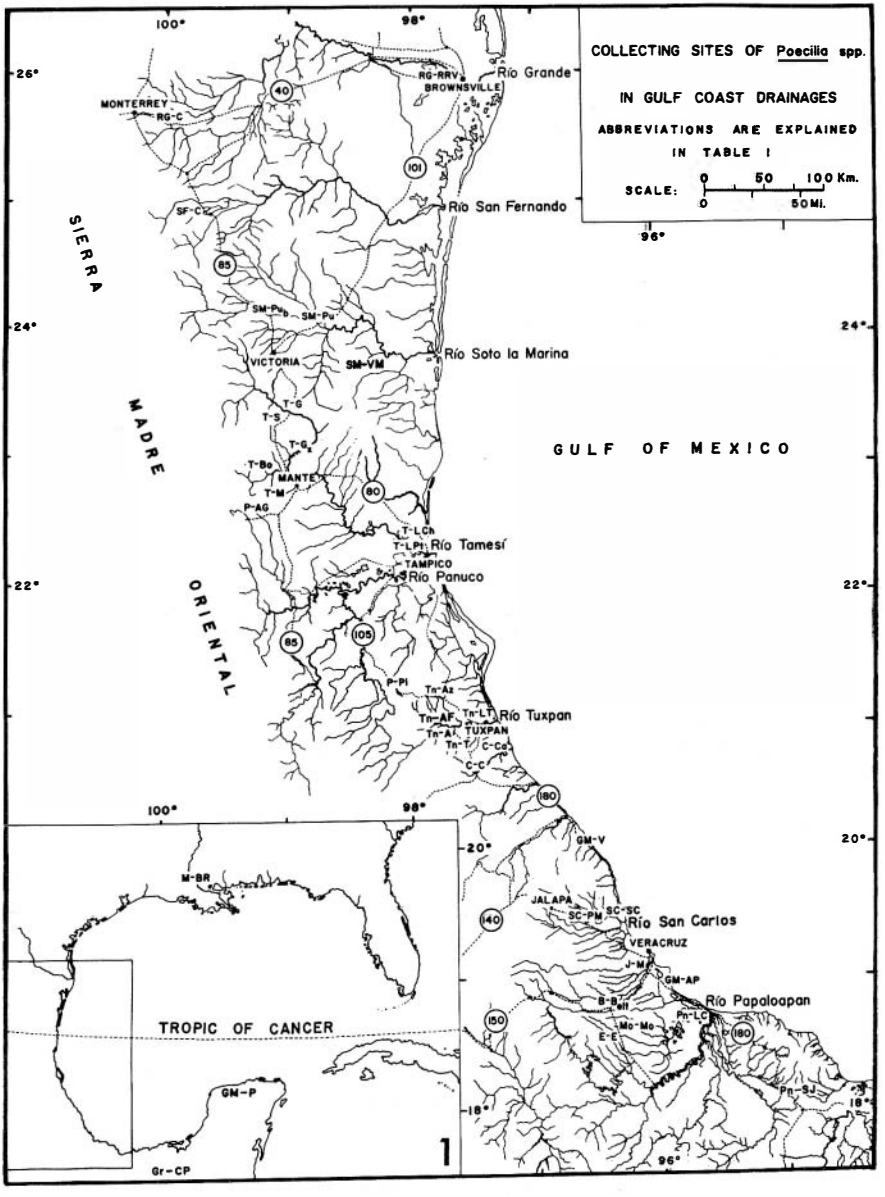
COLLECTING SITES OF Poecilia spp.
IN GULF COAST DRAINAGES
ABBREVIATIONS ARE EXPLAINED IN TABLE 1
SCALE: 0 50 100 Km. 0 50 Mi.
Río Grande
RG-RRV
BROWNSVILLE
MONTERREY
RG-C
Río San Fernando
SF-CZ
SIERRA MADRE ORIENTAL
SM-Pu
VICTORIA
SM-VM
Río Soto la Marina
T-G
T-S
T-Bo
MANTE
T-M
P-AG
GULF OF MEXICO
T-LCh
T-LPl
Río Tamesí
TAMPICO
Río Panuco
P-Pl
Tn-Az
Tn-AF
Tn-LT
Río Tuxpan
TUXPAN
Tn-T
C-Co
C-C
GM-V
JALAPA
SC-PM
SC-SC
Río San Carlos
VERACRUZ
J-M
GM-AP
B-B
Río Papaloapan
Pn-LC
Mo-Mo
E-E
Pn-SJ
M-BR
TROPIC OF CANCER
GM-P
Gr-CP
1
100°
98°
96°
26°
24°
22°
20°
18°

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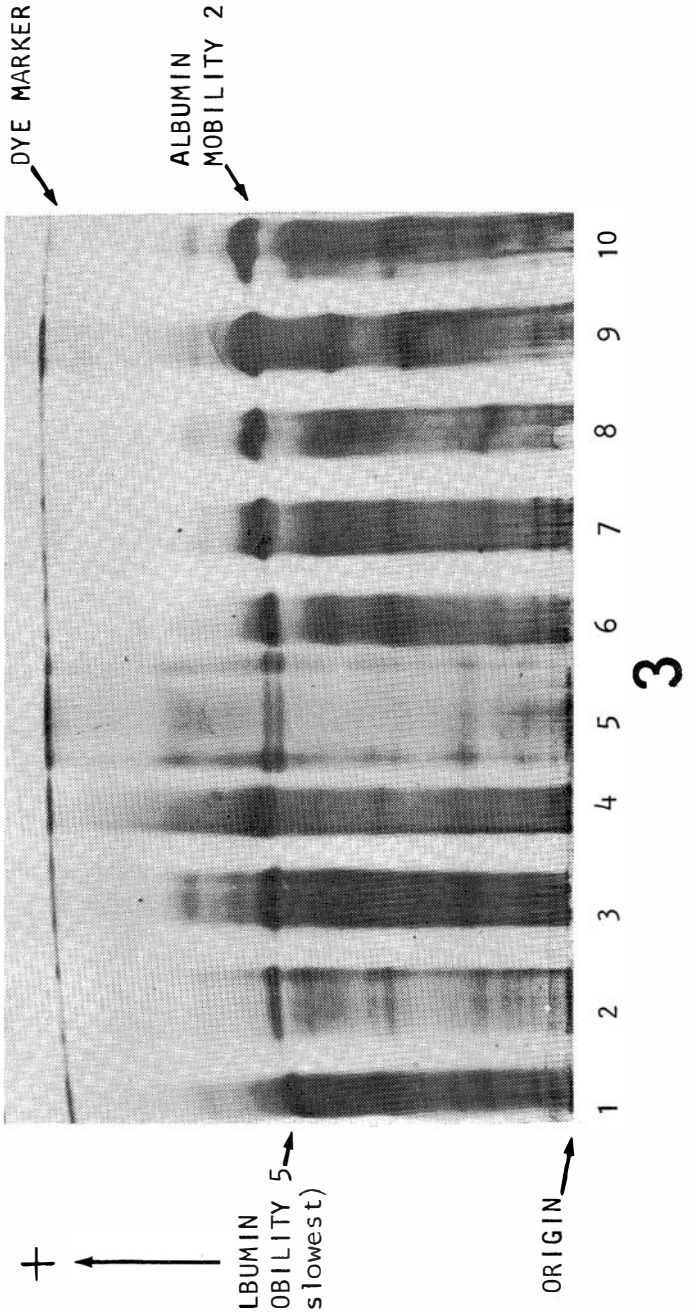
Fig. 2. Generalized Electropherogram of *Poecilia* spp. plasma. The electropherograms shown in this figure were stained for total protein. Albumin is the only specified protein that corresponds to the bands shown on the electropherograms. A direct relationship between the other bands on the electropherograms and the specified enzymes does not necessarily exist. The figure is designed to show some of the enzymes that occur in different regions of the electropherogram and to illustrate the variation observed in albumin phenotypes.


DYE MARKER
ALBUMIN MOBILITY 2
1 2 3 4 5 6 7 8 9 10
3
+
ALBUMIN MOBILITY 5 (slowest)
ORIGIN

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1963. The poeciliid fishes (Cyprinodontiformes), their structure, zoogeography, and systematics. *Bull. Amer. Mus. Nat. Hist.*, 126: 1-176.

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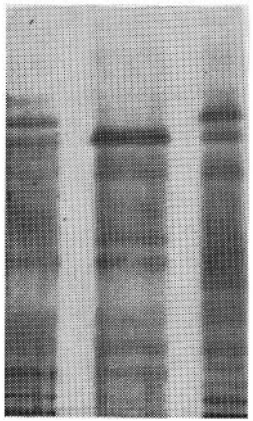
1965. Electrophoretic variation in enzymes. *Science*, 149: 936-943.

Fig. 3. Variation in albumin mobilities observed in seven species of *Poecilia*. Electropherogram is shown with the anode at the top and origin at the bottom. The line across the top of the gel indicates the position of the marker dye. 1: *P. latipunctata* (slowest albumin mobility, i.e., 5); 2: *P. mexicana* (albumin 4 phenotype from southern coastal populations); 3: *P. latipinna* (western phenotype, albumin 4); 4: *P. velifera* (albumin 3½); 5: *P. formosa* (double-banded phenotype of 3 + 4); 6: *P. butleri* (west coast, albumin 3½); 7: *P. mexicana* (northern inland, albumin 3); 8: *P. sphenops* (east coast, albumin 2½); 9: *P. sphenops* (west coast, albumin 2½); 10: *P. mexicana* (southern populations, albumin 2). The southern inland phenotype of *P. mexicana* (not included here) has the fastest albumin mobility, i.e. albumin 1.

GENERALIZED ELECTROPHEROGRAM OF POECILIA SPP. PLASMA



Lactate Dehydrogenase (1 or 2 bands)

Peroxidase (wide, diffuse band)

Albumin (1 to 4 bands)

Esterase (1 to 6 bands)

Lactate Dehydrogenase (1 band)

Esterase (1 band)

Peroxidase (2 or 3 bands)

Peroxidase (1 band)

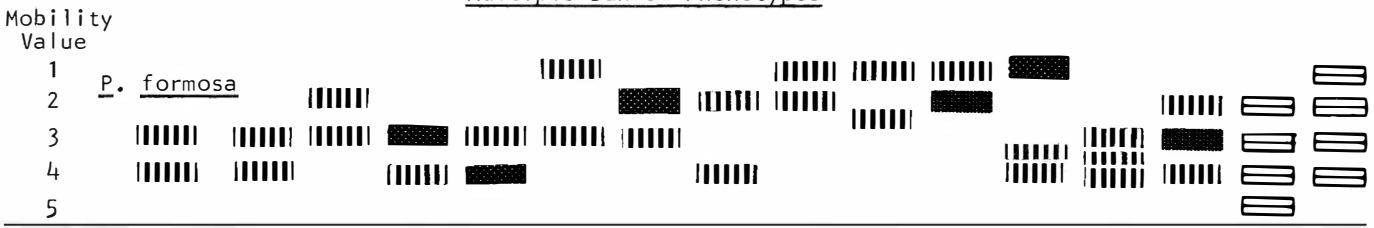
Lactate Dehydrogenase (1 band)

Origin

Single Banded Phenotypes

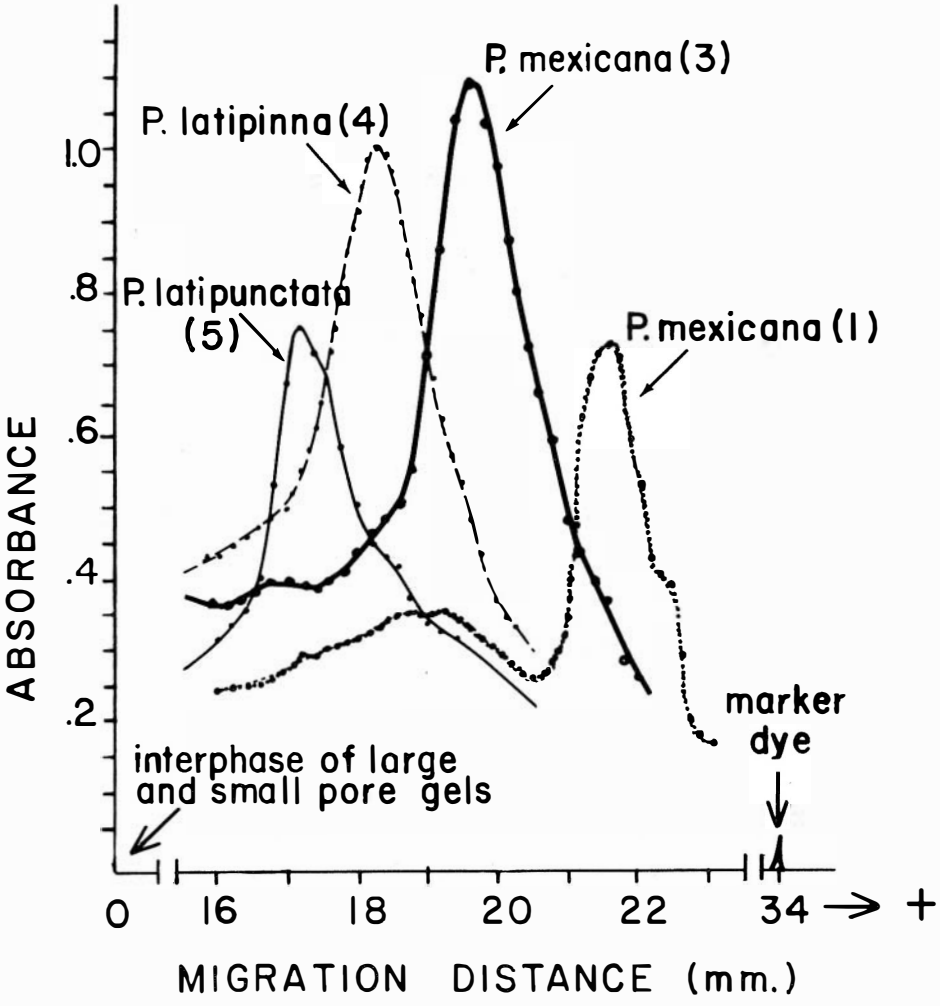
Mobility Value	Species
1*	P. mexicana (southern inland)
2	P. mexicana (southern)
$2\frac{1}{2}$	P. sphenops
3	P. latipinna (eastern) and P. mexicana (northern)
$3\frac{1}{2}$	P. butleri, P. mexicana and P. velifera
4	P. latipinna (western) and P. mexicana (southern coastal)
5	P. latipunctata and P. sp.(southern)

Multiple Banded Phenotypes



*Fastest anodal migration.

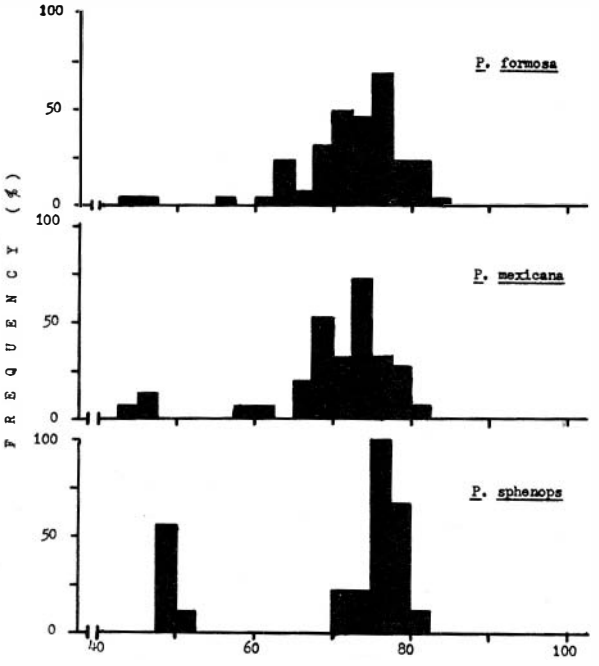
Fig. 4. Densitometric scans of plasma albumin regions of different *Poecilia* species. In this and subsequent figures, all scans in each figure were derived from a single electropherogram stained with 0.1% Coomassie Brilliant Blue; the sample origin and cathode are at the left of the scan track and the anode is at the right; migration distance in mm is measured from the interface of the large pore stacking gel and small pore separation gel, traversed in successive 0.1 mm intervals by a scanning spot 40.5 μ in diameter; maximum absorbance is based upon the means of 4 separate determinations at 560 nm; and numbers in parentheses after the species designation refer to the albumin phenotype. This series of plasma samples were run in triplicate on a single 8 % polyacrylamide gel which yields a migration distance for albumin bands equal to about one-half the migration distance observed in 7.5% gels as shown in subsequent figures.


P. mexicana (3)
P. latipinna (4)
P. latipunctata (5)
P. mexicana (1)
marker dye
interphase of large and small pore gels
ABSORBANCE
1.0
.8
.6
.4
.2
0
16
18
20
22
34
+
MIGRATION DISTANCE (mm.)

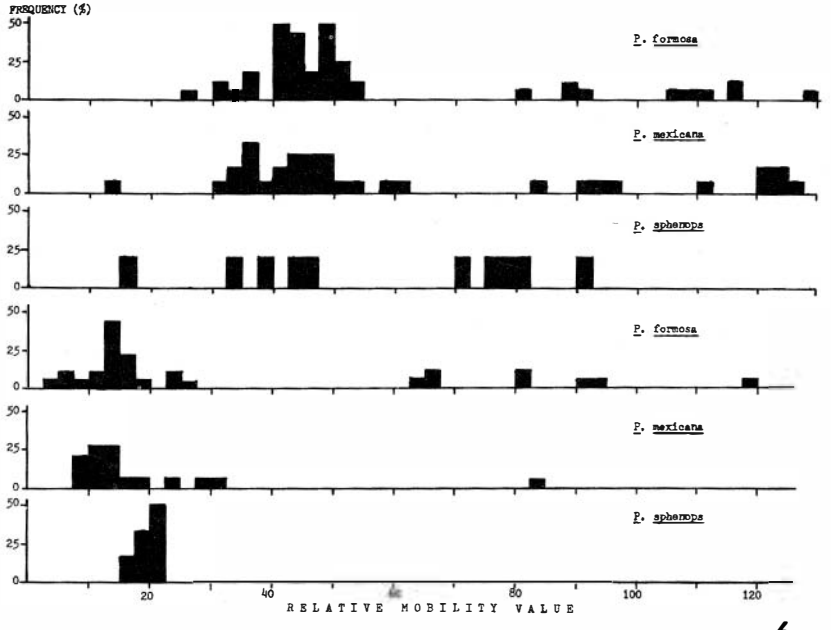
4

Fig. 5. Frequency of esterases in three species of *Poecilia*. The data for *P. formosa* are based upon 26 plasmas with an average of 2.9 esterase bands per plasma. The data for *P. mexicana* are based upon 15 plasmas with an average of 2.8 esterase bands per plasma. The data for *P. sphenops* are based upon 9 plasmas with an average of 2.9 bands per plasma. Relative mobility values were calculated in relation to albumin mobility normalized to 85.0 for *P. formosa* and *P. mexicana* and to 89.0 for *P. sphenops*.

Fig. 6. Frequency of peroxidases and lactic dehydrogenases in three species of *Poecilia*. The top half includes the peroxidase data: 16 plasmas of *P. formosa* with an average of 3.1 bands per plasma, 12 plasmas of *P. mexicana* with an average of 2.8 bands per plasma, and 5 plasmas of *P. sphenops* with an average of 2.4 bands per plasma were used. The bottom half includes the lactic dehydrogenase data: 18 plasmas of *P. formosa* with an average of 1.7 bands per plasma, 14 plasmas of *P. mexicana* with 1.2 bands per plasma, and 6 plasmas of *P. sphenops* with an average of 1.0 bands were used. All relative mobility values were calculated in relation to albumin mobility normalized to 85.0 for *P. formosa* and *P. mexicana* and at 89.0 for *P. sphenops*.


100
50
0
P. formosa
P. mexicana
P. sphenops
F R E Q U E N C Y ($)
40
60
80
100
R E L A T I V E M O B I L I T Y V A L U E

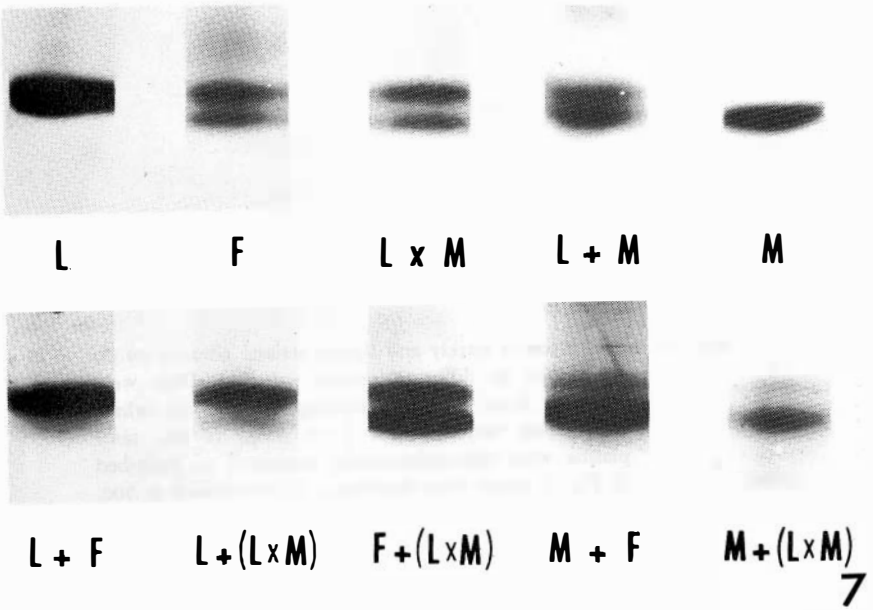
5


FRSQUENCY ($)
P. formosa
P. mexicana
P. sphenops
P. formosa
P. mexicana
P. sphenops
20
40
60
80
100
120
RELATIVE MOBILITY VALUE

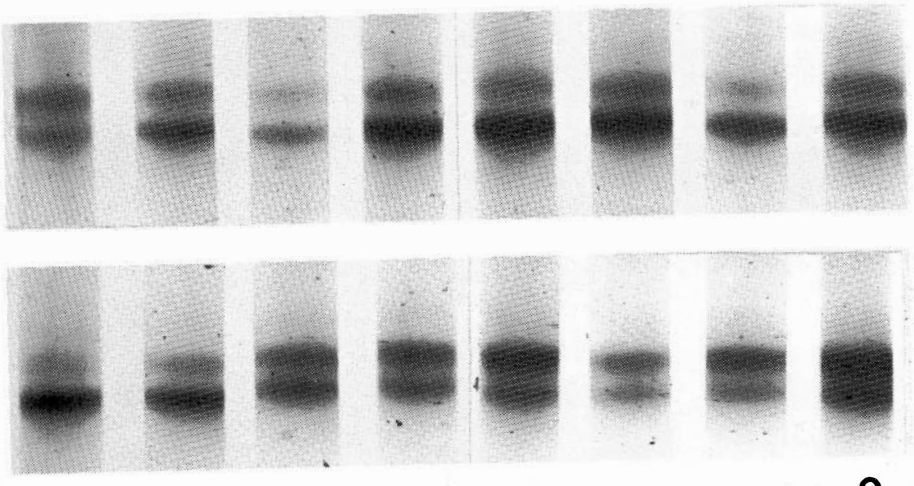
6

Fig. 7. Validation of plasma mixing procedure (only albumin region shown). *P. mexicana* and *P. latipinna* used in this breeding experiment have albumins of different electrophoretic mobility. L: *P. latipinna* (western phenotype, slower migrating albumin); F: *P. formosa,* wild-caught; L x M: laboratory F_1 hybrid of *P. latipinna* (western) x *P. mexicana* (northern); L + M: isoaliquot mixture of *P. latipinna* (western) plus *P. mexicana* (northern); M: *P. mexicana* (northern phenotype, faster migrating albumin). Bottom row illustrates isoaliquot mixtures of above plasmas.

Fig. 8. Mixing two plasmas that contain different albumin mobilities in varying protein concentrations. Top row: 136 μg *P. latipunctata* plasma (upper band) plus 55 to 442 μg *P. mexicana* plasma (lower band), arranged from left to right. Bottom row: 138 μg *P. mexicana* plasma (lower band) plus 54 to 435 μg *P. latipunctata* plasma (upper band) arranged from left to right.

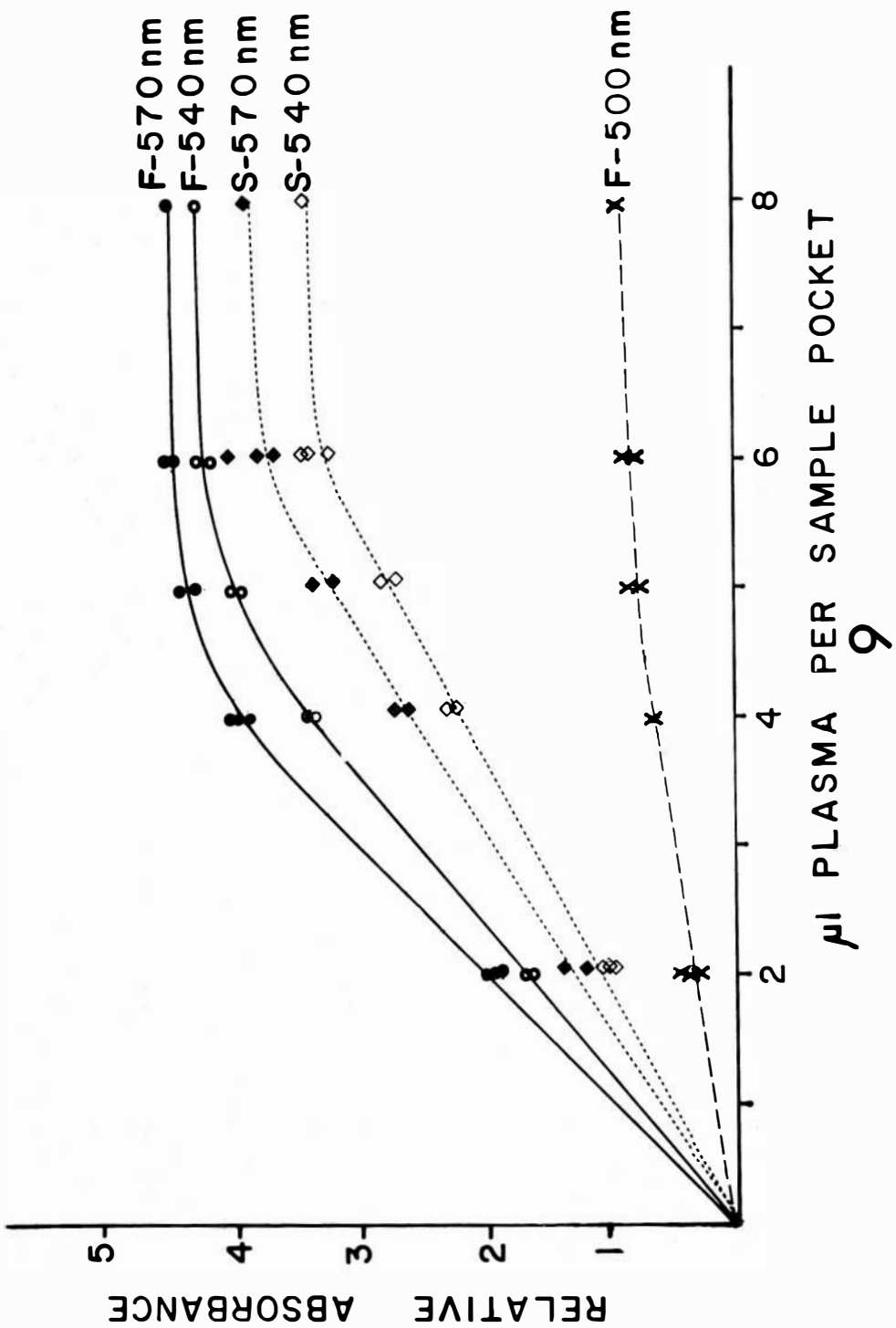


7



8

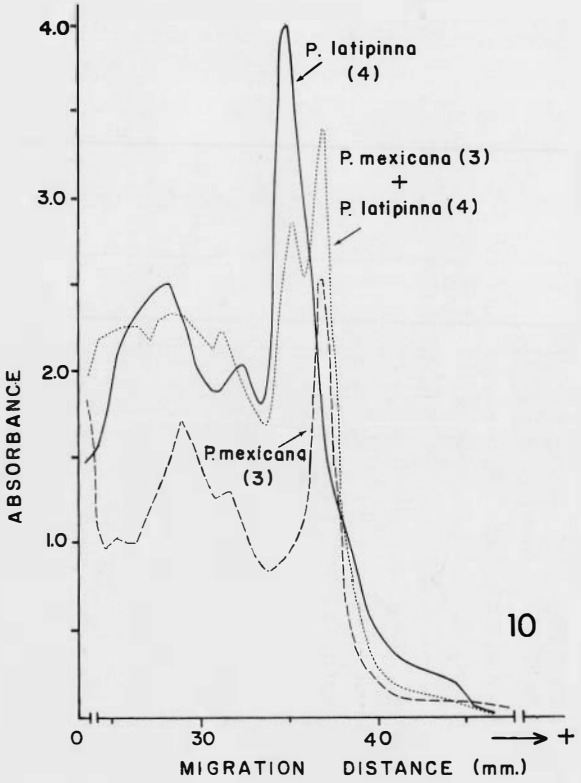
Fig. 9. Absorption of darkly and lightly stained albumin bands in relation to different sample volumes. Data were obtained from a single electropherogram in which 10 samples varying from 2 to 8 μl of the same plasma were electrophoretically separated as described in Fig. 4 except that absorbance is determined at 500, 540 and 570 nm. The plasma was obtained from a presumed triploid (albumin phenotype: 3 dark + 4 light) in which the staining intensity of the faster migrating, albumin 3 band (designated F) is about 1.5 times darker than the staining intensity of the slower migrating, albumin 4 band (designated S).


F-570nm
F-540nm
S-570nm
S-540nm
F-500nm
RELATIVE ABSORBANCE
1
2
3
4
5
2
4
6
8
µl PLASMA PER SAMPLE POCKET

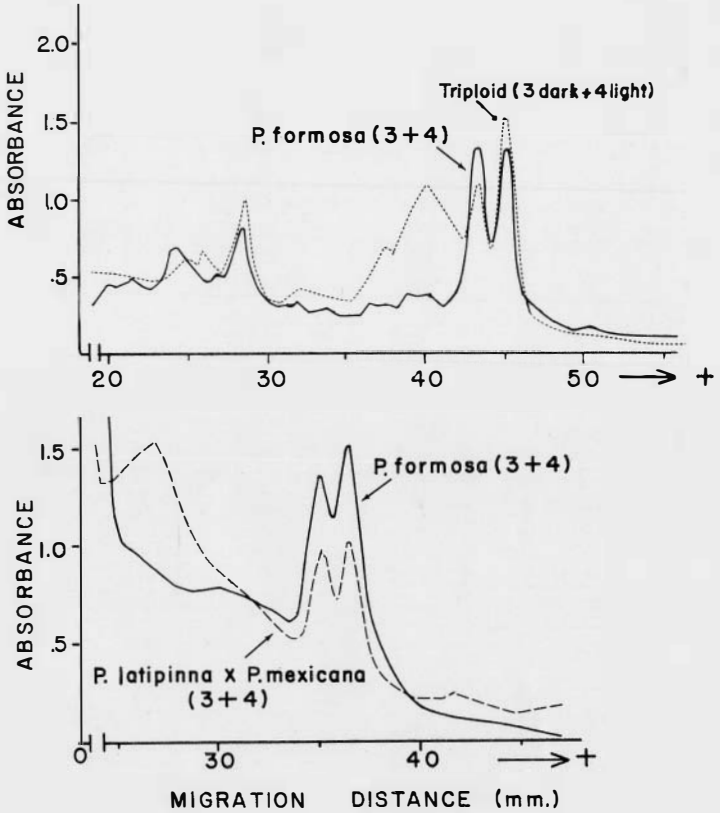
9

Fig. 10. Densitometric scans of plasmas of ***P. latipinna*** and ***P. mexicana*** and of a sample from a 1:2 aliquot mixture of these two plasmas, respectively.

Fig. 11. Densitometric scans of plasmas of *P. formosa* (3 + 4), of the laboratory hybrid, ***P. latipinna*** (4) x ***P. mexicana*** (3), and of the triploid hybrid (3 dark + 4 light).


4.0
3.0
2.0
1.0
ABSORBANCE
P. latipinna (4)
P. mexicana (3) + P. latipinna (4)
P. mexicana (3)
0
30
40
+
MIGRATION DISTANCE (mm.)

10


2.0
1.5
1.0
.5
ABSORBANCE
Triploid (3 dark + 4 light)
P. formosa (3+4)
20
30
40
50
+
1.5
1.0
.5
ABSORBANCE
P. formosa (3+4)
P. latipinna x P. mexicana (3+4)
0
30
40
+
MIGRATION DISTANCE (mm.)

11