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Population differentiation in Trinidadian guppies (*Poecilia reticulata*): patterns and problems

P. W. SHAW*, G. R. CARVALHO*, A. E. MAGURRAN† AND B. H. SEGHERS†
**Marine, Environmental and Evolutionary Research Group, School of Biological Sciences, University of Wales Swansea, Singleton Park, Swansea SA2 8PP and †Animal Behaviour Research Group, Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, U.K.*

Populations of the guppy, *Poecilia reticulata*, in N. Trinidad exhibit marked population differentiation in allozyme frequencies. Here we investigate six further populations electrophoretically at 25 enzyme-coding loci to examine patterns in geographical structuring, genotypic distributions and genetic diversity. With one exception, possibly related to an experimental introduction, populations divided broadly (dendrogram of Nei's mean genetic identity, $\bar{I}$) in accordance with proposed ancestral colonization. Most populations were in Hardy–Weinberg equilibrium, though some significant deficits in heterozygotes were detected. Incorporating information from published data, markedly higher levels of genetic diversity (mean observed heterozygosity, $\bar{H}_0$) were recorded in lowland [$\bar{H}_0 = 0{\cdot}0382 \pm 0{\cdot}006$ (S.E.), $n = 9$] compared with upstream populations [$\bar{H}_0 = 0{\cdot}0112 \pm 0{\cdot}0034$ (S.E.), $n = 9$]. Patterns are discussed in relation to historical and present-day evolutionary forces.

Key words: guppy; *Poecilia reticulata*; allozymes; population differentiation; genetic diversity.

I. INTRODUCTION

One of the criticisms often levelled against electrophoretic studies in population biology is that genetic data are interpreted without sufficient recourse to the biology and environment of the species under study. Realistic interpretations of population structure derived from allozymes require information on mating patterns and life histories, together with the possible effects of putative selection pressures and population size. Such knowledge has accumulated in recent years for the live-bearing teleost, *Poecilia reticulata* Peters, particularly from the rivers of N. Trinidad (e.g. Seghers, 1974; Liley & Seghers, 1975; Endler, 1978; Reznick *et al.*, 1990; Carvalho *et al.*, 1991). Relationships between environment and genetic structure can also be examined since *P. reticulata* inhabits widely contrasting habitats, ranging from rapidly flowing shallow headwaters, to slowly moving deep water lowland rivers. In addition to examining the effects of present day processes, the influence of historical factors can be assessed since local geological events are documented (Boos, 1984; Briggs, 1984; Kenny, 1988), especially in relation to landlinks with South America.

Although marked population differentiation in morphology, life histories and behaviour has been widely documented, there is surprisingly only one published account of genetic structure in natural populations of *P. reticulata* using allozymes (Carvalho *et al.*, 1991). Electrophoretic examination from six locations in N. Trinidad (Carvalho *et al.*, 1991) demonstrated marked genetic divergence among populations, with a positive correlation between degree of isolation and extent of differentiation. Allele frequencies from all six rivers were significantly different,

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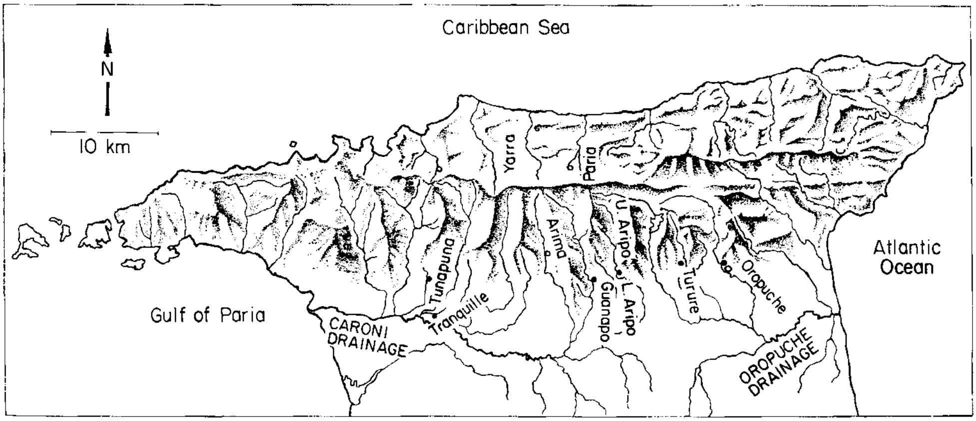

FIG. 1. Location of rivers in N. Trinidad from which samples of *P. reticulata* were obtained. ●, 1990 samples (present study); ○, 1989 samples (Carvalho *et al.*, 1991).

but populations could be separated broadly into two groups (Yarra-Paria-Caroni *v.* Oropuche drainages), supposedly related to colonization by divergent ancestral stocks (Boos, 1984). Most populations were in Hardy–Weinberg equilibrium, with deviations exclusively due to heterozygote deficiencies.

The present study extends the electrophoretic analysis of guppy populations in N. Trinidad in order to test whether historically-based patterns of population differentiation are characteristic of *P. reticulata* in this region. Furthermore, two populations sampled in 1989 (Lower Aripo and Oropuche) were re-sampled in 1990 for a between-year comparison of allele frequencies, together with a survey of the distribution of genetic diversity among sites.

II. MATERIALS AND METHODS

SAMPLING METHODS

Samples of *P. reticulata* were obtained using seine and dipnets during February 1990 from six rivers in N. Trinidad encompassing two drainage basins ('Caroni' drainage = Guanapo, Lower Aripo, Tranquille and Tunapuna; 'Oropuche' drainage = Turure and Oropuche; Fig. 1). Samples were collected from the upper Tunapuna, and all other sites from lowland rivers. Fish were humanely killed in iced water, and returned frozen in an insulated flask to the U.K. for storage at −70° C.

ELECTROPHORESIS

Variability at 25 enzyme-coding loci was assayed using standard horizontal starch gel electrophoresis (12·5% gels; Connaught Laboratories) (Harris & Hopkinson, 1976). Full experimental details are given in Carvalho *et al.* (1991). Two additional loci were screened in the present study: lactate dehydrogenase (*LDH**; E.C. 1.1.1.27) and dipeptidase (*PEPA**; E.C. 3.4.-.-).

Allele frequencies are presented only for those loci with clearly resolved and interpretable polymorphisms. Genetic nomenclature is in accordance with recent recommendations (Shaklee *et al.*, 1990). The relative mobility of alleles are compared with the common allele (100 units) in a previously studied population ('Yarra'; Carvalho *et al.*, 1991). Genetic data were analysed using version 1.7 of the BIOSYS-1 package (Swofford & Selander, 1981).

TABLE 1. Allele frequencies in eight samples of *P. reticulata*. Only loci with clearly resolved and interpretable polymorphisms are presented. For locations of sample sites see Fig. 1

Allele	1990						1989§	
	TU	TR	LA	GU	TE	OR	LA	OR
n	39	47	50	59	69	49	26‡	53
*PGM*119*	0·09	0·47	0·39	0·73	0·61	0·02	0·65	0·03
*PGM*110*	0·91	0·49	0·06	0·07	0·25	0·53	0	0·39
*PGM*100*	0	0·04	0·55	0·20	0·14	0·45	0·33	0·58
*PGM*81*	0	0	0	0	0	0	0·02	0
H_o‖	0·08*	0·43	0·42	0·46	0·51	0·37*	0·42	0·38
H_e	0·16	0·54	0·54	0·42	0·54	0·52	0·47	0·51
n	48	50	26	55	56	32	24†	44
*SOD*100*	0·94	0·15	0	0·97	0·93	0·27	0	0·24
*SOD*69*	0·06	0·85	1·00	0·03	0·07	0·73	0·96	0·76
*SOD*30*	0	0	0	0	0	0	0·04	0
H_o‖	0·08	0·10**	0	0·02	0·04**	0·22*	0·08	0·39
H_e	0·12	0·26	0	0·05	0·13	0·39	0·08	0·37
n	48	50	50	60	69	51	26	55
*FH*100*	1·00	0·87	1·00	1·00	0·97	0·50	1·00	0·42
*FH*57*	0	0·13	0	0	0·03	0·50	0	0·58
H_o‖	0	0·22	0	0	0·06	0·45	0	0·44
H_e	0	0·23	0	0	0·06	0·50	0	0·49
n	48	50	50	59	69	47	26‡	53
*G3PDH*126*	0	0	0·07	0·03	0	0·32	0	0·43
*G3PDH*100*	1·00	1·00	0·93	0·97	1·00	0·68	1·00	0·57
H_o‖	0	0	0·10	0·03	0	0·34	0	0·30**
H_e	0	0	0·13	0·07	0	0·44	0	0·49
n	33	50	50	60	69	52	26	55
*AAT*128*	0·56	0·95	1·00	1·00	1·00	1·00	1·00	1·00
*AAT*100*	0·44	0·05	0	0	0	0	0	0
H_o‖	0·33	0·10	0	0	0	0	0	0
H_e	0·49	0·10	0	0	0	0	0	0
n	48	50	50	60	69	52	26	55
*EST-1*100*	1·00	1·00	1·00	1·00	1·00	0	1·00	0
*EST-1*87*	0	0	0	0	0	1·00	0	1·00
$\bar{H}_o$	0·021	0·040	0·024	0·022	0·024	0·058	0·020	0·062
$\bar{H}_e$	0·033	0·052	0·030	0·023	0·029	0·076	0·022	0·080
P	0·12	0·20	0·08	0·04	0·08	0·16	0·04	0·20

1990 samples: TU = Tunapuna; TR = Tranquille; LA = Lower Aripo; GU = Guanapo; TE = Turure; OR = Oropuche. 1989 samples (data from Carvalho *et al.*, 1991): LA = Lower Aripo; OR = Oropuche.

n = sample size. H_o = observed heterozygosity. H_e = expected heterozygosity. $\bar{H}_o$ and $\bar{H}_e$ = mean heterozygosity values across 25 loci. **P** = proportion of loci polymorphic (0·95 criterion for common allele).

§Significant differences in allele frequencies between years (Nass' χ^2): †$P<0{\cdot}05$; ‡$P<0{\cdot}01$.

‖Deviations from Hardy–Weinberg expectations: *$P<0{\cdot}05$; **$P<0{\cdot}01$.

III. RESULTS

POPULATION DIFFERENTIATION

Allele frequencies differed markedly among populations, with one fixed difference at *EST** (E.C. 3.1.1.–) in fish from the Oropuche (Table I). Nei's (1972) mean genetic identities ($\bar{I}$) and distances ranged from 0·999–0·907 and 0·001–0·064

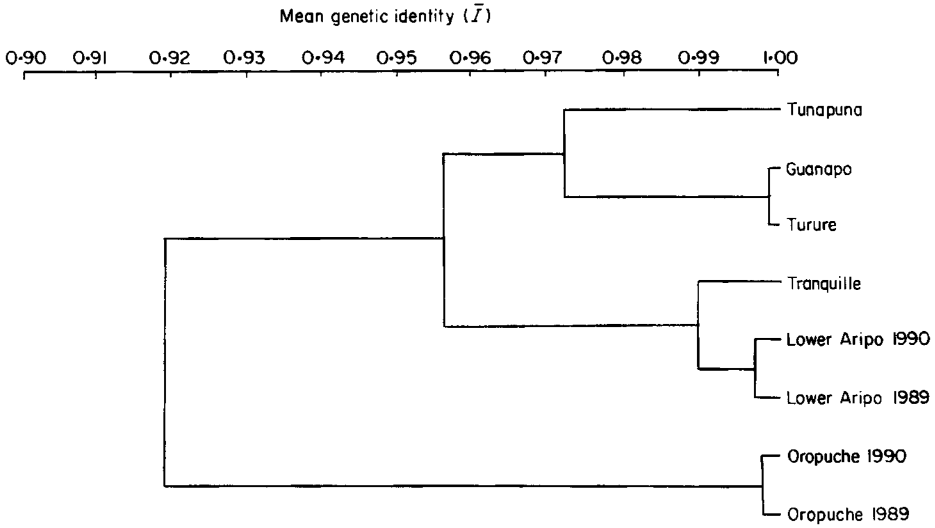

FIG. 2. Dendrogram of mean genetic identities between eight samples of *P. reticulata* from six rivers in N. Trinidad. Calculations (UPGMA–cophenetic correlation = 0·923) are based on frequencies of 37 alleles at 25 gene loci. Sample sites are presented in Fig. 1.

respectively, indicating marked population differentiation. A dendrogram of $\bar{I}$ values (Fig. 2) shows, with one exception (Turure), that populations from the Caroni and Oropuche drainages cluster separately.

A comparison of 1989 and 1990 allele frequencies from the Lower Aripo and Oropuche indicate high similarity between years in the Oropuche, but significant differences between years in the Lower Aripo (Nass' (1959) χ^2 1 d.f., $P < 0{\cdot}05$; Table I). Nevertheless, when Nei's (1972) mean genetic identities ($\bar{I}$) between the 1989 and 1990 samples were calculated, the pairs of annual samples clustered together (Fig. 2), indicating broad similarity.

POPULATION STRUCTURE

Genotypic distributions were in Hardy–Weinberg equilibrium in the majority of populations, though some significant departures were detected [χ^2 with Levene's (1949) correction; Table I]. Deviations were due exclusively to a deficit of heterozygotes, and were distributed across loci and populations.

DISTRIBUTION OF GENETIC DIVERSITY

Estimates of genetic diversity as indicated by observed ($\bar{H}_0$) and expected heterozygosities ($\bar{H}_e$), and proportion of polymorphic loci (P) differed markedly among populations (Table I). The highest mean observed heterozygosities were recorded in the Oropuche [$\bar{H}_0 = 0{\cdot}058 \pm 0{\cdot}027$ (S.E.)] and Tranquille [$\bar{H}_0 = 0{\cdot}040 \pm 0{\cdot}019$ (S.E.)] rivers, while the lowest value was found in the Tunapuna [$\bar{H}_0 = 0{\cdot}021 \pm 0{\cdot}014$ (S.E.)].

IV. DISCUSSION

Interpretations of population differentiation in space are at best difficult, especially where sites are sampled once only. There is a need to estimate the relative degree of temporal variation in allele frequencies, although problems are eased if genetic diversity can be partitioned into meaningful geographical patterns (Carvalho *et al.*, 1991). Preliminary data presented here indicate the maintenance of allozymic differences among *P. reticulata* populations over 1 year (Table I), time sufficient for three to four generations of guppies. There was, not unexpectedly, some variation between years within a site.

Present findings support our earlier data (Carvalho *et al.*, 1991) showing marked genetic divergence in N. Trinidad guppies. The extent and patterns of divergence suggest that historical factors (Boos, 1984; Ali, 1989; J. S. Kenny, personal communication) have played a major role in determining the extent of differentiation. The broad genetic separation of guppies from the Caroni and Oropuche drainages supports the ' two arcs ' hypothesis (Boos, 1984) which asserts that the freshwater fauna from these drainages derive from divergent ancestral stocks. There was, however, one exception to this pattern: the clustering of the Turure population from the Oropuche drainage with the Guanapo River of the Caroni system (Fig. 2). The level of identity (Nei's $\bar{I}=0{\cdot}999$) was as high as previously found between sites within rivers (Carvalho *et al.*, 1991). The remarkable similarity of the Turure and Guanapo samples may be due to an experimental introduction of guppies by C. P. Haskins (personal communication to BHS, 1990) in 1957. Approximately 200 adult guppies (roughly equal numbers of males and females) were collected from the lower Arima River (Caroni drainage, Fig. 1) near its confluence with the Guanapo, and released in the Upper Turure River. The introduction was highly successful and the Upper Turure, previously free of guppies, was soon populated with large numbers of Caroni-drainage fish. Although our Turure site was 2 km downstream of the introduction site, an intriguing possibility is that the fish in our collection were descendants of the Arima guppies in Haskins' experiment. Clearly this hypothesis requires further testing, and to this end we collected additional samples from the Oropuche and Caroni drainages in April 1991.

Population structure of *P. reticulata* as described by genotypic distributions indicates that most populations conform to Hardy–Weinberg predictions. Such observations appear surprising in view of the intense sexual selection and strong mate preference characteristic of many guppy populations (Houde & Endler, 1990). Such non-random mating would be expected to distort genotypic distributions, especially since the cues for mate choice are genetically controlled (Houde, 1988). Conformity could arise from the counterbalancing effects of sampling error, or local variation in mate frequency and intensity of sexual selection. The heterozygote deficiencies detected in some populations do suggest the operation of non-random mating, though sampling bias due to the Wahlund effect (Richardson *et al.*, 1986) may also contribute. The extent of microgeographic differentiation within rivers (Carvalho *et al.*, 1991) must be more closely addressed.

When present populations are added to those analysed previously (Carvalho *et al.*, 1991: six rivers x two sub-samples per river), and the sites classified broadly as upland or lowland rivers (Fig. 1), a strong association between mean observed heterozygosities and river type emerges. *P. reticulata* from lowland habitats (Guanapo, Lower Aripo, Oropuche, Tranquille, Turure sites) have higher mean heterozygosities [$\bar{H}_0=0{\cdot}0382\pm0{\cdot}006$ (S.E.), $n=9$] than their upland counterparts (Arima, Upper Aripo, Paria, Yarra, Tunapuna sites; [$\bar{H}_0=0{\cdot}0112\pm0{\cdot}0034$ (S.E.), $n=9$]. The downstream movement of fish during the rainy season, and one-way gene flow across barrier waterfalls could contribute to the increased heterozygosities of lowland guppy populations. Field observations on site fidelity (Haskins *et al.*, 1961, and our own unpublished mark–recapture studies of April 1991) do not, however, provide strong support for this idea. Alternatively, other factors likely to enhance genetic diversity, such as increased heterogeneity of the environment (Valentine, 1976) could operate. It is interesting that there is a highly significant relationship between the biological diversity of sites (measured as number of fish species present) and the mean observed heterozygosity of guppy populations (Spearman rank-order correlation coefficient: $r_s = 0{\cdot}83$, $P < 0{\cdot}01$, $n = 10$; fish species lists provided by Liley & Seghers, 1975; Magurran & Seghers, 1992; J. A. Endler, unpublished). In more diverse communities, guppies are exposed to higher levels of predation, and must cope with specialized fish predators such as the pike cichlid, *Crenicichla alta* Eigenman. Behavioural responses to predation include increased levels of schooling (Seghers, 1974; Magurran & Seghers, 1992) and risk-sensitive courtship (Magurran & Seghers, 1990). Higher rates of sneaky mating (gonopodial thrusting) in predated populations (Luyten & Liley, 1985) are likely to reduce opportunities for female choice and result in less intense sexual selection.

Studies on allozymic differentiation in *P. reticulata* populations from Trinidad have uncovered a number of patterns related to ancestral colonization, population structure and levels of genetic diversity. The detailed biological background available for these populations, taken together with an integrated genetic and behavioural approach should reveal much about the evolutionary determinants of population differentiation.

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