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Genetic Differences among Strains of the Guppy, *Poecilia reticulata*

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Summary

The inheritance of colour patterns, electrophoretic markers, and quantitative traits are presently being studied in nine strains of the guppy, *Poecilia reticulata*. Homozygote excesses observed at electrophoretic loci deviating from Hardy-Weinberg equilibrium suggests an inbreeding structure that has accompanied decrease in effective population size. The levels of heterozygosity observed in the 9 strains are typical of cultured populations where adequate or inadequate number of parents have contributed to their propagation. Genetic characterization of the nine strains identifies two major groupings according to electrophoretic and body size characteristics, one group typifying the wild type with normal size, and the other typifying the selected fancy type with larger body size. The greater genetic differentiation within the fancy-type strains reflect the bottlenecking and founder effects that accompanied selection in producing these strains.

The guppy, *Poecilia* (*Lebistes*) *reticulata*, is useful as a pilot fish for the study of genetics and breeding, because of its short life cycle, capacity to reproduce, and ease of breeding as an aquarium fish. Various breeds of the guppy, differing in body colour patterns as well as in the shape and colour of fins, have been created by aquarists. Moreover, sexual dimorphism and availability of several exotic strains in which the males show striking colour patterns can be used to study the mode of inheritance of sex-linked or autosomal genes (1).

The establishment of several strains and strain differences can provide useful information on distances from electrophoretic and quantitative data that are relevant to the evaluation of genetic resources or are applicable to breeding programs. This paper presents a genetic investigation of strain differences in terms of enzyme variation and body size character in the guppy.

Materials and Methods

Nine guppy strains, S, S3, S2, F, M, T, T1, SC, and SA, have been maintained in closed colony in 60-liter aquaria at a density of 300-500 individuals per

aquarium depending on the average size of the strain. The fishes are maintained at a temperature of $23 \pm 2^{\circ}C$ and fed with ground carp pellets twice a day; dried Daphnia is given as a supplementary diet. Each container is provided with the water plant, willow moss, which acts primarily as a hiding place for newborn guppies.

For quantitative analysis, fertilized females were obtained at random from the different stocks and transferred to 2.5-liter aquaria for litter production. The litters produced were subsequently used for quantitative trait measurements. At least 7 litters per strain were measured from Day 0 to 180 at 15-day intervals. Standard body lengths were measured from the tip of the snout to the hypural bone just before the tail. For each sample, the von Bertallanfy growth equation, $L = L_{\infty} [1 - \exp(-K_t + K_{to})]$, was fitted to these data and estimates of asymptotic length, L_{∞}, computed. Differences between strains were evaluated by t-test while Spearman's rank correlation (2) was used to test the reliability of measurements in males and females at 60 and 180 days.

For electrophoretic analysis, individuals were sampled at random from each strain and whenever possible, with equal numbers of males to females. Each whole body was homogenized in distilled water, frozen overnight, thawed, and centrifuged to obtain the supernatant. Electrophoretic and staining procedures were based on Fujio (3). To determine the number of loci actually being scored, exudates from individual tissues (eye, liver, and muscle) were also used for electrophoresis to visualize tissue-specific exzymes. Loci were numbered from the most anodal as 1 and so on, while alleles were designated *A*, *B*, *C*, from the most anodal downwards.

Results

Morphological Characteristics of Strains

The guppy female is usually monotonously grey in body colour while the males have bright, diverse colour patterns; thus, the male colour characterizes the strain. The history and development as well as colour patterns of nine strains are summarized as follows:

S — The oldest strain being maintained in this laboratory is the Standard-type which was purchased from a hobby shop in 1975. This strain typifies the wild-type guppy, the male having a bluish-green body colour with red and black spots, and a variable tail shape ranging from spade, sword, to flag-shaped. The presence of black and red spots on the body of the males is a manifestation of the Maculatus (*Ma*) gene which has been reported by Winge and Ditlevsen (1) to be in the Y chromosome and is transmitted by the male parent only to the male offspring.

S3 — In 1983, a portion of the S strain was isolated and maintained but has been unused for any experiment until now.

S2 — In 1984, a population of guppies was found proliferating in a pond in Okinawa, probably discarded by a hobbyist. They resemble the S strain morphologically but have a larger black spot in the body and are usually round-tailed with ocassional occurrence of sword tails.

F — The first fancy-type guppy to be maintained in this laboratory was purchased in 1982 ; the present laboratory strain, characterized by males displaying a flamingo-red delta tail, was propagated from a single pair. The light pink body colour is due to scanty melanin formation caused by a recessive gene and is manifested in both sexes.

T — Another fancy-type, characterized by its black delta tail, was purchased in 1985 under the name, Tuxedo. The gene for melanin formation is homozygous in males that display the black colour from the latter half of the body to the caudal fin and is heterozygous in males that display the black colour only in the caudal fins. Even the female which is usually drab grey in other strains, displays a black caudal fin.

T1 — This strain was produced in this laboratory by selecting from the offspring of the original Tuxedo those that did not exhibit the typical black tail but a light pink body and a light blue delta tail. The scanty melanin formation on the body is probably controlled by the same gene in the F strain.

SC — In 1982, 2 or 3 S females were crossed with a single cobra-type male and subsequently propagated ; the cobra features are displayed by the males but the red spots on the body and the shape of the caudal fin remain to be that of the S strain. The genes for the cobra pattern are absolutely located in the Y chromosome and found to be dominant, from the results of reciprocal crosses with other strains.

SA — In 1982, another cross was made between S females and a single albino-like male ; albino-like features appeared in the F2 generation at a ratio of 1 (albino) : 3 (wild) and these individuals were segregated and propagated as a source of the strain. Both sexes have a pinkish-orange body and a whitish-orange flag tail. Melanin formation in F1 hybrids between SA and F or T1 strains indicates that the recessive albino-like gene found in the former is in a different locus from that of the recessive gene found in the latter strains.

The strains were characterized according to body length from Day 0 to 180. The Day 0 measurements were not significantly different between strains. At Day 60, the sex could usually be distinguished and growth curves were observed to be linear within this period ; by Day 180, the growth has plateaued for all the strains. Thus, 60 and 180-day body lengths for both males and females were used for comparison between strains. Table 1 presents standard lengths$\pm$SD at 60 and 180 days and Bertallanfy's L_∞ values for both male and female guppies in 8 strains, not including S3 which has been left unstudied until this time. Using Spearman's rank correlation test, male and female measurements were found to

TABLE 1. *Standard length at 60 days, 180 days, and Bertallanfy's L^{∞} for 8 guppy strains*

STRAIN	Body L at 60 days (mm)		Body L at 180 days (mm)		L^{∞}* (mm)	
	♀	♂	♀	♂	♀	♂
S	18.99±2.50 (28)	15.48±1.01 (25)	25.32±6.04 (10)	16.21±0.58 (9)	26.88	16.37
S2	16.94±1.99 (19)	15.75±1.41 (13)	23.97±1.97 (9)	15.99±1.59 (6)	25.63	16.22
SC	15.96±3.00 (15)	14.88±1.20 (11)	26.35±2.68 (12)	17.30±0.83 (7)	32.61	17.59
SA	15.90±2.41 (28)	15.09±0.66 (21)	27.52±2.14 (7)	17.58±1.42 (5)	36.59	17.70
F	18.90±2.39 (37)	17.92±1.49 (20)	28.67±3.05 (15)	21.36±1.40 (18)	34.29	22.24
T	17.49±2.05 (28)	17.60±0.85 (16)	29.72±1.00 (8)	20.57±1.33 (6)	37.57	21.33
T1	15.17±2.66 (24)	16.62±1.37 (11)	24.96±1.70 (4)	18.80±1.15 (5)	31.25	19.52
M	16.88±2.35 (53)	17.18±1.68 (32)	27.48±3.45 (20)	20.41±1.46 (10)	31.74	20.95

(n)—numbers in parentheses indicate the number of individuals measured.

*L^{∞}—asymptotic length obtained from the Bertallanfy equation, $L=L^{\infty}\ [1-\exp(-Kt+Kto)]$ after plotting body length measurements from Day 0 to 180 at 15-day intervals for every strain.

have low correlation at 60 days ($r_s=0.381$) but high at 180 days ($r_s=0.786$) and in the Bertallanfy L_{∞} values ($r_s=0.690$). Female length ranking at 60 days had low correlation with the 180 day length ($r_s=0.238$) and with L_{∞} ($r_s=-0.07$).

TABLE 2. *T-tests for size differences between 8 strains of the guppy, Poecilia reticulata*

STRAIN	S	S2	SC	SA	F	T	T1	M
S		0.681	1.551	1.517	6.532***	6.958***	2.794**	4.463***
S2	0.384		1.610	1.854	4.173***	4.371***	1.526	2.702**
SC	3.100**	1.907		0.643	5.798***	6.912***	3.169***	4.174***
SA	2.594*	1.731	0.433		7.929***	10.121***	4.295***	5.418***
F	10.511***	7.880***	7.143***	5.326***		0.764	2.389**	1.612
T	8.782***	5.412***	5.411***	3.602***	1.210		2.299*	0.938
T1	5.694***	3.288***	2.639*	1.493	3.735***	2.332*		1.007
M	8.058***	5.677***	5.061***	3.569***	1.695	0.219	2.142*	

Asterisks indicate significant differences: *$P\leqq 0.05$; **$P\leqq 0.01$; ***$P\leqq 0.005$. Size differences at 60 days are presented above the diagonal while size differences at 180 days are presented below the diagonal.

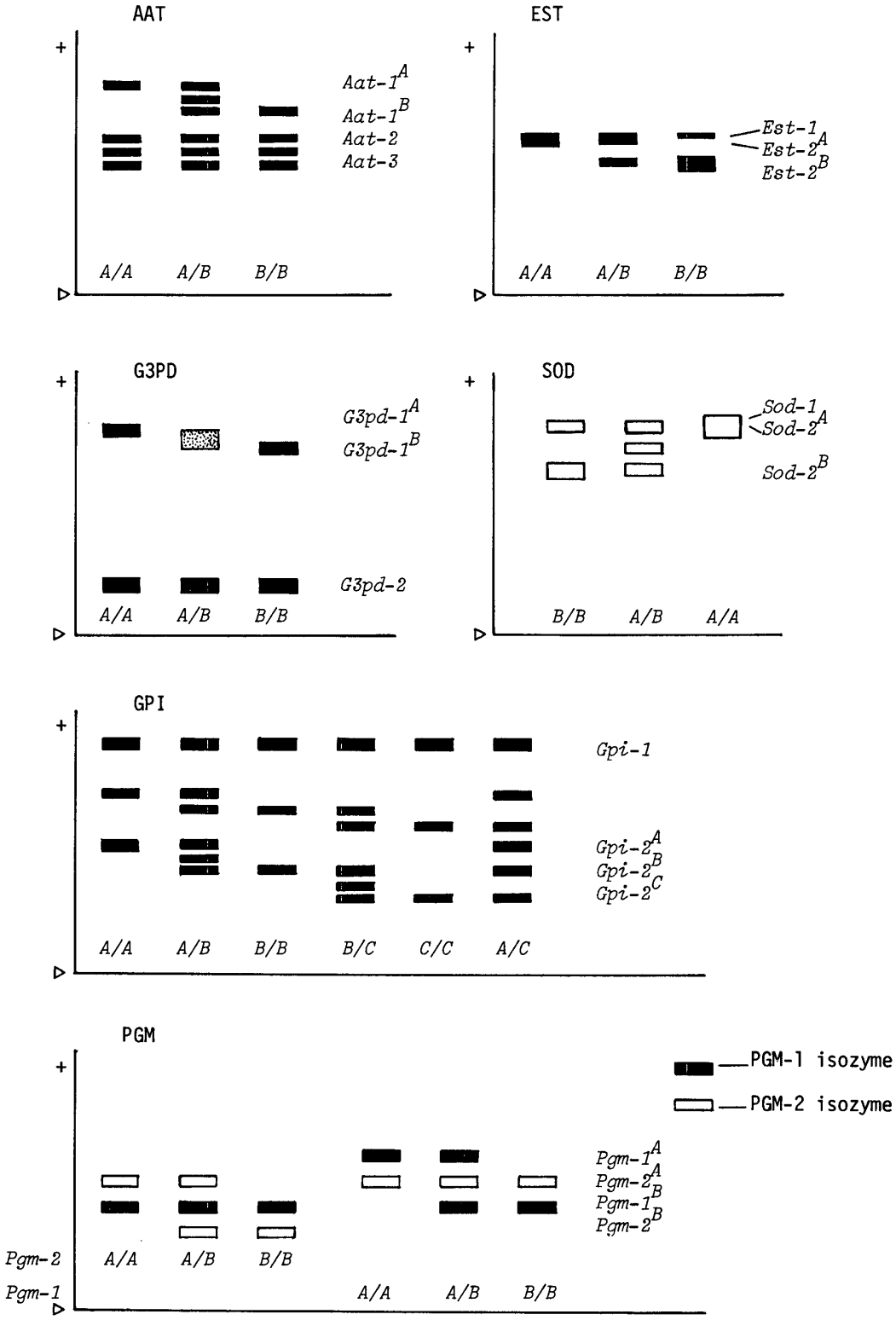

FIG. 1. Allozyme expressions at 7 polymorphic loci in the guppy, *Poecilia reticulata*.

TABLE 3. *Observed gene frequencies for 9 strains of the guppy, P. reticulata*

LOCUS	Allele	Strain								
		S	S3	S2	SC	SA	F	T	T1	M
Aat-1	*A*	0.414	0.580	0.570	0.226	0	0.560	1.000	1.000	0.462
	B	0.586	0.420	0.430	0.774	1.000	0.440	0	0	0.538
	(N)	(99)	(100)	(93)	(115)	(79)	(109)	(110)	(94)	(104)
Est-2	*A*	0.209	0.255	0.417	0.533	0.560	0.248	0.778	0.548	0.630
	B	0.791	0.745	0.583	0.467	0.440	0.752	0.222	0.452	0.370
	(N)	(115)	(100)	(30)	(30)	(75)	(109)	(81)	(42)	(54)
G3pd-1	*A*	1.000	1.000	1.000	1.000	1.000	0.805	1.000	1.000	0.728
	B	0	0	0	0	0	0.195	0	0	0.272
	(N)	(115)	(100)	(97)	(130)	(79)	(123)	(110)	(94)	(79)
Gpi-2	*A*	0	0	0.227	0	0	0.118	0	0	0.010
	B	1.000	1.000	0.737	1.000	1.000	0.567	1.000	1.000	0.990
	C	0	0	0.036	0	0	0.315	0	0	0
	(N)	(115)	(100)	(97)	(130)	(79)	(89)	(110)	(94)	(104)
Pgm-1	*A*	0.658	0.805	0.821	0.846	0	0	0	0	0
	B	0.342	0.195	0.179	0.154	1.000	1.000	1.000	1.000	1.000
	(N)	(95)	(100)	(95)	(130)	(79)	(123)	(110)	(94)	(134)
Pgm-2	*A*	1.000	1.000	1.000	1.000	0.987	0.890	0.782	0.622	0.959
	B	0	0	0	0	0.013	0.110	0.218	0.378	0.041
	(N)	(95)	(100)	(95)	(130)	(79)	(123)	(110)	(94)	(134)
Sod-2	*A*	0	0	0.130	0.435	0	0.053	0.267	0.317	0.260
	B	1.000	1.000	0.870	0.565	1.000	0.947	0.733	0.683	0.740
	(N)	(115)	(100)	(50)	(31)	(49)	(57)	(103)	(41)	(54)

Twenty-seven loci were scored; 20 were monomorphic for the same allele—*Aat-2*, *Aat-3*, *Adh*, *Est-1*, *αGpd-1*, *αGpd-2*, *G3pd-2*, *G6pd*, *Gpi-1*, *Idh-1*, *Idh-2*, *Ldh-1*, *Ldh-2*, *Ldh-3*, *sMdh-1*, *sMdh-2*, *Me*, *6Pgd*, *Sdh*, and *Sod-1*. (N) refers to the number of individuals sampled.

However, female measurements at 180 days agreed well with L_∞ ($r_s=0.929$). On the other hand, measurements for males agreed well between 60 and 180 days ($r_s=0.786$), between 60 days and L_∞ $r_s=0.786$), and between 180 days and L_∞ ($r_s=1.0$). Based on these results, size differences among strains was evaluated by univariate statistics using the t-test on male measurements. The matrix shown in Table 2 presents the t-values and degrees of significance for size differences at 60 and 180-day length measurements in male guppies. Results showed a significant difference between the S, S2, SC and SA from the F, T, T1, and M strains. The T1 strain also displayed a significant difference in growth rate from its T relative and from the other fancy-type strains.

Genetic Variability and Differentiation Among Strains

Of the 27 loci scored, polymorphism was observed at 7 loci, namely, *Aat-1*, *Est-2*, *G3pd-1*, *Gpi-2*, *Pgm-1*, *Pgm-2*, and *Sod-2*. Except for *Gpi-2* which has 3 alleles, the rest consist of 2 alleles. The allozyme expressions at these loci are illustrated in Fig. 1. Table 3 presents the allele frequencies at the polymorphic

loci observed in 9 guppy strains. G tests (4) for goodness of fit to Hardy-Weinberg equilibrium revealed significant deviations at the *Aat-1* locus in the F strain ($G=7.17$; $P<.01$), *Sod-2* locus in T1 ($G=5.96$; $P<.05$) and F ($G=9.72$; $P<.01$) strains, *Est-2* in S ($G=27.23$; $P<.001$), S3 ($G=23.00$; $P<.001$), M ($G=10.54$; $P<.01$), and F ($G=30.90$; $P<.001$) strains, *G3pd-1* in the M strain ($G=7.92$; $P<.01$), *Gpi-2* in the S2 strain ($G=12.34$; $P<.01$), and *Pgm-1* locus in the S strain ($G=4.66$; $P<.05$). Except for a heterozygote excess at the *Sod-2* locus in the T1 strain and *Gpi-2* locus in the S2 strain, all deviations were due to homozygote excess. Genetic variability within each strain could be further estimated by observed and expected heterozygosities, Ho and He, calculated from the observed number of heterozygotes and from the expected frequency of homozygotes, respectively. As shown in Table 4, the SA strain exhibited the lowest heterozygosity (He=0.019) while the F strain exhigited the highest (He=0.076). The mean expected heterozygosity is He=0.053±0.006. A further measure of deviation from Hardy-Weinberg equilibrium, d, showed a general picture of homozygote excess, with only one exception, the T1 strain.

There is an observed heterogeneity of allele frequencies at polymorphic loci among the populations but one interesting observation was the significant divergence of S, S3, S2, and SC from the SA, F, T, T1, and M strains at the 2 PGM loci. While the former group shows polymorphism for the *A* and *B* alleles in *Pgm-1*, the latter group exhibits monomorphism for the *B* allele. In *Pgm-2*, the former group is monomorphic for the *A* allele while the latter group shows polymorphism for the *A and B* alleles. Genetic divergence in gene frequencies among populations was revealed by Nei's genetic distance between population pairs (5) where the smallest D was that between S and S3 (0.0019) and the largest was that

TABLE 4. *Genetic variation in 9 strains of the guppy, Poecilia reticulata*

STRAIN	AVERAGE HETEROZYGOSITY		d*=Ho−He/He
	Ho	He	
S	0.035	0.047	−0.255
S3	0.037	0.044	−0.159
S2	0.064	0.070	−0.086
SC	0.058	0.059	−0.017
SA	0.018	0.019	−0.053
F	0.062	0.076	−0.184
T	0.037	0.040	−0.075
T1	0.060	0.052	+0.154
M	0 056	0.068	−0.176

* d is the deviation from Hardy-Weinberg's expectations wherein a + value denotes an excess of heterozygotes while a— value an excess of homozygotes.

TABLE 5. *Matrix of genetic distances among 9 strains of the guppy, Poecilia reticulata*

STRAIN	S	S3	S2	SC	SA	F	T	T1	M
S									
S3	0.0019								
S2	0.0067	0.0040							
SC	0.0143	0.0154	0.0113		Mean D±SE=0.0312±0.0030				
SA	0.0281	0.0420	0.0431	0.0373					
F	0.0262	0.0339	0.0332	0.0510	0.0236				
T	0.0490	0.0488	0.0453	0.0589	0.0459	0.0289			
T1	0.0452	0.0459	0.0449	0.0594	0.0483	0.0244	0.0028		
M	0.0300	0.0377	0.0356	0.0357	0.0137	0.0142	0.0169	0.0193	

between T1 and SC (0.0594). The mean genetic distance was 0.0312±0.0030 (Table 5). A dendrogram based on the UPGMA method of Sokal and Sneath (6) is shown in Fig. 2, revealing the following relationships. Two major groups are identifiably separated, namely, the S, S3, S2, and SC from the T, T1, M, SA, and F groups. This is consistent with the observed genetic differences at the *Pgm-1* and *Pgm-2* loci. Based on the genetic distance range categorized by Nei (7) for a local race (≅0.01), S, its derivative (S3), and the released population in Okinawa (S2), constitute one local race, and the SC strain, another race. The 4 strains comprising Group 2, namely, T and its derivative T1, M, SA, and F are considered local races. Additional observations are the close genetic relationship of T1 to T strain and S3 to S strain from where each was derived respectively. It is interesting to note that SC and SA which were produced between the S strain and relatives of Group 2, diverged sufficiently to belong to 2 different groups, one genetically close to its S parent and the other, genetically close to its fancy-type parent. Following the grouping identified from the dendrogram, mean genetic distance values obtained within Groups 1 and 2 were 0.0089±0.0023 and 0.0238±0.0045 respectively. Between-group D was 0.0416±0.0021. A measurement of genetic differentiation, GST (7), among the guppy strains, calculated as GST=(HT-HS)/HT from expected heterozygosities, gave an over-all GST of 32.91%.

Discussion

The mode of inheritance of most genes affecting body colour patterns as well as finnage are sex-limited and manifest themselves only in the male. With this knowledge, the guppy breeder has created a variety of mutant races from the wild-type guppy. Most of the fancy-type guppies are the result of selection and inbreeding for recessive traits such as giantism, albinism, veiltail, *etc*··· Thus, with the availability of both wild-type and fancy-type guppies in this laboratory, the genetics of colour patterns as well as finnage characteristics could be under-

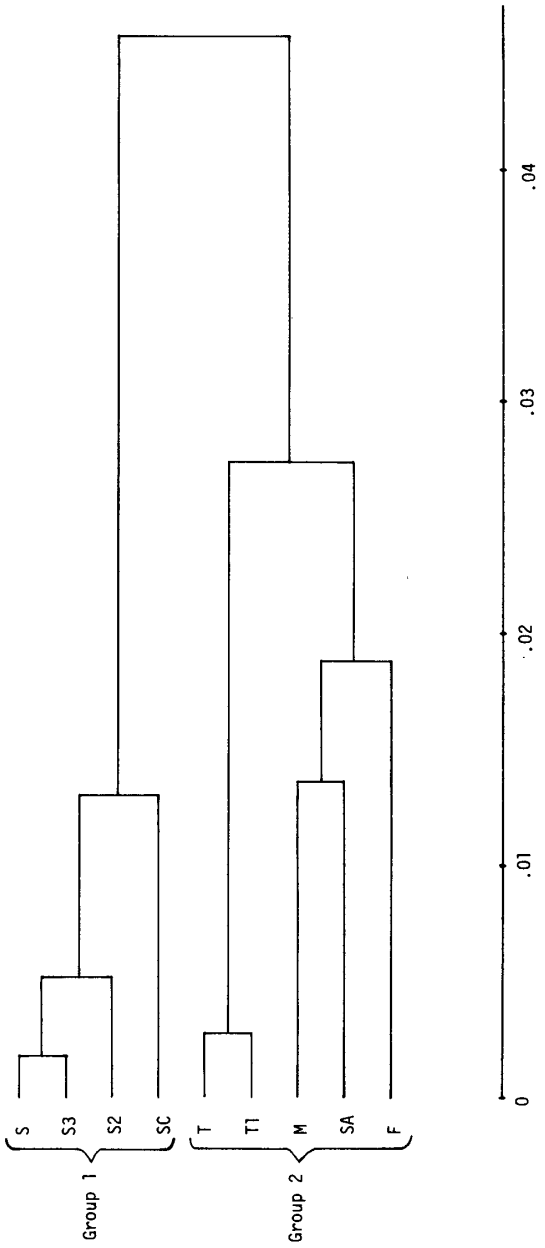

FIG. 2. Dendrogram (UPGMA, Sokal and Sneath 1963) constructed from genetic distance (D) values of Table 5.

stood in the course of producing new strains. The S strains (S, S3, and S2) typify the wild-type guppy with their various colours and polymorphic finnage shapes while the fancy-type guppies (F, T, and M) are more constant in their body colours as well as finnage. The T1 strain is a product of further selection and inbreeding for the recessive bluish tail colour caused by the inhibition of the black coloration typical of the T strain. Likewise, the SA strain is a product of selection for the recessive albino-like colour and veiltail. On the other hand, the SC strain typifies a product of the intrusion of Y-linked traits, particularly cobra patterns, into the

wild-type.

In body size characteristics, the S strains (S, S3, S2) were smaller than the fancy-types (F, T, M). The SC strain has inherited the normal size of the S parent and is grouped as such. The unexpected deviation of body size for T1 and SA from the other fancy-type guppies might be the effect of too much inbreeding, but this allegation will have to be supported by other traits.

The 9 strains of *Poecilia reticulata* exhibit significant levels of genetic variation at enzyme loci contributing to the local race level of genetic differentiation and subdivision into 2 major groups of guppies. This subdivision is shown by a significant genetic distance between Groups 1 and 2. The S strains (S, S2, and S3) comprising Group 1 exhibit very little genetic differentiation, reflected by low within-group genetic distance. The greater variability of the Group 2 strains, reflected by higher within-group D, demonstrate the effects of selection that led to producing the different guppy varieties. The grouping of SC into Group 1 and SA into Group 2 is consistent with the history of production of these respective strains. The genetic changes that can be produced in laboratory strains have implications for breeding programs where selection for desirable traits and maintenance of the hatchery stocks have consciously or unconsciously altered the genetic constitution of the species. As parent populations, the different strains qualify as randomly mating units. The homozygote excesses are most probably a result of inbreeding which could be expected from the history of selection that the guppy has undergone to produce the strains or varieties. It is remarkable, however, that the guppy populations have maintained different levels of polymorphism which could be a mechanism of balancing selection, a phenomenon which is still seeking sufficient explanation. The selective advantage of heterozygotes is particularly displayed by the F strain which has the highest genetic variability despite its propagation from a single pair (most probably heterozygous at most polymorphic loci). On the other hand, the low heterozygosity of the SA strain is due to homozygosity accompanying selection for the recessive albino-like individuals. The levels of heterozygosity observed here were also seen in a previous investigation of cultured populations of Japanese char (8) which were propagated from adequate or limited number of parents.

As a species, the level of differentiation in guppies, as reflected by genetic distance values, is comparable to land-locked species such as the Arctic char (9) (Mean D=0.0359; Range-0.0010-0.0780) and the brown trout (10) (Mean D=0.0155; Range-0.0000-0.0783) but is more differentiated than the Japanese carp (11) (Mean D=0.0115; Range-0.0012-0.0352) or the Japanese char (8) (Mean D=0.0081; Range-0.0000-0.0289) despite the presence of two different char forms. Likewise, the over-all gene diversity, GST, in guppies is comparable to land-locked or stationery freshwater species such as the blue gill (12) (GST=33.10%), the brown trout (10) (GST=36.8%), and the Arctic char (13) (GST=

36.4%).

There is a striking correlation between the subdivision of the guppies into 2 major groups based on electrophoretic data and body size characteristics. Reznick (14) has found interpopulation differences in body size as well as other life history traits that were associated with differences in predation ; these differences were found to have a genetic basis. The selective force that has influenced the life history evolution in Trinidadian guppies resulting in 2 size classes might have a bearing on the genetic separation of the guppies into the major groups described here. Proper evaluation of this correlation will have to wait for results from the other quantitative traits. Our preliminary investigations convey information which enhance the predictability and value of our present approach in the study of quantitative traits in various strains and hybrids. Other characters associated with morphology, survival, and maturity, are being investigated.

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