VARIATION, POPULATION STRUCTURE AND GENE FLOW IN THE NASSAU GROUPER, *EPINEPHELUS STRIATUS*.

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ABSTRACT

Preliminary results are presented of genetic variability and population structure in the commercially important grouper *Epinephelus striatus*, examined through enzyme electrophoresis. 21 enzyme systems were screened and clearly resolved enzyme phenotypes were obtained at 20 loci of which 5 exhibited polymorphisms; AH*, CK*, GPI-1*, GPI-2* and SOD*. Scoring at these 20 loci for 5 samples (264 individuals). revealed intermediate - low levels of genetic variability; mean heterozygosities per locus = 0.024, proportion of polymorphic loci = 0.15, and the mean effective number of alleles = 1.45. No evidence was found for population substructuring by sex or small-scale spatial distribution. Analyses of allelic frequencies, using samples from Belize, Bahamas, Turks and Caicos and Cayman Islands, provided no evidence of macrogeographic stock separation. As all samples conformed to Hardy-Weinberg expectations, these results are consistent with a single panmictic population within the northern Caribbean basin. Estimates of mean Nem, the effective number of migrants per generation, through standardized variances of allelic frequencies (Fst), and the frequencies of private alleles, indicated that gene flow in the region must be high. Recommendations for resource management are discussed.

Key words: Genetics, Epinephelus striatus, grouper, stock identification.

INTRODUCTION.

The serranid subfamily Epinephelinae, commonly known as groupers, are of considerable economic value, particularly in the coastal fisheries of tropical and sub-tropical regions. Perhaps 90% of world foodfish landings derive from artisanal fisheries, of which groupers are a major component. Total grouper landings in 1990

were estimated to be 91,141 metric tons, with a high proportion (29,505 tons) being caught in the western central Atlantic region (Heemstra and Randall, 1993).

The Nassau grouper, *Epinephelus striatus*, is one of the most important commercial species, with annual landings in the Caribbean region ranging from 200 - 490 tons over the last 10 years (Heemstra and Randall, 1993). However, serious declines have recently been recorded in a number of stocks, primarily as a result of intense fishing pressure. Aggregation sites have disappeared in Bermuda, Puerto Rico and the U.S. Virgin Islands, where E. striatus is now considered commercially extinct (Sadovy, in press). As adjacent stocks appeared to have weathered similar levels of exploitation, these localised stock crashes may have resulted from the processes of self-recruitment and recruitment overfishing. Alternatively, off-island recruitment to these islands, with their narrow insular platforms, may be more sporadic than recruitment to large extensive platforms such as Cuba or the Bahamas, or the long coastline of central America (Sadovy, in press; Colin, Shapiro and Weiler, 1987).

Grouper larvae appear to have the biological properties appropriate for wide-scale dispersal and recruitment. Bouyant eggs generally hatch within 24 hours and the pelagic larvae begin feeding on zooplankton approximately 2 - 4 days after hatching (Tucker and Woodward, in press). The larvae develop elongate dorsal and pelvic fin spines for bouyancy and protection which are reabsorbed prior to transformation. From hatchery studies, larval duration is estimated to range between 25 and 75 days (Leis, 1987; Tucker and Woodward, in press). As adults and juveniles are entirely limited to a benthic existence, widespread dispersal and gene flow within grouper species would be dependant on this relatively prolonged larval stage.

In view of the potential for long-distance recruitment, genetic studies are needed to delineate stocks of *E. striatus* within the Caribbean and thereby establish biologically meaningful management units. With this information, appropriate management and recovery strategies can subsequently be identified and implemented (Sadovy, in press). The aim of this study was to examine Nassau grouper stock relationships in the Caribbean and estimate gene flow through biochemical genetic techniques. The hypothesis tested was that E. striatus comprised a genetically homogeneous population, with differentiation interpreted as evidence for local stocks being dependent on local spawning events. Enzyme electrophoresis was employed, as investigations of grouper systematics identified suitable combinations of tissues, enzyme systems and buffers for inter and intra-stock comparisons (Lemus, 1988; Lee and Chao, 1990).

Considering the commercial importance of the grouper fisheries, it is surprising that the extensive application of biochemical genetic techniques to

investigate population structure in fish stocks has been slow in diffusing to grouper species. Other than one mtDNA restriction fragment length polymorphism (RFLP) study on red grouper (*E. morio*), demonstrating relatively low levels of nucleon diversity and nucleotide sequence diversity (Richardson and Gold, 1993), little published information is currently available on basic genetic information such as levels of variability. In view of the paucity of such information and its value in grouper aquaculture and stock enhancement projects, this study also describes fully the electrophoretic methods, and levels of polymorphism and heterozygosities obtained, to provide an experimental basis for further studies.

MATERIALS AND METHODS.

Specimen collection: Sample sites are described and illustrated in Figure 1. All specimens were obtained from the catch of commercial fishermen, during local spawning periods and in the vicinity of presumed aggregation sites. At Grand Cayman, Little Cayman, Turks and Caicos and the Bahamas, tissue samples were dissected from fish at landing sites and immediately frozen; specimens from Belize were frozen intact. Intact individuals (full sibs) from a single cultured spawn, were obtained from Dr. John Tucker at Harbor Branch Oceanographic Institution.

50 - 200 mg sub-samples of eye, liver and white muscle tissues were subsequently dissected, placed in cryo-tubes and frozen in liquid oxygen for scoring experiments. Eye tissue was only available from the intact specimens.

The sex of all individuals from the Belize and Grand Cayman samples was determined by gonadal examination.

Screening studies: 25 animals from Belize and 9 from Tucker's cultured stock were used for the initial screening programme. Samples of eye, heart, liver and white muscle were analysed electrophoretically on 4 buffer systems utilised in earlier allozyme studies on groupers (Lee and Chao, 1990; Lemus, 1988. Table 1).

Tissue preparation and electrophoresis: Samples were mechanically homogenised in an equal volume of deionised water and centrifuged at approximately 5000 G in a sero-fuge for 5 min at room temperature. Filter paper wicks (Whatman No. 3) were soaked in the supernatent for 10 min at 4¡C.

All systems were run on horizontal starch gels (11.3 % Sigma starch). Gels were prepared in a microwave with vigorous stirring every 8 - 30 secs. All gels were run for approximately 16 hours at 4°C with ferritin used as an internal standard to compare relative mobilities between samples.

Enzyme activity was visualised according to standard staining procedures (Harris and Hopkinson,1976; Shaw and Prasad, 1970). Substrates for PEPB and PEPD were leu-gly and phe-pro respectively. All staining solutions other than ACP were incorporated in a 1% agar overlay.

Terminology used to describe the isozymes was based on the recommendations of the Fish Genetics Section of the American Fisheries Society to standardise genetic nomenclature (Shaklee et al, 1990). Allele products were numbered by migratory distance relative to the most common band in the congeneric species Epinephelus fulvus, nominally set at 100.

Statistics: All genetic analyses were conducted using BIOSYS-1 release 1.7 (Swofford and Selander, 1981). Fst, a standardised measure of variation in allelic frequencies, was employed to estimate Nem, the number of individuals exchanged between populations each generation using the formula (Slatkin 1985a, b, 1987);

$$Nem = (1/Fst - 1)/4 \quad (1)$$

where Ne is the population size and m is the migration rate. Private alleles, defined as allelic variants restricted to single populations (Neel, 1973), were used to provide an alternative estimate of Nem using the formula (Slatkin, 1985a);

$$Nem = e^{-[(\ln(p) + 2.44)/0.505]} \,/25 \quad (2)$$

where p is the average frequency of private alleles and „ is the average number of individuals sampled per population.

RESULTS

Screening programme: 21 enzyme systems were screened, of which 6 generated unreadable phenotypes under all test conditions. Combinations of system, buffer and tissue were selected to maximise activity, resolution and the number of scorable loci. The results are summarised in Table 1 with details of run conditions. All analyses were conducted on eye, muscle and liver tissues as heart tissue provided little additional information.

20 putative loci were selected from the remaining systems for the scoring programme; 5 loci were polymorphic (AH*, CK*, GPI-1*, GPI-2* and SOD*) and 15 monomorphic. Most loci retained acceptable activity after freezing, however prolonged storage produced blurred phenotypes at 3 of the monomorphic loci (ACP*, MEP* and PEPB*), and activity of LDH-1* and LDH-3* in white muscle was reduced when eye tissue was not available.

4 alleles were identified at AH*; exhibiting the highest heterozygosities of those loci examined. All 4 alleles were present in each of the samples, with the exception of *90 which was absent in the Bahamas sample. Heterozygotes produced double-banded phenotypes typical of a monomeric enzyme and activity remained strong after storage of >3 months at -20¼C.

CK* was weakly polymorphic, one rare allele was detected in the Belize sample (*118) at a frequency of 0.011. The heterozygote appeared to behave as a monomer and not as the predicted dimer, however CK extracted from the skeletal muscle of teleost fish is notable for not displaying heteromeric bands (Ryman and Utter, 1988) and the locus was included in all genetic analyses. The frequency of anodal satellite banding increased with storage time, but phenotypes remained readable for >1 month.

4 alleles were determined at GPI-1*, 3 of which were uncommon. *95 was found at a low frequency at most sites, *120 was only identified at Little Cayman and *100 at Turks and Caicos.

The second GPI locus was resolved later and results were not available for the Belize, Bahamas and cultured stocks. One rare allele (*150) was identified at a low frequency in all the remaining samples. For both GPI loci, three-banded heterozygotes were clearly identifiable in muscle tissue samples > 3 months after freezing.

SOD*, scored on the MPI gel, was clearly polymorphic although resolution was poor. 2 alleles were found, with the heterozygotes generating 3-banded phenotypes as predicted from the enzyme quarternary structure. No significant loss of activity or resolution was detected after 3 months of storage.

Sample degradation occasionally precluded scoring amongst the monomorphic loci; ACP*, MEP* and PEPB* were particularly vulnerable to age-related modification as enzyme activity declined rapidly. In addition, satellite banding in older samples mimicked genetic polymorphisms at 2 loci; MPI* and GLUDH*. Scoring at the latter locus was subsequently discontinued as phenotypes could not be identified with confidence. PGM* resolved as two series of bands, however as the equivalent loci in related species exhibited similar behaviour with correlated variation, the system was scored as a single locus.

Genetic variability: The polymorphic loci were tested for independence by homogeneity c2 test (Richardson, Baverstock and Adams, 1986). All pairwise comparisons were not significant, indicating no evidence for linkage disequilibrium between loci. Individual samples were tested for conformity to Hardy-Weinberg expectations by c2 and exact probability tests. None of the samples were determined to depart significantly from genetic equilibrium.

The 5 geographic samples were pooled and estimates of genetic diversity are summarised in Table 2. The close agreement between observed and expected heterozygosities at all loci, illustrated by very low values for SelanderÕs D statistic, demonstrates that pooling samples independently of sampling date and location generates no significant departures from Hardy-Weinberg expectations. Mean heterozygosity per locus, H, was estimated to be 0.024 and the proportion of

polymorphic loci, P = 0.15. The effective number of alleles, Ne* was calculated to be 1.45.

Microgeographic variation: Population substructuring was examined by sex and spatial distribution. Relevant allele frequencies are given in Table 3.

Information on the sex of individuals were only available from the Belize and Grand Cayman specimens. Skewed sex ratios (females > males) necessitated pooling of these two samples in order to produce an acceptable male sample size (n = 28) for statisical comparisons. As these geographic samples were subsequently determined to be genetically homogeneous by c2 test, and neither of the two pooled samples departed significantly from Hardy-Weinberg expectations, pooling was considered acceptable. c2 tests for heterogeneity revealed no significant differences between the two sexes at any locus and therefore no evidence for sex-linkage.

Spatial heterogeneity within a geographic stock was investigated through comparisons between adult E. striatus captured from two contempory spawning aggregations within the Cayman Island group. The two aggregation sites; 12-mile Bank (Grand Cayman) and Little Cayman, were approximately 130 km distant and separated by deep water, an effective barrier to migration by post-larval individuals. No significant differences between the two samples were found at any of the loci examined and the sum c2-value was also not significant (sum c2[6] = 2.177, P > 0.05). Consequently these two samples were pooled for all future genetic comparisons involving the Cayman Islands stock.

Comparisons were also to have been conducted between different size-classes to test for population substructuring by size. However, the range of total lengths obtained from the Belize sample (49.5 - 67.0 cm) was insufficient to permit groupings by size. Ideally, juvenile and sub-adult fish need sampling for such tests, but these are rarely abundant in the catch of commercial fishermen.

Macrogeographic variation: To determine the genetic relatedness of stocks distributed throughout the northern Caribbean basin, the genetic structures of sexually mature E. striatus from 4 sites; Bahamas, Turks and Caicos, Belize and pooled Cayman Islands, were compared by homogeneity c2 test. The allele frequencies for these samples are presented in Table 3. No statistical differences were identified at any of the 5 polymorphic loci and the sum c2-value was also not significant (sum c2[24] = 29.530, P > 0.05). Pairwise comparisons between the sites confirmed the general homogeneity, as only one test proved significant; pooled Cayman vs. Belize SOD* (c2[1] = 5.51, P < 0.05). As a total of 21 individual tests were conducted, 1 result would be expected to be significant at P < 0.05 by chance alone. Thus, there is no convincing evidence for differentiated E. striatus stocks within the distribution examined.

Gene flow: Fst and the derived estimates of Nem for each locus are presented in Table 4. Values of Nem are high when all 4 sites are included, ranging from 22.5 (AH*) to 41.4 (GPI-1*), with a mean value of 29.9. Genetic data from GPI-2* was limited to 2 sites; pooled Cayman and Turks and Caicos, but when Fst from this locus is included in the calculation, the estimate of mean Nem increases to 36.4.

3 alleles were considered to meet the criteria as private alleles; CK*118 found at a frequency of 0.011 at Belize, GPI-1*120 at a frequency of 0.004 in the pooled Cayman sample, and GPI-1*100 at a frequency of 0.011 in the Turks and Caicos. When the mean of these frequencies (0.0087) is inserted in Equation 2 („ = 64.9), the estimate of Nem is 36.9. Both this estimate and the estimate derived from Equation 1 indicate that gene flow is or has been high within the distribution of samples examined.

Cultured stock: 129 individuals were reared in the Harbor Branch Oceanographic Institution hatchery from a single spawning of 1 male and 1 female E. striatus from Cayman Islands stock (Tucker and Woodward, in press). In 1994, 27 of these were shipped to St. Thomas, U.S. Virgin Islands, and released as sub-adults. This pilot stock enhancement project was conducted without genetic information on the geographic stocks, or the released fish. Native specimens of E. striatus were not available from the Virgin Islands. However, 9 individuals of the cultured stock were sacrificed and examined electrophoretically, with the allele frequencies presented in Table 3. As the cultured fish were not a product of random matings, tests for genetic homogeneity with wild stocks cannot be conducted, and comparisons must be limited to allelic content. The sample was fixed for the commonest alleles found in Caribbean wild stocks at all loci, strongly indicating that both parents were also homozygous at these loci. (Assuming one parent was heterozygous at one of the polymorphic loci, the probability of 8 sibs exhibiting a single allele would be $P < 0.01$).

DISCUSSION.

Levels of electrophoretically detectable genetic variation in *E. striatus* appear to be intermediate - low when compared with other grouper species. Mean heterozygosity per locus ($H = 0.024$) was lower than estimates obtained from preliminary analyses of sympatric populations of red hind (E. guttatus; $H = 0.090$), coney (E. fulvus; $H = 0.031$) and creolefish (Paranthius furcifer; $H = 0.061$) (Hateley, unpublished results). Similar trends were recorded for two other measures of genetic diversity; the proportion of polymorphic loci ($P = 0.150$ vs. 0.333, 0.250 and 0.222 respectively), and the effective number of alleles ($Ne^* = 1.45$ vs. 1.83, 1.55 and 1.39 respectively). However, Nassau grouper generally exhibit more

variability than has been recorded for 4 species of Epinephelus from the Gulf of California, where H ranged from 0 - 0.028, P from 0 - 0.083 and Ne* from 1.0 - 1.1 (Lemus, 1988). Genetic diversity is also relatively low when compared with other tropical marine reef fish, where average heterozygosities were estimated to be 0.054 ± 0.019 when only enzymatic loci were included in the tests (Smith and Fujio, 1982). Despite these findings, sufficient detectable variability exists within E. striatus enzyme loci to permit genetic comparisons within and between stocks.

This study failed to demonstrate significant population substructuring at the microgeographic level when stocks were examined by sex and spatial distribution. Tests for sex-linkage are not only important to ensure that different sex-ratios in collections do not bias allele frequency analyses, but can provide useful information on the genetic basis of sex determination (Shaklee, 1983). Most groupers are believed to be protogynous hermaphrodites; sub-adults mature as females and then undergo sex reversal to become functional males (Shapiro, 1987). However, protogynous hermaphroditism has not been positively shown in E. striatus and primary males have been identified (Shapiro, 1987; Colin, 1992). The absence of sex-linkage at any of the polymorphic loci examined would not be unexpected in either protogynous or gonochoristic reproductive systems, but subsequent identification of a polymorphic sex-linked gene would erode the theory of protogyny.

Genetic homogeneity within the Cayman Island group must be maintained independently of the post-settlement stages. The distance between the 2 aggregation sites sampled (approximately 130 km), would not present a serious obstacle to gene flow through adult migration, as tagging studies have demonstrated large individuals are capable of travelling distances in excess of 100 km (Colin, 1992). However, tracts of deep water (>200 m) would be an effective barrier to migration by the shallow water benthic adult and juvenile stages. Similar results have been obtained in genetic comparisons of E. fulvus and E. guttatus from the Bermuda island chain, as isolated adult sub-populations from the Bermuda platform and an offshore bank were also determined to be genetically homogeneous (Hateley, unpublished results).

Sampling over an extended geographic range (Belize, Bahamas, Cayman, Turks and Caicos) also failed to demonstrate population differentiation. As these samples were also in equilibrium, the results are consistent with a single panmictic population of E. striatus throughout the northern Caribbean. This parallels the genetic structures of 2 commercially important shellfish species in the region; as stocks of queen conch (Strombus gigas) and spiny lobster (Panulirus argus) distributed throughout the Caribbean have been determined to be genetically

undifferentiated (Mitton, Berg and Orr, 1989; Silberman, submitted; Hateley and Sleeter, 1993).

The similarity of allelic frequencies amongst E. striatus populations is consistent with the presence, or recent occurrence, of considerable gene flow within the region. The estimates of Nem through Fst and the frequency of private alleles are concordant and indicate the order of 30 individuals exchanged among localities per generation. These estimates are higher than similar estimates for queen conch, where Nem » 10. This may reflect the relative estimated dispersal capabilities of the 2 species, as conch larvae are believed to be planktonic for 12 - 25 days, compared with 46 - 75 for Nassau grouper (Tucker and Woodward, in press). No similar estimates are available for P. argus, which probably has the highest dispersal capability of the 3 species with a larval duration exceeding 6 months (Sims and Ingle, 1967). As the estimates of Nem for E. striatus do not include the relatively isolated Bermuda population, gene flow throughout the grouper's northern range must be inferred from parallel studies on the congeners E. guttatus and E. fulvus. These electrophoretic surveys are not as complete as for E. striatus, but preliminary results indicate that Bermuda, Puerto Rico and Turks and Caicos populations of the more polymorphic *E. guttatus* are also genetically homogeneous (Hateley, unpublished results).

Recruitment of settlement-stage Nassau grouper larvae has been demonstrated to be highly episodic and dependent on unpredictable weather events (Shenker et al., 1993). During conditions unfavorable for local recruitment, larvae may be transported considerable distances before settlement is possible at a suitable nursery area. The ability to delay transformation would assist in the process of off-island recruitment. There is some circumstantial evidence for delayed metamorphosis, as length of settlement-stage larvae collected in the Bahamas ranged between 18 - 30 mm (Shenker et al., 1993), whereas an individual collected near Bermuda was 45.6 mm total length (Smith, 1971). Transformation of hatchery-reared Nassau grouper typically occurred between 46 and 75 days at lengths of 32 - 50 mm (Tucker and Woodward, in press).

As a single northern Caribbean stock has not been ruled out by this study, closer international co-operation in the management of Nassau grouper is strongly recommended. However, as an element of self-recruitment almost certainly occurs within the region, particularly for stocks inhabiting expansive platform areas (e.g. Bahamas; Shenker et al., 1993) or where local circulation patterns may be important in larval entrainment (e.g. Cayman Islands; Colin, Shapiro and Weiler, 1987), countries must continue to develop and implement local management policies. Examples of conservation measures currently employed are (from Sadovy, in press); minimum sizes (Bahamas), bag limits (Bermuda), prohibiting fishing at

aggregation sites (Bermuda, Dominican Republic), gear limitations (Cayman Islands, Mexico) and total protection (U.S. federal waters). One major limitation of gel electrophoresis is that only differences between samples can be proven, and not similarities. Differences in gene frequencies between samples may exist but were not detected by the experimental procedure. Therefore it is recommended that similar genetic studies employing more sensitive techniques are initiated to investigate E. striatus stock structure (e.g. mtDNA and single copy nDNA RFLP; Wirgin and Waldman, 1994).

The recent release of 27 Nassau grouper at St. Thomas, U.S.Virgin Islands, as part of a pilot stock enhancement project, was conducted without knowledge of the genetic relatedness of the stocks involved (Roberts et al., in press). There is increasing concern worldwide that the potential benefits of restocking may be offset by alterations in the genetic structure of recipient stocks. For example, hybridisation between introduced fish and residents from a different stock may cause the breakdown of locally adapted gene complexes, or levels of genetic variability may differ in hatchery and wild stocks (Stickney, 1994). Unfortunately, this study provides little additional genetic information on the merits of this introduction, as genetic analyses of the recipent stock were not possible due to the precarious state of the local population. Other than non-destructive DNA sampling of Nassau grouper (employing polymerase chain reaction methodology), the optimum approach to identify the genetic relatedness of the federally protected U.S.Virgin Islands Nassau grouper stock to the donor Cayman Islands stock, is through parallel stock identification studies on more variable congeners (e.g. E. guttatus; Hateley, unpublished results). As a product of a single mating pair, the cultured stock not unexpectedly demonstrated no genetic variability, being homozygous for the commonest alleles at all loci. Thus these fish could not be considered genetically marked, a useful property for introduced fish particularly when dart-tag loss exceeded 30% over 3 months (Tucker and Woodward, in press).

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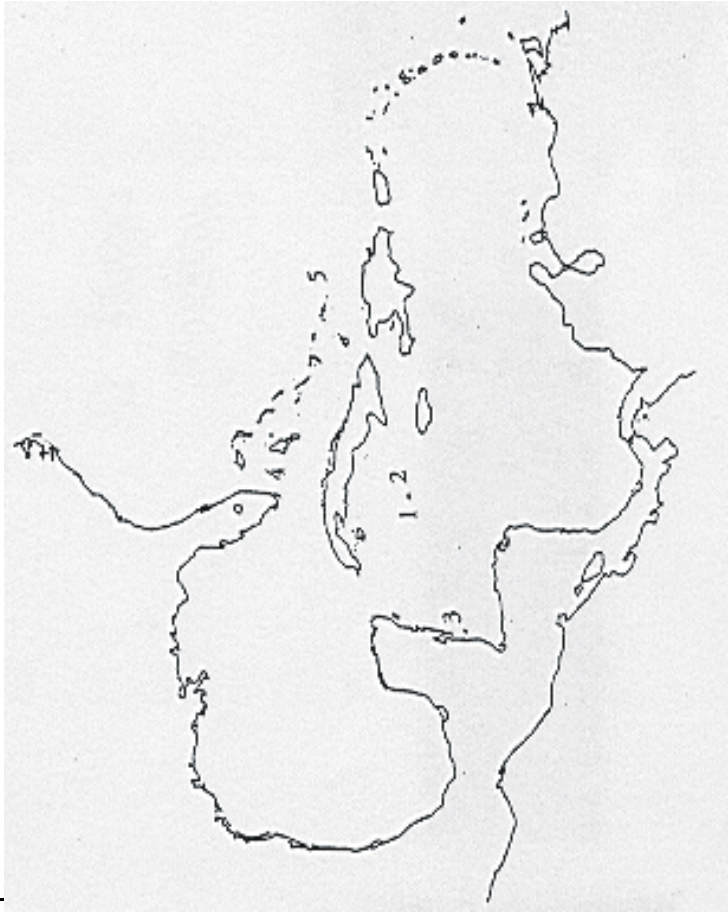

Figure 1. Collection localities for genetic studies of Nassau grouper:
1. 12-mile Bank, Grand Cayman; 2. Little Cayman; 3. Ambergris Cay, Belize; 4. New Providence, Bahamas. 5. Caicos Bank, Turks and Caicos.

Table 1. Results of screening programme. Enzyme systems tested, tissue and buffer combinations employed and activity recorded.

System	E.C. Number	Optimum buffer & tissue	No. of loci	Activity
AAT	2.6.1.1	TC (pH 6.9) Liver	2	Monomorphic
ACP	3.1.3.2	TC (pH 6.9) Liver	1	Monomorphic
ADH	1.1.1.1	- - Unreadable		
AH	4.2.1.3.	TC (pH 8.0) Liver	1	Polymorphic
CK	2.7.3.2	EBT (pH 8.6) Muscle	1	Polymorphic
FH	4.2.1.2	TC (pH 6.9) Liver	1	Monomorphic
G3PDH	1.1.1.8	- - Unreadable		
G6PDH	1.1.1.49	- - Uninterpretable		
GDH	1.1.1.47	- - Unreadable		
GLUDH	1.1.1.-	- - Uninterpretable		
GPI	5.3.1.9	EBT (pH 8.6) Eye	2	Polymorphic
IDDH	1.1.1.14	- - Unreadable		
LDH	1.1.1.27	EBT (pH 8.6) Eye	3	Monomorphic
MDH	1.1.1.37	TC (pH 8.0) Liver	1	Monomorphic
MEP	1.1.1.40	EBT (pH 8.6) Muscle	1	Monomorphic
MPI	5.3.1.8	TC (pH 8.0) Liver	1	Monomorphic
PEPB	3.4.-.-	TC (pH 6.9) Liver	1	Monomorphic
PEPD	3.4.13.9	TC (pH 6.9) Liver	1	Monomorphic
PGDH	1.1.1.44	TC (pH 6.9) Liver	1	Monomorphic
PGM	5.4.2.2	EBT (pH 8.6) Muscle	1	Monomorphic
SOD	1.15.1.1	TC (pH 8.0) Liver	1	Polymorphic

Buffer systems tested:

TC (pH 6.9); 0.75 M Tris/0.25M citrate (Whitt, 1970). 30 mA.gel-1,16 hours.

EBT (pH 8.6);0.02 M EDTA/0.5 M borate/0.9 M Tris (Shaklee, 1973). 6 mA.gel-1 16 hours.
TEMM (pH 7.4);0.1 M Tris/0.1 M maleate/0.01 M EDTA/0.01 M MgCl2 (Shaw and Prasad, 1970). 8 mA.gel-1 16 hours.
TC (pH 8.0);0.687 M Tris/0.157 M citrate (Shaw and Prasad, 1970). 10 mA.gel-1 16 hours.

Table 2. Mean heterozygosities (expected and observed) and deviation from Hardy-Weinberg proportions as estimated by SelanderÕs D statistic. Indices of genetic diversity follow.

	Heterozygosoties		
Locus	Expected	Observed	D
AH*	0.397	0.394	-0.006
CK*	0.004	0.004	0.000
GPI-1*	0.034	0.035	0.012
GPI-2*	0.036	0.036	0.015
SOD*	0.011	0.011	0.004

Mean heterozygosity per locus H = 0.024;
Proportion of polymorphic loci (commonest allele frequency < 0.99) P = 0.15;
Effective number of alleles Ne* = 1.45.

Table 3. Allele frequencies for population samples and pooled Grand Cayman / Belize samples partitioned by sex (N = number of individuals).

Locus	Allele	Female	Male	Grand Cayman	Little Cayman	Pooled Cayman	Belize	Bahamas	Turks & Caicos	Cultured
*AAT-1**	N	57	24	42	62	104	39	34	23	8
	*93	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*AAT-2**	N	57	23	42	62	104	39	34	23	7
	*95	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*ACP**	N	19	5	-	-	-	24	-	-	9
	*100	1.000	1.000	-	-	-	1.000	-	-	1.000
*AH**	N	68	28	50	67	117	46	34	49	8
	*110	0.044	0.000	0.050	0.037	0.043	0.010	0.029	0.010	0.000
	*103	0.735	0.696	0.680	0.724	0.705	0.772	0.736	0.837	1.000
	*100	0.199	0.286	0.250	0.224	0.235	0.196	0.235	0.143	0.000
	*90	0.022	0.018	0.020	0.015	0.017	0.022	0.000	0.010	0.000
*CK**	N	68	28	50	73	123	46	46	45	8
	*118	0.007	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000
	*100	0.993	1.000	1.000	1.000	1.000	0.989	1.000	1.000	1.000
*EN**	N	68	28	50	66	116	46	46	45	9
	*90	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*GPI-1**	N	67	28	50	73	123	45	46	45	8
	*120	0.000	0.000	0.000	0.007	0.004	0.000	0.000	0.000	0.000
	*112	0.985	0.982	0.980	0.973	0.976	0.989	1.000	0.978	1.000
	*100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000
	*95	0.015	0.018	0.020	0.020	0.020	0.011	0.000	0.011	0.000
*GPI-2**	N	31	17	49	73	122	-	-	44	-
	*150	0.000	0.029	0.010	0.027	0.020	-	-	0.011	-
	*100	1.000	0.971	0.990	0.973	0.980	-	-	0.989	-
*IDHP**	N	68	28	50	66	116	46	34	52	8
	*120	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*LDH-1**	N	19	5	-	-	-	24	-	-	6
	*97	1.000	1.000	-	-	-	1.000	-	-	1.000
*LDH-2**	N	68	28	50	73	123	46	46	45	8
	*98	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*LDH-3**	N	5	3	8	14	22	-	-	-	8
	*100	1.000	1.000	1.000	1.000	1.000	-	-	-	1.000
*MDH-1**	N	65	25	50	67	117	40	45	52	8
	*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Locus	Allele	Female	Male	Grand Cayman	Little Cayman	Pooled Cayman	Belize	Bahamas	Turks & Caicos	Cultured
*MEP**	N	36	10	-	-	-	46	46	-	7
	*95	1.000	1.000	-	-	-	1.000	1.000	-	1.000
*MPI**	N	32	18	50	67	117	-	46	52	8
	*100	1.000	1.000	1.000	1.000	1.000	-	1.000	1.000	1.000
*PEPB**	N	39	9	22	14	36	26	35	13	9
	*100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*PEPD**	N	68	28	50	66	116	46	46	52	9
	*103	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*PGDH**	N	66	28	49	66	115	45	34	52	9
	*108	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*PGM**	N	66	28	50	73	123	46	46	45	9
	*84	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*SOD**	N	68	28	50	76	126	46	46	52	8
	*100	0.993	0.982	1.000	1.000	1.000	0.978	1.000	0.990	1.000
	*40	0.007	0.018	0.000	0.000	0.000	0.022	0.000	0.010	0.000

Table 4. Estimates of Fst and Nem for each locus (only 2 sites).

Locus	Fst	Nem
AH*	0.011	22.5
CK*	0.008	31.0
GPI-1*	0.006	41.4
GPI-2*	0.004	62.3
SOD*	0.010	24.8