1976/7

FISHING INDUSTRY TRUST ACCOUNT

Final Annual Report

1. Title of Project

Determination of the geographical structure of breeding populations in several species of commercial fishes.

2. Name of Applicants

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Introduction:

This is the final annual report for this grant and consists of a short summary of the information obtained on each of the species studied. The detailed information will become available over the next few months in the seven manuscripts that are presently being prepared for publication and in Mr MacDonald's PhD thesis, which will be submitted for examination in March.

As well as these summaries we have included an appendix which comments on our experiences during this pilot study in a new research area, and we hope that these observations will be of use to the Committee.

Australian 'salmon' (genus Arripis)

As outlined in two previous annual reports, the problem of the relationship between stocks of western 'salmon' fished in Western Australian, South Australian, and Victorian waters was resolved mainly through a preponderance of negative evidence.

Liver tissue was collected from 670 western 'salmon' and 100 eastern 'salmon' in all southern States (Figure 1) with the aid of staff from the relevant State Fisheries departments, CSIRO Division of Fisheries and Oceanography, and commercial fishermen. Electrophoretic analysis of 22 enzymatic proteins, coded by 27 genetic loci, revealed very low levels of polymorphism (11.1%of loci polymorphic at the $p \leq .99$ level) and low levels of overall genetic variation (mean heterogygosity, $H = .008 \pm .003$) in western 'salmon'. Only a liver esterase locus (EST) was polymorphic at the $p \leq .95$ level, and as such was the only useful marker system for looking at the population structure of western 'salmon'.

A homogeneity $x^2$ test of EST allele frequencies in each of the nine western 'salmon' samples collected revealed no significant geographical heterogeneity. Tests of EST allele distributions between sexes and between different age classes also yielded no significant differences. If average coefficients of kinship between the nine samples are computed from the EST data and plotted against geographic distance between the samples (Figure 2), a useful visual summary of the spatial breeding structure of western 'salmon' is obtained. The coefficient of kinship is effectively a measure of breeding, as it determines the probability that two genes sampled from

Figure 1

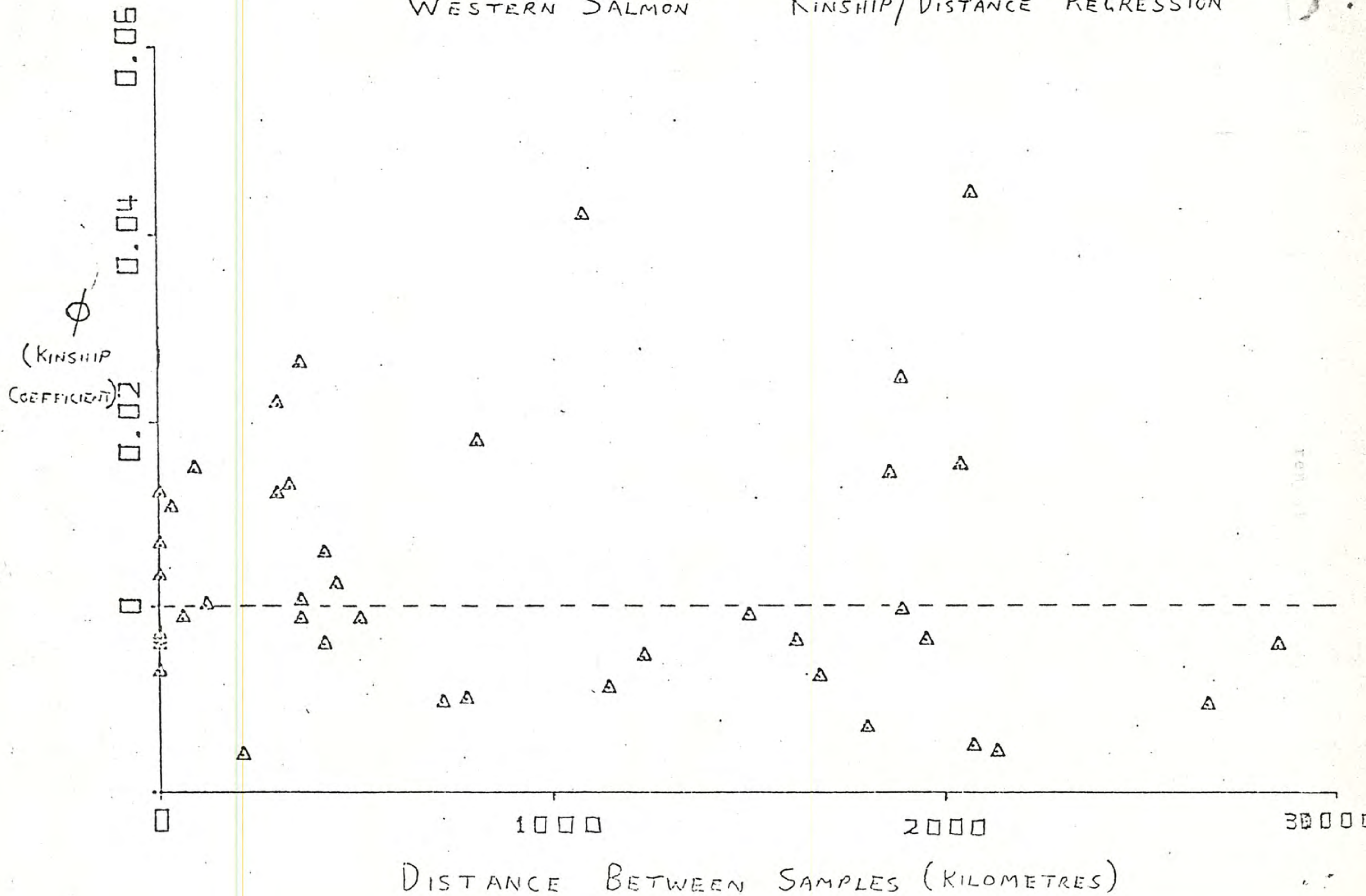
AUSTRALIAN 'SALMON' - CAPTURE LOCATIONS

1976 - 7

| SAMPLE | LOCATION | SIZE (n) |
|---|---|---|
| A | TWO PEOPLES BAY, W.A. | 100 |
| B | BREMER BAY, W.A. | 99 |
| C | VENUS BAY, S.A. | 52 |
| D | MT. DUTTON BAY, S.A. | 50 |
| E | FRANKLIN HARBOUR, S.A. | 78 |
| F | CAPE JERVIS, S.A. | 78 |
| G | "THE PAGES", S.A. | 55 |
| H | MURRAY R. MOUTH, GOOLWA, S.A. | 50 |
| I | PORT PHILIP BAY, VIC. | 110 + 25* |
| J | FIVE MILE BEACH, TAS. | 70* |
| K | TWOFOLD BAY, N.S.W. | 3* |
| L | ULLADULLA, N.S.W. | 3* |

* = SPECIMENS IDENTIFIED AS EASTERN 'SALMON'

Figure 2.



the same locus in different individuals are identical by descent. The 0 line on the graph represents zero inbreeding, i.e. the point at which 2 sampled genes are equally likely to be identical or different. The negative values indicate that the two sampled genes are more likely than not to be different.

It can be seen from Figure 2 that at any given distance between samples the scatter of kinship coefficient values about the 0 value is essentially similar, indicating no change in the relatedness of samples with distance. The high variance of kinship estimates at any given distance is due to the fact that only one locus was used to generate the regression. This lack of subpopulation structuring across the range of western 'salmon' is consistent with the hypothesis that all western 'salmon' stocks are part of a single, panmictic breeding population.

Analysis of the distribution of EST genotypes in each of the western 'salmon' samples, and in different age classes, reveals that there is a significant deficiency of heterogygous genotypes in the 1 + year class of juveniles, but this deficiency is not apparent in older year classes. It is suggested that the EST heterozygote deficiency arises through selective mortality during the early dispersal phases of eggs, larvae and juveniles, and is then counterbalanced in later years either by opposing selective forces or by random processes. The EST system is thus a potentially useful marker in monitoring the nature and extent of environmental influences on the survival of successive year classes of young western 'salmon'. However more work needs to be done to determine

which environmental parameters influence the distribution of the EST genotypes.

Eastern 'salmon' exhibit more genetic variation than western 'salmon' with six loci (22.2%) out of 27 polymorphic at the $p \leq .99$ level, and three of these loci polymorphic at the $p \leq .95$ level. Mean heterogygosity was estimated at $H = .049$, approximately five times the estimate for western 'salmon'. The historical, biogeographical and evolutionary implications of this result will be discussed in forthcoming scientific publications.

As previously reported, the electrophoretic evidence clearly shows that the western and eastern forms of 'salmon' are maintaining separate gene pools despite being sympatric over part of their ranges. The existence of apparently fixed allelic differences between the two forms at the lactic dehydrogenase and superoxide dismutase loci indicates that gene flow (interbreeding) is negligible. The demonstration of reproductive isolation, together with diagnostic morphological and ecological differences established by other workers, is sufficient to consider eastern and western 'salmon' as two separate species.

Liver tissue was recently obtained from 20 specimens of kahawai with the aid of staff from the New Zealand Fisheries Research Division for the purposes of a trans-Tasman comparison of 'salmon' stocks. By electrophoretically screening the kahawai samples for the same 27 loci examined in 'salmon' it was established that the New Zealand stock is genetically distinct from both of the Australian 'salmon' species. The most

diagnostic difference was at the adenosine deaminase locus, where an allele present in kahawai at a frequency of .25 is absent from both eastern and western 'salmon' populations. It seems therefore that there is negligible trans-Tasman migration of salmon stocks - at least from New Zealand to Australia.

While New Zealand kahawai can be considered an isolated breeding unit, they are not sufficiently distinct, either morphologically or genetically, to be considered a species separate from eastern 'salmon'. They share a number of polymorphic enzyme systems, they exhibit no fixed allelic differences at any locus, and they are morphologically distinguished only by characters with overlapping distributions. The taxonomy of the genus Arripis will be revised in forthcoming publications.

Snapper (Chyrsophrys auratus)

Further collections of snapper liver tissue were made in 1979 to complete a reasonable coverage of the species' range, resulting in a final total of 580 specimens in 15 sample sets (Table 3.1). Electrophoretic analysis of the extra samples for seven polymorphic loci confirmed the pattern of allele distribution described in our last annual report (Table 3.11). Two extra samples from Spencer Gulf in South Australia have confirmed the presence of a rare alcohol dehydrogenase allele which is not found elsewhere in Australia, suggesting a distinct resident population of snapper in Spencer Gulf. Tagging studies by Dr Keith Jones of the South Australian Fisheries Division have confirmed the sedentary nature of snapper in the Gulf.

As described in our last annual report, the clinal change in allele frequencies at the esterase, isocitrate dehydrogenase and alcohol dehydrogenase loci, and the heterogeneous distribution of rare alleles in several other loci (Table 3.11) can best be explained by postulating the existence of a series of partially isolated snapper populations around the Australian coastline, with a small but significant amount of gene flow (migration) between adjacent populations, but increasing isolation and genetic divergence with distance. This hypothesis can be visualised by calculating average coefficients of kinship between samples and plotting them against distance (Figure 3). It can be seen that the line of best fit for the regression passes through the 0 line at the 1100 to 1200 kilometer mark. This result implies that on

TABLE 3.1

# AUSTRALIAN SNAPPER

| Sample | Collecting Locality | Map Reference | Sample Size (n) | Collection Date | Length Range (LCF) | Capture Method | Sampled By |
|---|---|---|---|---|---|---|---|
| 1 | Moreton Bay, Qld. | 27°07'S; 153°21'E | 53 | August 1979 | 13-24cm | hand line | Qld. Uni. staff |
| 2 | Off Sydney Heads, NSW | 33°51'S; 151°20'E | 55 | May 1977 | 23-50 | fish traps | author |
| 3 | Off Sydney Heads, NSW | 33°51'S; 151°20'E | 33 | July 1978 | 28-52 | fish traps | author |
| 4 | Bannister Head, NSW | 35°20'S; 150°30'E | 29 | December 1976 | 38-61 | hand line | commercial fishermen |
| 5 | Narooma, NSW | 36°16'S; 150°08'E | 33 | March 1979 | 23-66 | hand line | author |
| 6 | Port Phillip Bay, Vic. | 38°18'S; 144°53'E | 62 | November 1976 | 36-81 | set lines | author |
| 7 | Port Phillip Bay, Vic. | 38°18'S; 144°53'E | 64 | November 1977 | 38-72 | set lines | author |
| 8 | Backstairs Passage, SA | 35°45'S; 138°08'E | 37 | March 1977 | 30-79 | hand line | author |
| 9 | Backstairs Passage, SA | 35°45'S; 138°08'E | 57 | November 1977 | 50-82 | hand line | author |
| 10 | Rosalind Shoal, SA | 34°58'S; 136°24'E | 56 | November 1978 | 29-64 | hand line | S.A. Fisheries Division staff |
| 11 | Cowled Landing, SA | 33°20'S; 137°30'E | 33 | November 1977 | 25-53 | hand line | author |
| 12 | Cowled Landing, SA | 33°20'S; 137°30'E | 70 | April 1979 | 27-67 | hand line | A.N.U. staff |
| 13 | Great Aust. Bight, WA | 33°12'S; 128°21'E | 20 | March 1978 | 31-71 | trawl net | WA Department of Fisheries & W'life |
| 14 | Wilson's Inlet, WA | 34°57'S; 117°22'E | 18 | May 1978 | 22-27 | gill net | author |
| 15 | Shark Bay, WA | 24°39'S; 113°12'E | 60 | May 1978 | 35-67 | hand line | author |

Table 3.11 Allele frequencies for seven polymorphic loci in Australian snapper

| Locus | Allele / Sample | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *FST* | *117* | – | – | – | – | – | – | – | – | – | – | – | – | – | – | .01 |
| | *108* | .04 | .05 | .06 | – | .04 | – | – | .07 | .14 | .08 | .17 | .16 | .10 | .14 | .10 |
| | *100* | .89 | .89 | .89 | .93 | .85 | .96 | .95 | .86 | .74 | .79 | .70 | .72 | .68 | .67 | .67 |
| | *95* | .02 | .04 | – | .02 | – | – | – | .03 | .09 | .08 | .07 | .04 | .02 | .14 | .10 |
| | *90* | .05 | .02 | .05 | .05 | .11 | .04 | .05 | .03 | .03 | .05 | .06 | .08 | .18 | .03 | .11 |
| | *83* | – | – | – | – | – | – | – | .01 | – | – | – | – | .02 | .02 | .01 |
| *IDH* | *124* | – | – | – | – | – | – | – | – | – | – | – | – | – | – | .01 |
| | *114* | .08 | .10 | .12 | .09 | .11 | .15 | .12 | .20 | .22 | .20 | .17 | .18 | .10 | .22 | .24 |
| | *111* | – | .03 | .02 | .05 | .01 | .01 | – | – | – | – | – | – | – | – | – |
| | *100* | .85 | .80 | .83 | .83 | .77 | .73 | .76 | .70 | .68 | .72 | .77 | .71 | .75 | .56 | .63 |
| | *80* | .07 | .06 | .03 | .03 | .11 | .11 | .12 | .10 | .10 | .08 | .06 | .11 | .15 | .22 | .12 |
| | *70* | – | .01 | – | – | – | – | – | – | – | – | – | – | – | – | – |
| *ADA* | *124* | – | – | – | – | – | – | – | – | – | – | – | – | – | – | .01 |
| | *112* | – | – | – | .03 | – | – | – | – | – | – | – | – | – | – | .01 |
| | *100* | .85 | .85 | .79 | .76 | .76 | .80 | .83 | .93 | .82 | .88 | .95 | .83 | .95 | .81 | .91 |
| | *88* | .15 | .15 | .21 | .21 | .24 | .20 | .17 | .07 | .18 | .12 | .05 | .17 | .05 | .19 | .07 |

TABLE 3.11 (cont.)

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FUM | 120 | – | – | – | – | .03 | – | – | – | – | – | – | .01 | – | – | – |
| | 100 | .96 | .96 | .97 | .90 | .95 | .95 | .95 | .97 | .99 | .99 | .98 | .99 | .97 | 1.0 | .95 |
| | 84 | .04 | .04 | .03 | .10 | .02 | .05 | .05 | .03 | .01 | .01 | .02 | – | .03 | – | .05 |
| ADH | 150 | – | – | – | – | – | – | – | .01 | .01 | .02 | – | .01 | – | – | .03 |
| | 125 | – | – | – | – | – | – | – | – | – | .01 | .09 | .05 | – | – | – |
| | 100 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | .96 | .98 | .96 | .94 | .95 | .91 | .92 | .95 | .92 | .93 |
| | 70 | – | – | – | – | – | .04 | .02 | .03 | .05 | .02 | – | .02 | .05 | .08 | .04 |
| GPI | 120 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | .01 |
| | 108 | – | .02 | .02 | – | .01 | .02 | .03 | .01 | .01 | .03 | .02 | .04 | .05 | .03 | .08 |
| | 100 | 1.0 | .97 | .98 | .93 | .97 | .95 | .95 | .97 | .97 | .94 | .95 | .92 | .92 | .94 | .88 |
| | 88 | – | .01 | – | .07 | .02 | .03 | .02 | .02 | .02 | .03 | .03 | .04 | .03 | .03 | .03 |
| PGM | 117 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | .01 |
| | 110 | .02 | .04 | – | .03 | .02 | .01 | – | – | .04 | .04 | .02 | .03 | – | – | .01 |
| | 100 | .98 | .95 | .98 | .97 | .92 | .98 | .98 | 1.0 | .95 | .96 | .95 | .96 | .97 | 1.0 | .97 |
| | 91 | – | .01 | .02 | – | .06 | .01 | .02 | – | .01 | – | .03 | .01 | .03 | – | .01 |

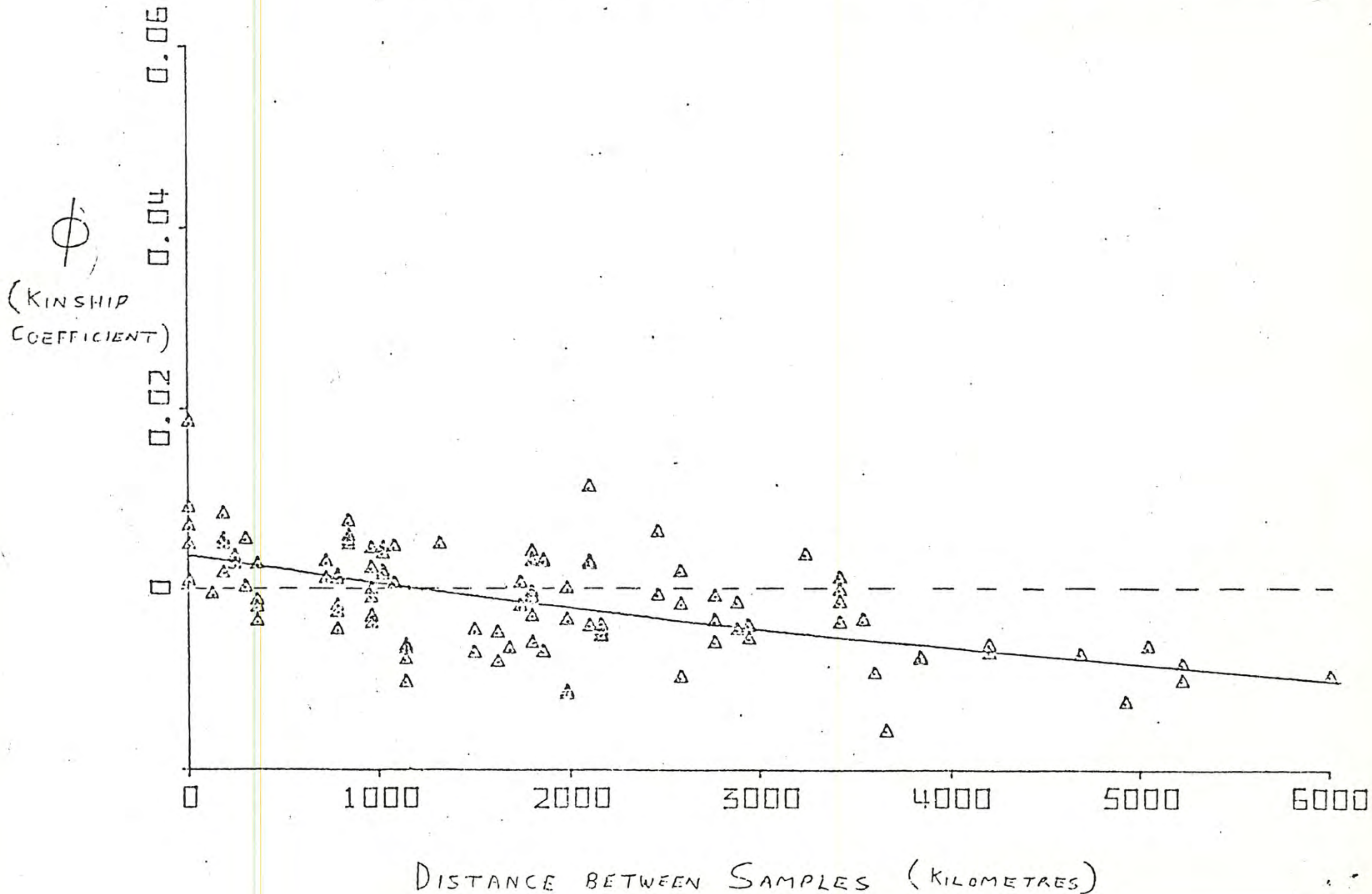



Figure 3.

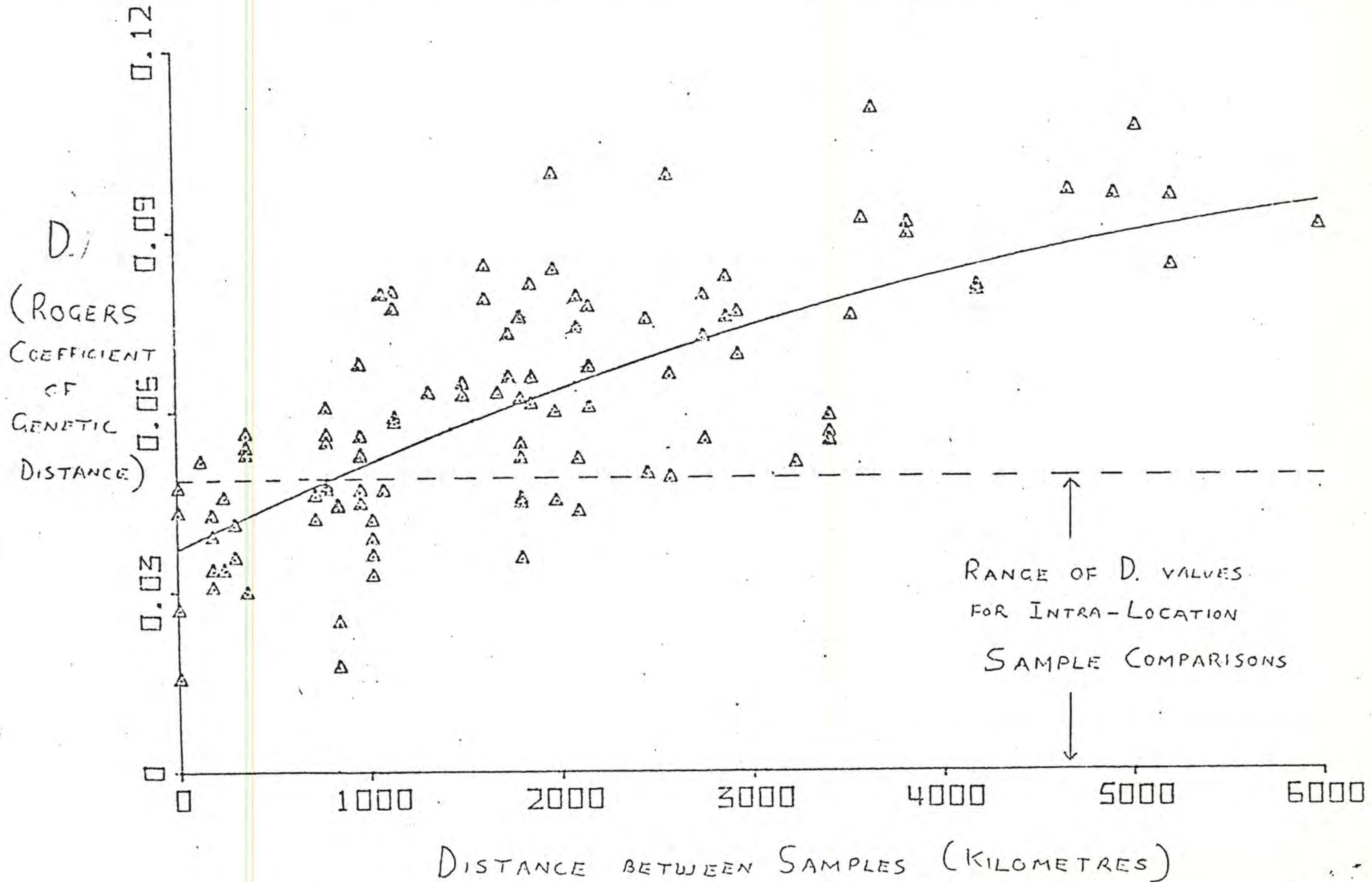
average an interbreeding group of snapper will cover up to 1200 km of coastline, but snapper caught at distances further apart than this are likely to be from distinct breeding populations.

The above results from coefficient of kinship measurements are supported by measuring genetic distance (accumulated differences in allele frequencies for the seven polymorphic loci) between pairs of samples and plotting these against geographic distance (Figure 4). A range of intra-location genetic distances can be obtained by comparing duplicate samples from the same location. This was done using repeat samples of snapper from Sydney, Port Phillip Bay, Cape Jervis (S.A.) and Spencer Gulf. Where genetic distance measurements exceed the maximum intra-location values, significant genetic divergence of samples can be assumed to exist. The line of best fit for the regression in Figure 4 intersects the limit of intra-location values at the 900-1100 km mark, showing close agreement with the result of the kinship analysis. The genetic distance/geographic distance method is less precise than kinship/distance, however, in reflecting the population structure, as spatial heterogeneity in allele frequencies within a species is not necessarily due to breeding isolation.

While the above results indicate that 900 km is the average coastal range of an interbreeding snapper population, other evidence suggests that the actual range of some populations may vary considerably from this mean. We have already described the presence of a small isolate of snapper in Spencer Gulf. On the other hand there appears to be some gene flow along the entire east coast range of snapper based on the homogeneity of allele frequency data from this study, and on long distance

Figure 4

AUSTRALIAN SNAPPER GENETIC/GEOGRAPHICAL DISTANCE



movements indicated by tagging studies conducted by Victorian State Fisheries staff in the 1960s. It appears that a number of environmental and behavioural factors will influence the distribution and breeding structure of snapper stocks, but in general snapper found along open coastlines will tend to be part of a population connected by gene flow over a range of about 1200 km, while those found in large bodies of comparatively sheltered water will tend to be part of a smaller resident population (e.g. Spencer Gulf, S.A. and Shark Bay, W.A.).

Skipjack Tuna (Katsuworus pelamis)

It has not proved possible to produce definitive answers to questions on the relationships of skipjack from Australia to those of other countries. However with the collection of six more sets of samples (608 specimens) from the east coast in 1979 it is possible to make some comments of the eastern Australian stock. The results for the sets from NSW waters (Table 4) were similar to those obtained in the previous year. The higher gene frequency schools are similar to those obtained from skipjack in Papua-New Guinea and Solomon Is. waters, and this association is supported by the fact that the one recaptured tagged fish from NSW was caught in the Solomon Is.

The skipjack fishery in NZ is based on a mixture of fish seasonally moving in from the north (Central Pacific subpopulation) and north-east (south-eastern Pacific subpopulation) of the country, and an analogous situation may occur in Australia. If this is true then the P = 0.57 Jervis Bay school (Table 4) could have come from the north-east and belong to the Central Pacific subpopulation. Fish of this type were caught at Lord Howe Is. in the previous year. If this hypothesis is true then one or more of the 700 fish tagged in this Jervis Bay school might end up in the Fijian fishery. Detailed studies relating size class modes to gene frequencies for a series of years would help clarify relationships in the region and determine the relative importance of the different subpopulation to the fishery.

The three Queensland samples were similar in gene frequency to PNG material, though the frequencies were lower than those

found in sets taken off Port Moresby.

Another unsuccessful attempt was made to collect skipjack tuna from Western Australia, this time in conjunction with the American purse seiner 'Frontier'. A further effort will be made in the next few months to collect Indian Ocean skipjack at University expense.

Further studies using the new guanine deaminase polymorphism show that there is an east west cline in frequency of alleles across the South Pacific analogous to that found for esterase. Other polymorphisms detected have not proved useful for discriminating between subpopulations when wide surveys have been carried out.

Table 4.

| Location | Esterase Gene Frequency |
|---|---|
| West Pacific subpopulations | .60 - .82 |
| Central Pacific subpopulation | .50 - .61 |
| South-eastern Pacific subpopulations | .38 - .47 |
| Montague Is. | .67 |
| Jervis Bay, NSW | .71 |
| Jervis Bay, NSW | .57 |
| Flinders Reef, Qld | .68 |
| Flinders Reef, Qld | .66 |
| Willis Islets Qld | .66 |

Jackass Morwong (Cheilodactylus macropterus)

Further sets of samples of Jackass Morwong have been collected from Eden and analysed for the five previously described enzyme polymorphisms. As well all the material available has been typed for variation in glucose phosphate isomerase. All data sets (11 stations 600 animals) have now been typed and the data prepared and analysed by computer. The data from all areas is homogeneous and in Hardy Wienburg Equilibrium. The isolation by distance analysis shows that fish from various areas are as similar as fish from different stations in the same areas (Figure 5 ). This result is at variance with the conclusions reported last year where errors in the computing programmes used lead to false chi square values being used in the homogeneity tests.

No evidence was found during this study of differentiation between the morwong stocks of southern NSW, Tasmania, eastern Victoria or western Victoria.

Gemfish (Rexea solandri)

A survey of gemfish was initiated as requested. A total of 620 fish consisting of 10 sets of samples were collected from eastern Tasmania, southern NSW and eastern and western Victoria. A total of 23 enzymes were surveyed for genetic variation and unfortunately no variable loci were found. As a result no further studies have been carried out. The range of loci examined will be extended at University expense in the future.

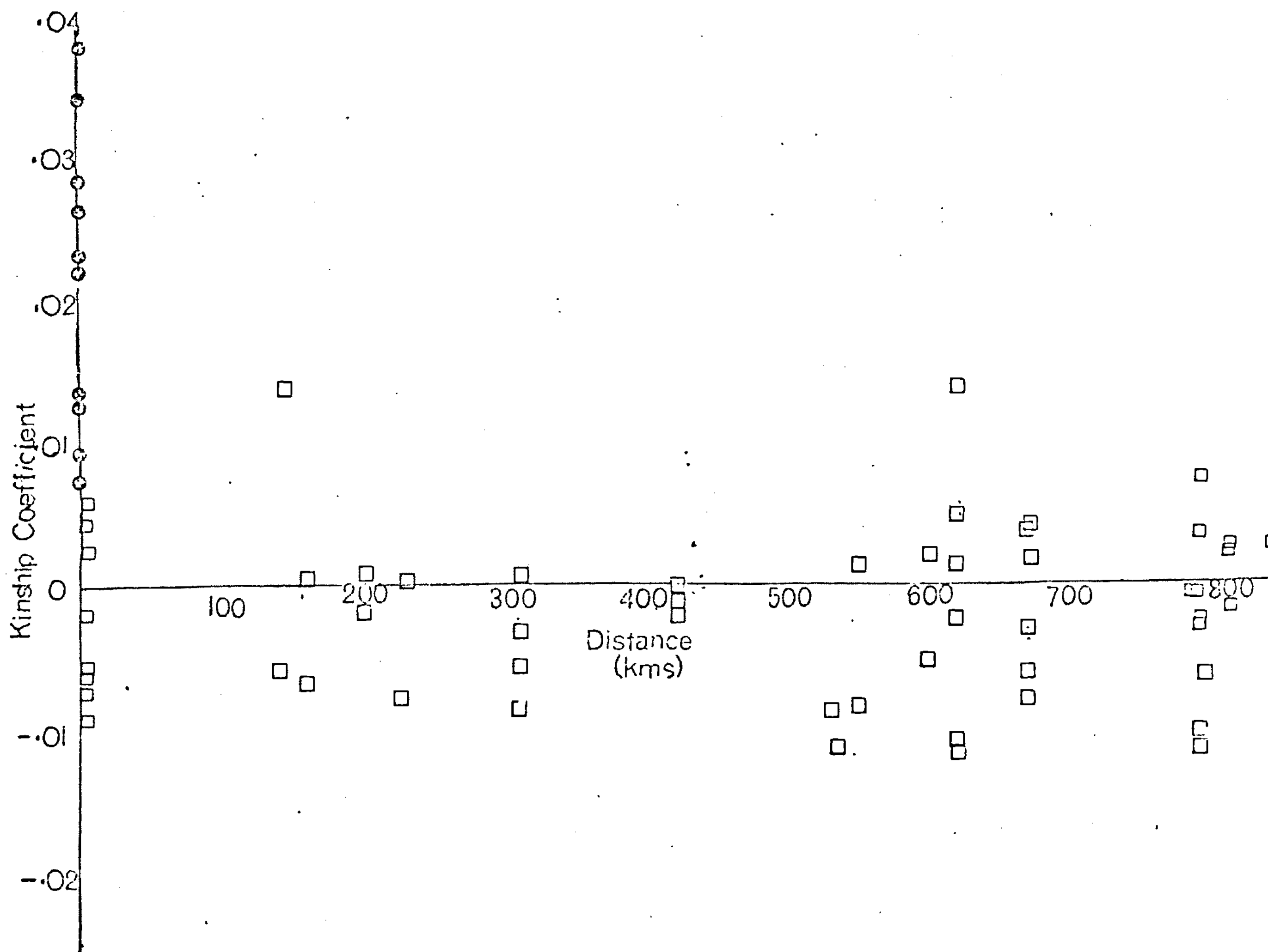



Figure 5. Genetic relationships between morwong populations collected at different geographical distances. Clearly populations from the same area are no more related than populations separated by 800 km.

Western King Prawn (*Penaeus latisulcatus*)

The presence of four-allele polymorphisms in the phosphoglucomutase and glucose phosphate isomerase loci were described in our last annual report. A total of six sample sets (530 animals) were collected from various locations in St. Vincent Gulf and Investigator Strait, S.A. These locations and the observed gene frequencies are given in Figure 4. Statistical analysis of the data showed that the gene and genotype frequencies observed were homogeneous throughout the areas sampled, thus providing no evidence of genetic differentiation between prawn stocks from St. Vincent Gulf and Investigator Strait.

Barramundi (*Lates calcarifer*)

Studies on the structure of barramundi populations were initiated in conjunction with CSIRO, Queensland State Fisheries and the Papua New Guinea government. Small sample sets were obtained from three rivers in the Northern Territory and a survey for genetic variation carried out. A total of 26 proteins were examined and only one, an esterase, was polymorphic. Further material from the Northern Territory was obtained and also a set of samples from Karumbin on the Gulf of Carpentaria. The same alleles were found in each area though more work is needed to properly define the relationships of these stocks (Table 5 ). Attempts to collect material on the east coast of Queensland were successful but the liquid nitrogen cylinder failed and the material denatured before it could be returned to Canberra. Further collections of material are being made in Queensland and material from PNG is also due shortly. This material will be typed at University expense to complete the programme.

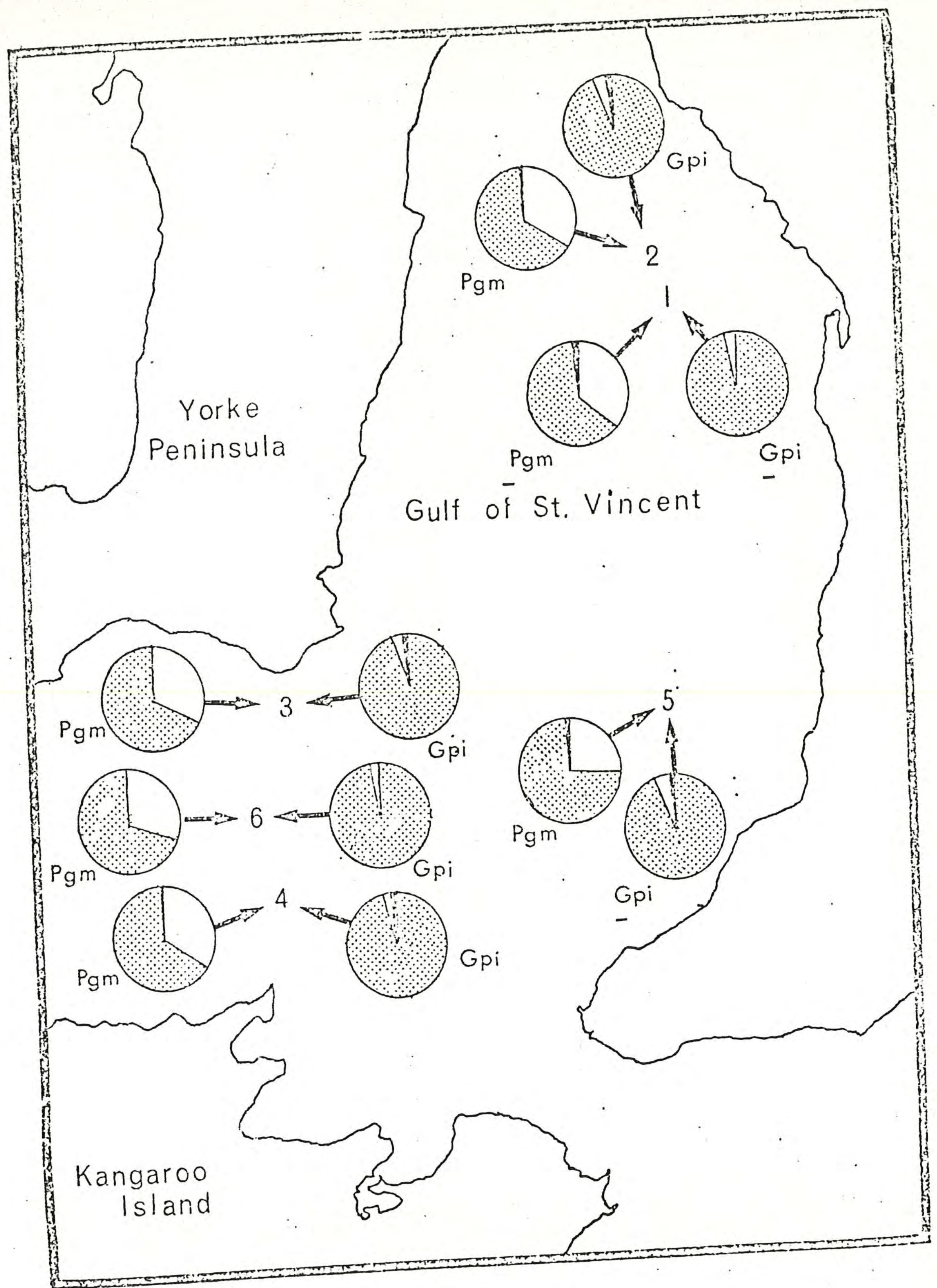



Figure 4. Location and gene frequencies for the six sets of prawns studied.

Table 5.

| | Esterase allele | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Northern Territory | .40 | .56 | .03 | .01 |
| Karumbin (Norman River Qld) | .51 | .47 | .01 | .01 |

Jack Mackerel (Trachurus declivis)

Marked differences in gene frequency between areas have been found for this species at each of the eight polymorphic loci studied. If this data is taken at face value the genetic relationships of the 1000 fish typed can be calculated and show the relationships summarised in Figure 6. However this simple interpretation of the data cannot stand as there is significant heterogeneity in gene frequency within areas and also all eight loci are out of Hardy Weinberg Equilibrium in each area. These results imply that the fish sampled were heterogeneous collections of two or more genetically distinct groups. However analysis of the data by size, age, time of year born or breeding condition did not allow the two or more classes present to be separated. Only for aconitase could part of the heterogeneity be explained and in this case it was due to genetic differences between year classes. However significant heterogeneity still remained at this locus after this year class effect was removed.

Comparison of enzymes from jack mackerel and the similar yellow tail (Trachurus novae-zealandae) showed that there were fixed genetic differences between the species at several loci and that this variation can be used to confirm that yellow tail were not accidentally included in the sample sets. To date no such confusion has been found and it seems unlikely the

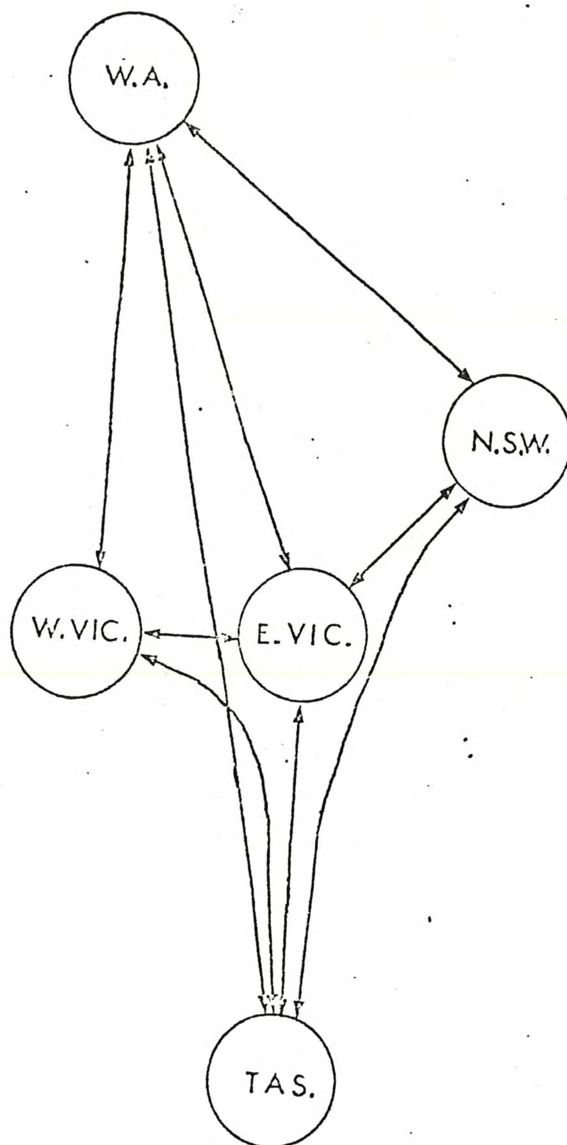



Figure 6. Apparent genetic distances between populations of Jack Mackerel. The length of the arrows are equivalent to the genetic distance between populations.

the heterogeneity is due to this effect.

If another unknown species is present (e.g. Trachurus trachurus from South Africa or Trachurus japonicus) it must be very closely related to the Jack Mackerel as no fixed differences were found in the data set (i.e. heterozygotes between the various alleles present in the material studied were always detected). Alternatively a series of genetically divergent subpopulations may be present in the areas studied but these were thoroughly mixed under the sampling conditions.

Five New Zealand Jack Mackerel were studied and these differed markedly from eastern Australia Jack Mackerel as they were homozygous for alleles that are rare in Australian fish, consequently part of the heterogeneity could be due to movement across the Tasman Sea.

The age class series from the east coast has been extended to seven year classes by the collection of more material from Eden. Attempts by CSIRO to collect more material from Western Australia were unsuccessful and consequently it was not possible to confirm the divergent character of the Western Australian stock.

Given this heterogeneity of unknown origin in the data it is not possible to assume that the divergene gene frequencies found in different parts of Australia are the result of different subpopulations occurring in different areas, as it may simply mean that different proportions of the various forms were caught in different places.

Appendix 1

The following points may be of use to the Committee when considering future genetic programmes.

i) Using cylinders of liquid nitrogen to snap freeze tissue samples in the field was superior to other methods in terms of sample quality and field storage time. However, this year three of the cylinders failed in the field (apparently due to metal fatigue) causing the loss of valuable material. Even so, this system is probably the best available under most circumstances.

ii) Upright -20°C commercial freezers were obtained for storing material in the laboratory. These have proved to be disasterous as they have failed on numerous occasions. Even though all freezers were fitted with alarms a great deal of untyped material was lost. It seems that all the cold air 'falls' out of upright freezers whenever the door is opened, causing the cooling unit to be overworked. Chest freezers seem to be much more dependable.

iii) Stored liver samples are not stable, even at -20°C, for more than a few months, and if material is to be held for long periods of time (e.g. because it cannot be typed immediately, or because it is to be compared with as yet uncollected material it should be stored at -80°C.

iv) Blood samples mixed with glycerol/citrate preservative can be collected on dry ice and are stable for long periods of time when stored at -20°C in the laboratory. The freezing of blood samples in liquid nitrogen actually reduced typability in many cases.

v) The typing facility has suffered from very few technical problems. This is the usual picture if a reasonably large number of people work together (here 10 people) using electrophoretic techniques. If a much smaller group were to carry out this type of work intermittently it could be expected that many technical problems would occur, and in our experience up to one third of available time and resources could be lost.

vi) The rapid turnover of technical staff was a problem during the first half of this programme, and can be expected to be a continuing problem in any project employing temporary staff. On the FIRTA grant inexperienced staff (Technical Assistance Grade 1) were employed and it was noticeable that extra salary funds outlayed to employ Technical Officers on an S.P.C. skipjack tuna study conducted in this laboratory were amply repaid in faster and more accurate typing.

vii) The statistical methods needed to calculate sample sizes and to detect significant heterogeneity in a genetic data set have been improved a great deal in the past few years. It is now possible to extract much more information from data sets and to calculate *a priori* sample sizes needed for specified levels of Type I and Type II sampling errors in genetic data of various kinds. At the same time we have been developing strategies for replicate and sequential sampling designed to identify the biological basis of detected genetic heterogeneity in space and time. Methods for testing isolation by distance models (e.g. kinship analysis) for use in fisheries research have also been developed. These can be used to study the nature of genetic divergence between samples and to infer population structure over the species' range.

As this field develops in the Australasian region the questions asked will become more and more explicit and will require stringent sampling procedures if they are to be properly answered. Already questions may be asked that cannot be answered using an opportunistic approach to sampling, e.g. the origins of the east coast skipjack stock or the seasonal range of the Spencer's Gulf snapper subpopulation.

viii) One difficulty in carrying out this type of project in cooperation with Fisheries agencies is the conflicting demands made on the collecting staff and ship time of these agencies and the Committee may want to establish these priorities with the relevant parties before supporting a project. At present it is often not feasible because of other duties for Fisheries agency staff to spend whatever time is necessary to collect adequate samples from critical locations where collection is uncertain, but unless the predetermined sampling strategy is carried out a genetic programme cannot be expected to answer the questions for which it was designed.

When available, it has proved very effective to place our own staff on commercial fishing vessels to obtain samples, and this should be done whenever possible. However as the complexity of programmes develop it will become increasingly necessary to use research vessels, or to hire commercial fishing boats, to visit particular non-commercial fishing localities or to visit regularly fished areas out of season.

ix) Recent experience has shown that the rate for typing up to six polymorphic systems should be set at about $3 per specimen. However, changes in the international value of Australian currency (all the electrophoresis chemicals come from overseas)

and domestic rates of inflation make it necessary to slightly overbudget on annual funding requests, unless some method is available for obtaining extra funds quickly to cover an unexpected shortfall in expendable research money.

x) In supporting future programmes of this type the Committee might like to consider using the following three stage pattern of funding.

a) Pilot programmes should be funded to determine whether suitable genetic variation is present in the species concerned and, if so, to determine approximate gene frequencies at each locus.

b) Assuming useful variation has been detected, grant applications should be formulated in consultation with potentially co-operating agencies and the Committee. These applications should explicitly define the question to be answered, the sample sizes and sampling strategy required and the type of outside co-operation needed to successfully complete the programme (ship time, collecting staff, computor access etc.).

c) In considering submitted applications it would be to the funding body's benefit to ensure that the specific outside co-operation needed for success is committed before supporting the project. It is also desirable that the projects be operated by experienced research and technical staff and that each project be limited in scope to the questions defined by agreement. If further research work is found to be needed then further funding can be considered by returning to step B.

We have used this pattern for a baitfish study in association with the PNG government and (except for combining steps A and B because of lack of time) for a recently established Australian squid programme. This structure safeguards the committee from funding unproductive work and the scientist from expectations he cannot fulfill.

Abstract: The results of a survey of 42 loci for electrophoretically detectable polymorphisms in the skipjack tuna are described. A new polymorphism in guanine deaminase was found as well as low frequency variation in glucose phosphate isomerase and adenosine deaminase. Average heterozygosity for the species was 0.032. The gene frequencies for 70 sample sets, each of approximately 100 animals, collected at locations throughout the South Pacific Commission region, are given. The genetic structure of the skipjack tuna population of the southern Pacific Ocean is discussed in the light of the results obtained.

The declaration of exclusive economic zones by the nations of the central and south-western Pacific region, combined with the recent rapid expansion of the skipjack tuna fishery in this area (Kearney 1979), has emphasized the need for information on the distribution, population structure and basic biology of this highly migratory species. Fundamental to sound management of the skipjack tuna stocks of the region is the identification of the number, distribution and degree of reproductive independence of any subpopulations of the species which might occur throughout the Pacific Ocean. A research programme combining genetic techniques and tagging to identify subpopulations is being carried out under the auspices of the South Pacific Commission (SPC) and this paper describes the genetic results obtained during this research. For the purposes of this paper a 'subpopulation' is defined as a selfsustaining genetic unit, that is, a group that does not exchange genes at random with other parts of the population, while a 'stock' constitutes the exploitable group of fish found in a particular geographical area at a particular time (Anon 1976).

In the past, genetic markers have been used to infer the subpopulation structure of a number of fish species (de Ligny 1968). Several factors need to be considered when genetic techniques are used in an attempt to detect genetic differentiation and population subdivision within a species.

1. Suitable genetically inherited markers must first be identified. Experience has shown that six or more polymorphic loci are desirable, to allow the importance of the results found at one locus to be independently confirmed at other loci.

2. If two stocks have large differences in gene frequencies and maintain these differences over several generations, then they cannot belong to the same randomly mating population, as improbably high selection pressures or differential migration would be needed to maintain detectable differences in gene frequency between different parts of a large randomly mating population (Crow and Kimura 1970).

3. The absence of significant differences in gene frequency between stocks does not prove that the stocks belong to the same subpopulation, as it is quite possible for different subpopulations to independently maintain the same gene frequency.

4. It cannot be assumed that one or two samples of gene frequencies from a stock adequately reflect the genetic differentiation present in that area. Replicate and time series sampling are needed before genetically detectable structuring present in an area can be accurately represented.

Genetic studies have been carried out on Pacific skipjack tuna for some years and, as a result, electrophoretically identifiable genetic polymorphisms have been described in serum transferrin (Fujino and Kang 1968a) and in an esterase (Fujino and Kang 1968b). Variation has also been reported in a second esterase (Fujino 1979) but this protein appears to be impossible to type in more than half of the samples of preserved blood.

Fujino (1970) using esterase polymorphisms, proposed that there are at least two discrete subpopulations of skipjack in the Pacific Ocean and that the boundary between the migratory ranges of these subpopulations in the northern Pacific shifts eastwards to about the International Dateline in the northern summer and westwards in the northern fall and winter. In the

south Pacific Fujino (1976) proposed that the boundary lay in the Tasman Sea all year, but moved closer to the south-eastern Australian coast early in the southern winter. These predictions were based on the analysis of variation at the esterase loci, which shows marked differences in gene frequency between the eastern ($Es^1$ = approximately .39 - .57) and the western ($Es^1$ = approximately .58 - .79) subpopulations. Fujino (1970) found no significant variation in the frequency of the transferrin alleles between these geographic groups.

Subsequent statistical analyses of the esterase gene frequencies estimated from sets consisting of 100 or more blood samples shows that the data from within a proposed subpopulation are not homogeneous and that the situation is therefore more complex than the two subpopulation model suggests (Sharp, 1978). Sharp (1978) suggested that each of Fujino's proposed sub-populations consist of several discrete subpopulations. In considering the evidence for two different spawning seasons in the western Pacific, Fujino (1970) also implied that the two subpopulation model is an oversimplification. More recently a preliminary analysis of the results generated by the SPC programme up to July 1979 indicated that the esterase gene frequencies within the SPC area were consistent with the existence of an east-west cline (Anon 1980). Undoubtedly the lack of adequate sampling from the area in the past has been one of the major obstacles to defining the subpopulation structure of the South Pacific region.

This paper firstly describes the results of a survey for new genetic variation in skipjack tuna and extends surveys carried out by other workers. Secondly, the phenotype numbers

and the gene frequency results for the sample sets collected during this study are given. Sampling for the present study was aimed at the whole of the central and western Pacific, but concentrated in areas from which no samples had previously been collected. Additional effort was made to take blood samples from schools for which large numbers of skipjack were tagged and released. It was likely that information generated by the recapture of these tagged fish would be valuable in assessing the extent of migrations between stocks when integrated with the genetic data. Detailed analysis of the data collected by previous workers, together with this data is continuing.

## METHODS

Blood samples were taken by heart puncture from tuna within an hour of capture. Each sample was mixed with an equal volume of preservative which consists of three parts of water, containing five per cent trisodium citrate, and two parts glycerol (Fujino 1966). The samples were then stored at -20°C in the ship's freezer until shipped to Australia on dry ice, where the samples were again stored at -20°C.

The sample size used in a programme of this type is critical to the success of the study. Obviously increasing the sample size increases the accuracy of the estimate of gene frequency obtained, but for practical reasons decreases the number of aggregations that can be sampled; some compromise strategy must be used. As the primary strategy of the SPC programme was to tag large numbers of skipjack tuna in as many areas as possible, blood sampling received secondary consideration. Blood samples were therefore not as large as may have been desired if genetic analyses was the only tool. However, as the difference in average esterase gene frequency between the

central and Western Pacific had been reported to be about 0.19 (Fujino 1976), it was anticipated that a level of discrimination of $\Delta P = 0.1$ was required. Consequently the sampling strategy of taking a number of blood samples from at least 100 fish of each size mode present in feeding aggregations was accepted. In practice, not all sample sets were from a single size mode, and occasional sets of fewer samples were typed from unusual groups or when collected from areas for which little data were previously available. It was appreciated that sample sets of much less than 100 animals would often give rise to little useful information.

Bloods from 25 individual fish from a range of locations across the Pacific were surveyed for electrophoretic variation at a total of 42 loci. The material was typed on cellulose acetate gels using techniques previously described (Richardson et al. 1980). The enzymes studied, electrophoresis running conditions, and stains used are listed in Table 1. All material collected was typed for esterase and transferrin and some sample sets were typed for other proteins that were found to be variable.

Homogeneity chi squared tests on gene numbers were used to measure the significance of differences in gene frequency and Smith's H statistic (Smith 1970) was used to estimate deviation from Hardy Weinberg Equilibrium. H is an unbiased estimate of half the difference between the expected and observed proportion of heterozygotes. Consequently it is zero when the observed distribution of phenotypes is that predicted, positive when there are too many homozygotes and negative when there are too many heterozygotes. Smith's H has an advantage over the usual chi squared test of observed and expected distributions of genotypes in that estimates of H from a series of sample sets may be combined to give an estimate of H with

much narrower confidence limits (due to the larger sample size) without increasing the Wahlund Effect (Wahlund, 1928; Crow and Kimura 1970).

RESULTS

The results obtained in the survey for genetic variation are given in Table 1. Six loci showed variation of the type usually inherited, nine showed variation that could be ascribed to post-transcriptional changes in structure and twenty seven were monomorphic. As well as the transferrin and esterase polymorphisms previously described, a five allele polymorphism was found in guanine deaminase (GDA). Low frequency variation was also detected in glucose phosphate isomerase (GPI) (nine alleles), and adenosine deaminase (ADA) (five alleles). Four of the five ADA alleles were very rare, but all were found throughout the region. At the GPI locus eight of the nine alleles found were so rare that statistical analysis of the gene frequency data was not feasible. Patterns similar to those previously described in 6-phosphogluconate dehydrogenase from Atlantic tuna muscle (McCabe et al. 1970) were also found in the blood of Pacific skipjack. However the system was unstable and it was not possible to study the distribution of this variation. The patterns observed for each of the enzymes showing genetic variation are diagrammatically represented in Figure 1.

The sampling locations are given in Table 2 and Figure 2, together with the esterase and transferrin gene frequencies and the Smith's H values for these sample sets. The phenotype data are given in Appendix 1. The equivalent data for the other variable loci studied are given in Tables 3 and 4.

To allow comparisons to be made between results from

different parts of the tropical Pacific, the area between $10^{\circ}N$, roughly the position of the northern edge of the equatorial countercurrent (Gorshkov 1976), and $25^{\circ}S$, marking the change to cooler water, has been divided into three (Figure 2). Area A is the region west of $170^{\circ}E$ and includes Fujino's western Pacific subpopulation. Fujino's eastern subpopulation is divided into two sections; area B which lies between $170^{\circ}E$ and $165^{\circ}W$, and Area C between $165^{\circ}W$ and $130^{\circ}W$. Table 5 shows the average frequency, sample size, Smith's H value and homogeneity $\chi^2$ values for selected alleles in each of the areas, in temperate waters (i.e. south of $25^{\circ}S$), and in the combined data.

Allele frequencies of the new polymorphism in GDA are heterogeneous over the region (Table 5) and examination of the distribution of the alleles shows that this result is due to significant differences in gene frequency between Area A and Areas B and C (Table 5, Figure 3) and to heterogeneity within area A. The average difference in gene frequency between the areas is only 0.05. The approximate 95% binomal confidence limits, assuming the data from an area are homogeneous would be 0.10 - 0.20 for area A and 0.07 - 0.16 for areas B and C, given sample sizes of 200 genes. Consequently with the sample sizes used in this study it is not possible to make any comment on gene frequencies between 0.10 and 0.16, as such gene frequencies will occur regularly in all areas. In each area there is a significant excess of homozygotes to that expected under Hardy Weinberg Equilibrium conditions, implying that either mixing of subpopulations with different gene frequencies (e.g. Wahlund Effect), inbreeding, or disruptive selection is occurring in the population.

The transferrin gene frequencies are similar to those found previously by other workers and are homogeneous throughout the area (Table 5). There was no evidence of a change in the proportion of transferrin heterozygotes with size, as was reported by Fujino and Kang (1968a) in data from Hawaii.

The esterase data confirm and extend the results of previous workers. There is a marked change in gene frequency between area A and Area B (Table 5), as previously reported by Fujino (1976), and the gene frequencies are similar to those he reports, thus confirming the stability of the system over a twelve year period. The esterase gene frequencies obtained from Area A aggregations are heterogeneous and out of Hardy Weinberg Equilibrium with an excess of heterozygotes, as was found with GDA. However, unlike the situation with GDA sample sets from areas B and C were in equilibrium. Heterogeneity in Area A has previously been described by Sharp (1978) and more detailed information on the genetic structure of stocks in this region will shortly be available (Lewis, unpublished). The combined esterase data for Areas B and C are also heterogeneous and an eastward reduction in esterase gene frequency is apparent in the data (Tables 2 and 5, Figure 4). Homogeneity tests of the data from Areas B and C individually show that Area C data are homogeneous but Area B data are heterogeneous (Tbale 5). However, the area C data were all collected at one time of the year and heterogeneity may have increased if samples from other periods were available.

The esterase data for the temperate area is also heterogeneous (Table 5) and the relationship of New Zealand and Australian stocks to each other and to the situation in the tropics will be considered elsewhere (Richardson and Habib

in prep., Lewis and Richardson, in prep.).

An average heterozygosity value (Selander, 1976) of 0.032 was determined from all of the systems studied. This is in the low normal range for fish (.005-.110, Somero and Soulé 1974). The ecological basis for differences in heterozygosity levels are presently unknown but subject to much debate (e.g. Ayala and Valentine 1979).

## DISCUSSION

The results of the survey for new useful polymorphisms are disappointing as only one new polymorphism was found. Consequently, the discriminatory power of the genetic approach to the identification of skipjack tuna subpopulations is still very limited.

There are a number of observations that can be made about the variation seen in gene frequencies. Firstly, the esterase data from the tropical Pacific may be viewed as a continuous east-west cline in esterase gene frequency (Figure 4) (Anon, 1980). A linear regression of longitude against gene frequency explains 73 per cent of the variation present in the esterase data. Similarly the $GDA^1$ frequency changes with longitude (Figure 3) though the change is not as marked as was found with the esterase data. A linear regression of gene frequency against longitude explains only 37% of the variance and a second order regression 54% of the variance. Because the change in gene frequency is much less than that seen for esterase the error involved in estimating the gene freuqency is much more significant and reduces the effectiveness of the regression lines.

A plot of the esterase gene frequency of each sample set against the GDA frequency for the same set are shown in Figure 5 and it can be seen that these variables are significantly correlated (r = .55). This correlation is quite high when it is realized that both variables used are estimates with wide confidence limits. Many environmental variables e.g. dissolved phosphate levels, temperature and salinity, change in an east-west direction in the region and could directly or indirectly supply the physical basis for the observed changes in gene frequencies.

While a detailed examination of previously collected data is outside the scope of this paper, it should be noted that the esterase gene frequency cline does not continue eastwards, and may not be supported by data sets from some areas north of the equatorial countercurrent. The data sets collected from fish off Ecuador (Sharp, 1978), (i.e. well to the east of our sampling area), are similar to those found in Area C. This is not surprising as there is thought to be very little skipjack breeding east of Area C, and it seems reasonable to assume that the South American stock comes, at least in part, from the same subpopulation as the French Polynesian stock.

The relationship of the stocks in Areas B and C to those in equivalent waters north of the equatorial countercurrent is presently unknown. However, no tags released by the SPC skipjack programme in Areas B and C have yet been returned from northern areas. The relationship between the tropical skipjack found in areas A, B and C, and between these and those in southern temperate waters will be discussed elsewhere (Richardson in prep., Richardson and Habib in prep., Lewis and Richardson, in prep.).

Continuous stepped, or unstepped, clines can be the result of a variety of explanations (Endler, 1977) and it is not possible at this stage to propose a single model of the population structure of the region with any confidence. Identification of the number and location of discrete breeding grounds if they exist, or alternatively, the genetic analysis of egg and larval populations, would add enormously to our understanding of skipjack population structure in the region.

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BIBLIOGRAPHY

Anon (1976) Ad hoc meeting of scientists to discuss skipjack fisheries developments and research requirements. Report. South Pacific Commission pp17.

Anon (1980) Review of Preliminary Results from genetic Analysis of skipjack blood samples collected by the skipjack survey and assessment programme. Skipjack Survey and Assessment Programme Technical Report No. 1 pp22.

Ayala, F.J. and Valentine, J.W. (1979) Genetic variability in the pelagic environment. A paradox? Ecology 60, 24-29.

Crow, J.F. and Kimura, M. (1970) "An introduction to population genetics theory". Harper and Row N.Y. pp 591.

de Ligny, W. (1968) Serological and biochemical studies on fish populations. Oceanogr. Marine Biol. Annu. Rev. 7; 411-513.

Endler, J.A. (1977) "Geographic Variation, Speciation and Clines. Princeton University Press, Princeton N.J. pp246.

Fujino, K. (1966) Instructions for collecting blood and serum samples from Tuna fishes. FAO Fisheries Circular No. 26, UN. Rome 1-5.

Fujino, K. (1970) Range of the Skipjack Tuna Subpopulation in the western Pacific Ocean. Prac. of the 2nd CSK Symposium, Tokyo; 373-384.

Fujino, K. (1976) Subpopulation Identification of Skipjack Tuna Specimens from the Southwest Pacific Ocean. Bull. Jap. Soc. Sci. Fish. 42; 1229-1235.

Fujino, K. (1979) Genetic Isolation of Skipjack Tuna in the western Pacific Ocean. Proc. of the 4th CSK Symposium, Tokyo.

Fujino, K. and Kang, T. (1968a) Transferrin Groups of Tuna. Genetics 59; 79-91.

Fujino, K. and Kang, T. (1968b) Serum Esterase Groups of Pacific and Atlantic Tunas. Copeia 1968(1); 56-63.

Gorshkov, S.G. (1976) World Ocean Atlas. Volume 1, Pacific Ocean Pergamon Press Oxford pp302.

Kearney, R.E. (1979) An overview of recent changes in the Fisheries for Highly Migratory Species in the Western Pacific Ocean and Projections for Future Developments. South Pacific Bureaux for Economic Cooperation. Publication No. 17.

McCabe, M.M., Dean, D.M. and Olson, C.S. (1970) Multiple forms of 6 phosphogluconate dehydrogenase and alpha-glycerophosphate dehydrogenase in the skipjack tuna, Katsuwonus pelamis. Comp. biochem. physiol. 34; 755-757.

Richardson, B.J., Rogers, P.M. and Hewitt, G.M. (1980) Ecological genetics of the wild rabbit in Australia II. Protein variation in British, French and Australian rabbits and the geographical distribution of the variation in Australia. Aust. J. biol. Sci. 33; 371-383.

Selander, R.K. (1976) Genetic variation in natural populations. In Molecular Evolution. F.J. Ayala Ed. Sinauer Assoc. Inc. Sunderland Mass.

Sharp, G. (1978) Behavioural and Physiological Properties of Tunas and their effects on vulnerability to fishing gear. In "The physiological ecology of tunas". G.D. Sharp and A.E. Dizon editors. Academic Press N.Y. pp397-450.

Smith, C.A.B. (1970) A note on testing the Hardy-Weinberg Law. Ann. Hum. Genet. 33; 377-383.

Somero, G.N. and Soulé, M. (1974) Genetic Variation in marine fishes as a test of the niche-variation hypothesis. Nature 249; 670-672.

Wahlund, S. (1928) Zuzammensetzung von Populationen and Korrelationserscheinungen vom Standpunkt der Vererbungslehre aus betrachtet. Hereditas 11; 65-106.

Table 1. The proteins studies, results obtained, running conditions and stains used in the survey for genetic variation. The results obtained are summarised using the following abbreviations; P, genetic variation present; V, nongenetic variation only; M, monomorphic; TM, 50 mMTris maleate pH7.2; TEB, 15 mMTris, 5 mM EDTA borate pH8.2, X, tested on the previous buffers plus several others (Richardson et al., 1980).

| Protein | Result | Running System | Stain |
|---|---|---|---|
| ACON (EC4.2.1.3) | M | X | 1 ml 0.1M Tris HCl pH8.6, 0.1ml cis-Aconitate (25mg/ml), 0.1ml NADP (10mg/ml), 0.1ml 0.1M $MgCl_2$, 0.1ml PMS (2mg/ml) 0.1ml MTT (4mg/ml) 2I.U. isocitrate dehydrogenase. |
| ADA (EC3.5.4.4) | P | TM | 1ml 0.05M Phosphate buffer pH7.5, 0.1ml Adenosine (25mg/ml), 0.1ml PMS (2 mg/ml), 0.1ml MTT (4mg/ml), 0.6I.U. Xanthine Oxidase, 0.3I.U. Nucleoside phosphorylase. |
| AK (EC2.7.4.3) | V | X | 1ml 0.1M Tris HCl pH8.0, 0.1ml ADP (10mg/ml), 0.1ml 0.1M $MgCl_2$, 0.1ml Glucose (40mg/ml), 0.1ml NADP (10mg/ml), 0.1ml MTT (4mg/ml), 0.1ml PMS (2mg/ml), 2I.U. Glucose-6 phosphate dehydrogenase, 2I.U. Hexokinase. |
| ALD (EC4.1.2.13) | M | X | 0.6ml 0.1M Tris HCl pH8.0, 0.1ml Fructose 1, 6 diphosphate (50mg/ml), 0.1ml NAD (10mg/ml), 0.1ml Sodium Arsenate (15mg/ml), 0.1ml 0.1M $MgCl_2$, 0.1ml PMS (2mg/ml), 0.1ml MTT (4mg/ml), 2I.U. Triose phosphate Isomerase, 2I.U. Glyceraldehyde 3 phosphate dehydrogenase. |
| ALB (Albumin) | M | X | Nonspecific protein stain; 2.5g Brilliant Blue R/litre of 7% acetic acid, 25% methanol; Destain in 7% acetic acid 25% methanol. |
| CA (EC4.2.1.1) | M | X | Dip gel in 0.2% bromothymol blue and 0.4% NaOH, blot and blow $CO_2$ over gel until yellow bands appear. |
| CAT (EC1.11.1.6) | V | X | Soak in 1 in 1000 dilution of $H_2O_2$ for 15 minutes, rinse and stain in a mixture of equal volumes of 2% potassium ferricyanide and 2% Ferrie chloride. Yellow bands of activity appear on a blue green background. |
| DIA (EC1.6.4.3) | M | X | 1.0ml 0.1M Tris HCl pH8.5, 0.2ml NADH (10mg/ml), 0.1ml dichlorophenol (10mg/ml), 0.1ml MTT (2 mg/ml). |

| | | | |
|---|---|---|---|
| E' (EC4.2.1.11) | M | X | 1.0ml 0.1M Tris HCl, pH7.6, 0.1ml 3 Phosphoglycerate (20mg/ml), 0.1ml NADH (10mg/ml), 0.1ml ADP (25mg/ml), 0.1ml 0.1M $MgCl_2$, 2I.U. Phosphoglyceromutase, 2I.U. Pyruvate Kinase, 2I.U. Lactate dehydrogenase, moniter under UV light. |
| ES (EC3.1.1.1) | P | TEB | 100ml 0.05M Phosphate buffer, pH6.5, 4.0m 1% αnaphthyl acetate in 50% acetone, 40mg Fast Blue R.R. |
| ESD (EC3.1.1.1) | M | X | 5mg methylumbelliferyl acetate dissolved in acetone, 10ml 0.05M Phosphate buffer pH6.5, moniter under UV light. |
| FUM (EC4.2.1.2) | M | X | 1ml 0.05 Phosphate buffer pH7.5, 0.2ml Fumeric acid (neutralized, 25mg/ml), 0.2ml NAD (10mg/ml), 0.2ml PMS (2mg/ml), 0.2ml MTT (4mg/ml), 2I.U. Malate dehydrogenase. |
| GAPD (EC1.2.1.12) | V | X | 1.0ml Tris HCl pH7.5, 10μl Glyceraldehyde 3 Phosphoric acid, 0.2ml NAD (10mg/ml), 0.1ml Sodium Arsenate (15mg/ml), 0.1ml PMS (2mg/ml), 0.1ml MTT (4mg/ml). |
| GDA (EC3.5.4.3) | P | TEB | 1.0ml 0.1M Tris HCl pH7.6, 0.04ml Guanine (25mg/ml 0.5M Na04), 0.6I.U. Xanthine Oxidase, 0.1ml PMS (4mg/ml), 0.1ml MTT (8mg/ml). |
| GLUD (EC1.4.1.3) | M | X | 1ml 0.1M Tris HCl pH8.0, 0.1ml Sodium glutamate (75mg/ml), 0.1ml NADP (10mg/ml) 0.1ml 0.1M $MgCl_2$, 0.1ml PMS (2mg/ml), 0.1ml MTT (4mg/ml). |
| GOT (EC2.6.1.1) | V | X | 1.0ml 0.1M Phosphate buffer pH7.0, 0.1ml Aspartic acid (70mg/ml), 0.1ml αKetoglutarate (25mg/ml), 0.1ml Pyridoxal phosphate (5mg/ml), 0.1ml Fast Violet (20mg/ml). |
| GPD (EC1.1.1.49) | V | X | 1.0ml Tris HCl pH8.0, 0.1ml Glucose-6 phosphate (25mg/ml), 0.1ml NADP (10mg/ml), 0.1ml 0.1M $MgCl_2$, 0.1ml PMS (2mg/ml), 0.1ml MTT (4mg/ml). |
| GPI (EC5.3.1.9) | P | TM | 1.0ml Tris HCl pH7.0, 0.1ml Fructose 6 phosphate (25mg/ml), 0.1ml NADP (10mg/ml) 0.1ml PMS (2mg/ml), 0.1ml MTT (4 mg/ml), 2I.U. Glucose-6 phosphate dehydrogenase. |
| Hb (Haemoglobin) | V | TM | By colour |

| | | | |
|---|---|---|---|
| H (EC2.7.1.1) | M | X | 1.0ml 0.1M Tris HCl pH8.0, 0.1ml glucose (20mg/ml), 0.1ml ATP (30mg/ml), 0.1ml NADP (40mg/ml), 0.1ml 0.1M $MgCl_2$, 0.1ml PMS (4mg/ml), 0.1ml MTT (4mg/ml), 2I.U. Glucose-6 phosphate dehydrogenase. |
| ICDH (EC1.1.1.42) | M | X | 0.7ml 0.1M Tris HCl pH9.0, 0.1ml sodium isocitrate (25mg/ml), 0.1ml NADP (10mg/ml), 0.1ml 0.1M $MgCl_2$, 0.1ml PMS (2mg/ml), 0.1ml MTT (4mg/ml). |
| LAP | M | X | 1.0ml 0.1M phosphate buffer pH6.6, 0.2ml Fast Black (potassium salt, 5mg/ml), 0.1ml L-Leucyl-βnapthylamine. |
| LDH (EC1.1.1.27) | V | X | 1.0ml 0.1M Tris HCl pH8.0, 0.1ml Lactic acid (25mg/ml), 0.1ml NAD (10mg/ml) 0.1ml PMS (2mg/ml), 0.1ml MTT (4mg/ml). |
| MOD (EC1.1.1.40) | M | X | 0.7ml 0.1M Tris HCl pH8.0, 0.1ml Malic acid (25mg/ml), 0.1ml NADP (10mg/ml), 0.1ml 0.1M $MnCl_2$, 0.1ml PMS (2mg/ml), 0.1ml MTT (4mg/ml). |
| MOR (EC1.1.1.37) | M | X | 0.8ml 0.1M Tris HCl pH8.0, 0.1ml Malic acid (25mg/ml), 0.1ml NAD (10mg/ml), 0.1ml PMS (2mg/ml), 0.1ml(4mg/ml). |
| MPI (EC5.3.1.8) | M | X | 1.0ml 0.1M Tris HCl pH7.5, 0.1ml Mannose phosphate (25mg/ml), 0.1ml 0.1M $MgCl_2$, 0.1ml NADP (10mg/ml), 0.1ml PMS (2mg/ml), 0.1ml MTT (4mg/ml), 2I.U. Glucose phosphate Isomerase, 2I.U. Glucose 6 phosphate Dehydrogenase. |
| NP (EC2.4.2.1) | M | X | 1.0ml 0.1M Phosphate buffer pH7.5, 0.1ml Inosine (25mg/ml), 0.1ml MTT (4mg/ml), 0.6I.U. Xanthine Oxidase. |
| OCT (EC2.1.3.3) | M | X | 100ml 0.5M Tris maleate pH7.2, 3mM Carbamoyl phosphate, 300mg Ornithine Hydrochloride, 5ml 2% lead nitrate. Incubate for 30 min. rinse in water, immerse in 1% ammonium sulphide, rinse again, blot and incubate until black band appear. |
| PEP (EC3.4.11. ) | M | X | 0.5ml 0.1M Tris HCl pH7.4, 0.1ml peptide (25mg/ml), 0.1ml Amino acid Oxidase (5mg/ml), 0.1ml peroxidase (5mg/ml), 0.1ml 0-dianisidine HCl (25mg/ml) different peptides may be used e.g. Val-leu (Pep A), leu-gly-gly (Pep B) leu-ala (Pep C). |

| | | | |
|---|---|---|---|
| I[illegible] (EC1.1.1.44) | P | TM | 1.0ml 0.1M Tris HCl pH8.0, 0.1ml $MgCl_2$, 0.1ml NADP (10mg/ml), 0.1ml PMS (2mg/ml), 0.1ml MTT (4mg/ml). |
| PGK (EC2.7.2.3) | M | X | 1.0ml 0.5M Tris HCl pH7.8, 0.1ml 3 phosphoglycerate (50mg/ml), 0.2ml ATP (30mg/ml), 0.1ml 0.1M $MgCl_2$, 0.1ml NADH (20mg/ml), 2I.U. Glyceraldehyde 3 phosphate dehydrogenase. Moniter under UV. |
| PGM (EC2.7.5.1) | M | X | 1.0ml Tris HCl pH8.0, 0.1ml Glucose-1-phosphate (25mg/ml + 0.1mg Glucose 1, 6 diphosphate), 0.1ml NADP (10mg/ml), 0.1ml 0.1M $MgCl_2$, 2I.U. Glucose 6 phosphate dehydrogenase, 0.1ml PMS (2mg/ml), 0.1ml MTT (4mg/ml). |
| PK (EC2.7.1.40) | V | X | 0.4ml 0.1M Tris HCl pH8.0, 0.1ml 1M KCl, 0.1m 0.1M $MgCl_2$, 0.1ml NADH (20mg/ml) 0.1ml ADP (20mg/ml), 0.1ml phosphoenol pyruvate (25mg/ml). Moniter under UV. |
| SOD (EC1.15.1.1) | M | X | 1.0ml 0.1M Tris HCl pH8.0, 0.2ml PMS (2mg/ml), 0.2ml MTT (4mg/ml) after incubation white bands appear on a blue background. |
| Tf (Transferrin) | P | TM | Nonspecific protein stain. 2.5gm Brilliant Blue R/litre of 7% acetic acid, 25% methanol. Destain in 7% acetic acid, 25% methanol. |
| XO (EC1.2.3.2) | V | X | 1ml 0.1M Tris HCl pH7.2, 0.2ml Hypoxanthine (25mg/ml), 0.1ml PMS (2mg/ml), 0.1ml MTT (4mg/ml). |

Table 2. Geographical location of sampling stations and results for Esterase and Transferrin.
*Sample set significantly out of Hardy Weinberg Equilibrium. †From Lewis (pers. comm.).

| Country | Station Identifier | Latitude | Longitude | Date | Esterase | | | | | Transferrin | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1 | 2 | 3+4 | $H_1$ | 2N | 1+4 | 2 | 3 | $H_2$ | 2N |
| AREA A | | | | | | | | | | | | | | |
| Palau, TTPI | R | 7°06'N | 134°54'E | 20.10.78 | 0.684 | 0.305 | 0.004 | 0.018 | 256 | 0.004 | 0.689 | 0.307 | 0.010 | 244 |
| " | BL | 7°41'N | 134°10'E | 18.8.80 | 0.639 | 0.348 | 0.013 | 0.036 | 230 | 0.013 | 0.746 | 0.241 | -0.001 | 224 |
| " | BM | 7°48'N | 134°18'E | 19.8.80 | 0.668 | 0.332 | 0 | 0.025 | 238 | 0.005 | 0.676 | 0.319 | 0.002 | 188 |
| Helen Reef | BK | 3°00'N | 131°36'E | 9.8.80 | 0.771 | 0.217 | 0.012 | -0.003 | 166 | 0.006 | 0.763 | 0.231 | -0.005 | 160 |
| Truk | P | 7°42'N | 151°44'E | 10.8.78 | 0.615 | 0.370 | 0.015 | 0.003 | 200 | 0.005 | 0.707 | 0.288 | 0.024 | 184 |
| " | AO | 8°41'N | 152°08'E | 10.11.79 | 0.555 | 0.432 | 0.014 | 0.023 | 146 | 0.014 | 0.692 | 0.295 | 0.071* | 146 |
| " | AP | 7°38'N | 155°22'E | 11.11.79 | 0.563 | 0.429 | 0.008 | 0.002 | 126 | 0 | 0.738 | 0.262 | 0.048 | 122 |
| " | AQ | 7°39'N | 155°20'E | 11.11.79 | 0.625 | 0.362 | 0.013 | 0.032 | 152 | 0.007 | 0.687 | 0.307 | -0.003 | 150 |
| Ponape | AR | 7°02'N | 158°25'E | | 0.630 | 0.358 | 0.012 | 0.010 | 330 | 0.020 | 0.686 | 0.294 | 0.017 | 296 |
| " | BH | 7°07'N | 158°18'E | 18.7.80 | 0.684 | 0.309 | 0.008 | -0.006 | 256 | 0 | 0.752 | 0.248 | 0.026 | 254 |
| " | BJ | 7°03'N | 157°55'E | 20.7.80 | 0.562 | 0.425 | 0.012 | 0.048 | 160 | 0 | 0.665 | 0.335 | 0.016 | 158 |
| Papua New Guinea | Red A | 3°20'S | 150°50'E | 19.5.77 | 0.682 | 0.299 | 0.013 | 0.019 | 318 | 0.006 | 0.716 | 0.278 | -0.030 | 162 |
| " | Unlab | 2°38'S | 149°20'E | 29.4.77 | 0.711 | 0.284 | 0 | 0.021 | 232 | 0.045 | 0.643 | 0.313 | 0.018 | 112 |
| " | MAS-A† | 1°19'S | 146°04'E | 23.5.79 | 0.652 | 0.324 | 0.010 | -0.005 | 210 | 0.005 | 0.714 | 0.281 | 0 | 210 |
| " | KVP† | 3°13'S | 150°57'E | 17.5.79 | 0.716 | 0.255 | 0.019 | 0.007 | 208 | 0.029 | 0.697 | 0.274 | -0.043* | 208 |
| " | KVQ† | 3°13'S | 150°57'E | 17.5.79 | 0.755 | 0.226 | 0.014 | 0.008 | 208 | 0.010 | 0.673 | 0.317 | -0.019 | 208 |

| Papua New Guinea | KVT† | $3^{o}16'S$ | $150^{o}55'E$ | 3.7.79 | 0.767 | 0.228 | 0 | 0.016 | 202 | 0.010 | 0.716 | 0.275 | -0.0[illegible] | 204 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| " | SW | $9^{o}40'S$ | $147^{o}15'E$ | 27.2.77 | 0.823 | 0.169 | 0.008 | 0.018 | 124 | 0 | 0.686 | 0.314 | 0.057* | 118 |
| " | SC | $9^{o}42'S$ | $147^{o}15'E$ | 27.2.77 | 0.763 | 0.221 | 0.016 | -0.013 | 190 | 0 | 0.744 | 0.256 | 0.014 | 78 |
| " | AM | $7^{o}36'S$ | $149^{o}47'E$ | 20.5.79 | 0.673 | 0.313 | 0.014 | -0.031 | 214 | 0.005 | 0.752 | 0.243 | 0.005 | 214 |
| " | AN | $4^{o}04'S$ | $151^{o}01'E$ | 3.6.79 | 0.700 | 0.295 | 0.005 | 0.011 | 220 | 0 | 0.692 | 0.307 | -0.002 | 218 |
| NE Australia | AJ | $17^{o}56'S$ | $148^{o}22'E$ | 1.5.79 | 0.692 | 0.293 | 0.014 | 0.007 | 218 | 0.009 | 0.665 | 0.325 | 0.008 | 218 |
| " | AK | $17^{o}31'S$ | $148^{o}05'E$ | 2.5.79 | 0.663 | 0.337 | 0 | -0.010 | 196 | 0 | 0.663 | 0.367 | 0.019 | 196 |
| " | AL | $16^{o}22'S$ | $150^{o}12'E$ | 3.5.79 | 0.673 | 0.309 | 0.018 | 0.030 | 220 | 0.005 | 0.699 | 0.296 | -0.016 | 216 |
| Solomon Is | D | $9^{o}09'S$ | $158^{o}43'E$ | 11.5.77 | 0.652 | 0.338 | 0.010 | 0.001 | 198 | 0.008 | 0.656 | 0.336 | -0.002 | 122 |
| " | H | $9^{o}09'S$ | $158^{o}22'E$ | 12.5.77 | 0.654 | 0.330 | 0.011 | 0.013 | 182 | 0.012 | 0.639 | 0.349 | 0.015 | 166 |
| " | E | $9^{o}09'S$ | $158^{o}43'E$ | 11.5.77 | 0.623 | 0.359 | 0.014 | 0.059* | 220 | 0.019 | 0.696 | 0.285 | 0.012 | 214 |
| " | G | $9^{o}15'S$ | $159^{o}00'E$ | 12.5.77 | 0.713 | 0.287 | 0 | -0.032 | 122 | 0 | 0.602 | 0.398 | -0.065 | 88 |
| " | BG | $8^{o}40'S$ | $159^{o}36'E$ | 18.6.80 | 0.712 | 0.270 | 0.017 | -0.011 | 226 | 0.009 | 0.689 | 0.302 | -0.024 | 222 |
| New Caledonia | A | $21^{o}46'S$ | $166^{o}42'E$ | 8.1.78 | 0.538 | 0.442 | 0.019 | 0.032 | 156 | 0.020 | 0.678 | 0.303 | 0.029 | 152 |
| " | B | $20^{o}58'S$ | $164^{o}24'E$ | 15.1.78 | 0.612 | 0.366 | 0.013 | -0.003 | 232 | 0 | 0.699 | 0.296 | -0.010 | 226 |
| Vanuatu | D | $16^{o}15'S$ | $167^{o}51'E$ | 21.1.78 | 0.753 | 0.240 | 0 | 0.023 | 146 | 0.014 | 0.699 | 0.288 | -0.062* | 146 |
| AREA B | | | | | | | | | | | | | | |
| Fiji | E | $18^{o}55'S$ | $178^{o}24'E$ | 31.1.78 | 0.550 | 0.450 | 0 | 0.039 | 200 | 0.019 | 0.709 | 0.272 | -0.033 | 158 |
| " | F | $17^{o}13'S$ | $179^{o}17'W$ | 10.2.78 | 0.600 | 0.383 | 0.008 | 0.008 | 240 | 0.004 | 0.708 | 0.288 | -0.008 | 236 |
| " | B | $17^{o}20'S$ | $179^{o}10'E$ | 3.3.77 | 0.500 | 0.490 | 0.010 | 0.018 | 98 | 0.045 | 0.705 | 0.250 | 0.055 | 44 |
| " | A | $18^{o}10'S$ | $178^{o}50'E$ | 2.3.77 | 0.565 | 0.429 | 0.005 | 0.021 | 382 | 0.010 | 0.741 | 0.248 | 0.011 | 286 |
| " | BD | $18^{o}37'S$ | $177^{o}49'E$ | 10.4.80 | 0.524 | 0.476 | 0 | 0.037 | 206 | 0 | 0.718 | 0.[illegible]2 | -0.010 | 206 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tonga | T1 | 18°19'S | 174°24'W | 21.4.78 | 0.595 | 0.389 | 0.016 | -0.047 | 190 | 0.006 | 0.815 | 0.179 | 0.006 | 160 |
| Wallis Is | W1 | 13°26'S | 176°02'W | 6.5.78 | 0.504 | 0.478 | 0.018 | -0.034 | 228 | 0 | 0.736 | 0.264 | -0.00[illegible] | 216 |
| " | W2 | 13°09'S | 176°22'W | 15.5.78 | 0.511 | 0.443 | 0.017 | 0.013 | 176 | 0 | 0.686 | 0.314 | 0.042 | 172 |
| " | W3 | 13°29'S | 176°07'W | 17.5.78 | 0.504 | 0.475 | 0.013 | 0.041 | 238 | 0.011 | 0.747 | 0.242 | 0.025 | 182 |
| " | W4 | 13°30'S | 176°05'W | 19.5.78 | 0.510 | 0.485 | 0.005 | 0.024 | 198 | 0 | 0.753 | 0.247 | -0.008 | 190 |
| " | W6 | 14°13'S | 178°03'W | 29.5.78 | 0.590 | 0.396 | 0.010 | -0.035 | 288 | 0 | 0.714 | 0.286 | 0.030 | 252 |
| " | BE | 14°20'S | 178°16'W | 22.5.80 | 0.533 | 0.450 | 0.016 | 0.031 | 242 | 0.008 | 0.736 | 0.256 | 0.029 | 246 |
| Samoa | H | 13°42'S | 171°45'W | 14.6.78 | 0.619 | 0.371 | 0.010 | -0.049 | 210 | 0.005 | 0.766 | 0.229 | -0.002 | 192 |
| " | BB | 14°20'S | 169°23'W | 21.2.80 | 0.466 | 0.525 | 0.009 | 0.041 | 234 | 0.008 | 0.736 | 0.256 | 0.029 | 246 |
| Tuvalu | J | 10°23'S | 178°48'W | 25.6.78 | 0.594 | 0.396 | 0.009 | 0.006 | 318 | 0.033 | 0.702 | 0.265 | -0.002 | 302 |
| " | K | 8°40'S | 179°13'W | 27.6.78 | 0.560 | 0.426 | 0.014 | 0.002 | 216 | 0.005 | 0.737 | 0.258 | 0.016 | 190 |
| " | L | 8°42'S | 179°19'W | 1.7.78 | 0.646 | 0.340 | 0.010 | 0.002 | 206 | 0.005 | 0.696 | 0.293 | -0.026 | 184 |
| Kirabati | M | 2°57'N | 172°45'E | 16.7.78 | 0.488 | 0.492 | 0.016 | 0.031 | 246 | 0.008 | 0.683 | 0.308 | 0.001 | 240 |
| " | N | 3°00'N | 172°48'E | 22.7.78 | 0.480 | 0.516 | 0 | -0.001 | 254 | 0.013 | 0.654 | 0.333 | -0.023 | 228 |
| Phoenix Is | AS | 3°35'S | 174°15'W | 2.12.79 | 0.467 | 0.525 | 0.008 | 0.030 | 122 | 0.008 | 0.656 | 0.336 | 0.081* | 122 |
| AREA C | | | | | | | | | | | | | | |
| Penrhyn Is | T | 9°07'S | 157°43'W | 4.12.78 | 0.457 | 0.531 | 0.006 | -0.047 | 162 | 0.019 | 0.637 | 0.344 | -0.018 | 160 |
| Tuamotu Gp, F.P. | U | 15°38'S | 145°34'W | 19.12.78 | 0.402 | 0.570 | 0.023 | 0.055* | 214 | 0.019 | 0.650 | 0.330 | 0.015 | 206 |
| " | Z | 16°15'S | 145°58'W | 22.1.79 | 0.429 | 0.561 | 0.005 | -0.001 | 198 | 0.010 | 0.704 | 0.286 | 0.016 | 196 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| " | AA | 16°07'S | 146°06'W | 24.1.79 | 0.439 | 0.543 | 0.007 | 0.015 | 280 | 0 | 0.682 | 0.318 | -0.0[illegible] (handwritten: -0.002) | 280 |
| " | AZ | 15°44'S | 146°55'W | 12.2.80 | 0.500 | 0.493 | 0.007 | 0.005 | 150 | 0.014 | 0.671 | 0.315 | -0.025 | 146 |
| Marquesas Gp | X | 8°58'S | 140°20'W | 11.1.79 | 0.382 | 0.598 | 0.015 | 0.022 | 204 | 0 | 0.709 | 0.291 | -0.006 | 206 |
| " | Y | 12°35'S | 143°26'W | 13.1.79 | 0.466 | 0.523 | 0.006 | -0.034 | 176 | 0.006 | 0.703 | 0.285 | -0.017 | 172 |
| " | AV | 10°02'S | 139°30'W | 23.12.79 | 0.428 | 0.548 | 0.024 | -0.013 | 166 | 0 | 0.720 | 0.280 | 0.020 | 164 |
| " | AW | 8°53'S | 139°56'W | 16.1.80 | 0.423 | 0.549 | 0.028 | -0.008 | 142 | 0 | 0.655 | 0.345 | 0.024 | 142 |
| " | AX | 8°53'S | 139°51'W | 17.1.80 | 0.411 | 0.580 | 0.009 | 0.011 | 224 | 0.009 | 0.698 | 0.293 | 0.009 | 222 |
| Gambier Is | AY | 23°49'S | 133°49'W | | 0.349 | 0.613 | 0.038 | 0.013 | 106 | 0.019 | 0.783 | 0.198 | 0.058* | 106 |
| TEMPERATE | | | | | | | | | | | | | | |
| New Zealand | AB | 35°51'S | 175°30'E | 6.3.79 | 0.538 | 0.458 | 0.005 | -0.024 | 212 | 0 | 0.724 | 0.276 | -0.005 | 228 |
| " | AC | 37°41'S | 177°26'E | 8.3.79 | 0.450 | 0.528 | 0.017 | -0.023 | 180 | 0.005 | 0.694 | 0.301 | -0.018 | 186 |
| " | AE | 35°47'S | 175°20'E | 20.3.79 | 0.480 | 0.505 | 0.015 | 0.003 | 198 | 0.005 | 0.665 | 0.330 | 0.002 | 212 |
| Norfolk Is | BC | 29°28'S | 168°20'E | 26.3.80 | 0.477 | 0.500 | 0.023 | -0.029 | 132 | 0 | 0.674 | 0.326 | 0.020 | 144 |
| S.E. Australia | AF | 36°04'S | 150°24'E | 5.4.79 | 0.661 | 0.328 | 0.006 | 0.001 | 174 | 0 | 0.722 | 0.278 | -0.031 | 176 |
| " | AG | 35°06'S | 151°04'E | 8.4.79 | 0.712 | 0.288 | 0 | 0.008 | 288 | 0.004 | 0.716 | 0.281 | -0.001 | 278 |
| " | AH | 34°58'S | 151°05'E | 9.4.79 | 0.574 | 0.411 | 0.005 | -0.002 | 202 | 0 | 0.712 | 0.288 | -0.052* | 198 |

T le 3. Results for guanine deaminase. A number of rare alleles has been combined for GPI. *Sample set significantly out of Hardy Weinberg Equilibrium.

| Country and Station | | GDA 1 | 2 | 3 | 4+5 | $H_1$ | 2N |
|---|---|---|---|---|---|---|---|
| Palau, TTP1 | R | 0.165 | 0.752 | 0.083 | 0 | 0.047* | 242 |
| " | BL | 0.174 | 0.716 | 0.101 | 0.009 | 0.025 | 218 |
| " | BM | 0.213 | 0.676 | 0.103 | 0.008 | 0.005 | 244 |
| Helen Reef | BK | 0.233 | 0.644 | 0.123 | 0 | 0.016 | 146 |
| Truk | AO | 0.139 | 0.771 | 0.009 | 0 | -0.005 | 144 |
| " | AP | 0.145 | 0.742 | 0.105 | 0.008 | -0.004 | 124 |
| " | AQ | 0.083 | 0.785 | 0.125 | 0.007 | 0.008 | 144 |
| Ponape | AR | 0.130 | 0.808 | 0.059 | 0.003 | -0.010 | 308 |
| " | BH | 0.119 | 0.791 | 0.090 | 0.004 | 0.027* | 244 |
| " | BJ | 0.084 | 0.863 | 0.052 | 0 | 0.006 | 154 |
| Papua NG | MAS-A | 0.136 | 0.772 | 0.087 | 0.005 | -0.003 | 206 |
| " | KVP | 0.225 | 0.695 | 0.065 | 0.010 | 0.018 | 200 |
| " | KVQ | 0.167 | 0.770 | 0.121 | 0 | 0.016 | 198 |
| " | KVT | 0.129 | 0.777 | 0.094 | 0 | 0.001 | 202 |
| " | SC | 0.146 | 0.764 | 0.090 | 0 | -0.027 | 144 |
| Solomon Is | BG | 0.173 | 0.717 | 0.106 | 0.004 | 0.024 | 226 |
| New Caledonia | A | 0.112 | 0.761 | 0.112 | 0.015 | -0.012 | 134 |
| Fiji | F | 0.083 | 0.859 | 0.057 | 0 | 0.002 | 192 |
| " | BD | 0.137 | 0.760 | 0.098 | 0.005 | 0.001 | 204 |
| Wallis Is. | W2 | 0.071 | 0.807 | 0.121 | 0 | -0.008 | 140 |
| " | W4 | 0.124 | 0.780 | 0.097 | 0 | -0.005 | 186 |
| " | W6 | 0.108 | 0.757 | 0.128 | 0.007 | -0.004 | 148 |
| " | BE | 0.115 | 0.803 | 0.072 | 0.009 | -0.004 | 234 |
| Samoa | BB | 0.098 | 0.791 | 0.102 | 0.009 | -0.001 | 234 |
| Kirabati | N | 0.116 | 0.823 | 0.061 | 0 | -0.006 | 164 |
| Tuvalu | L | 0.105 | 0.796 | 0.093 | 0.006 | 0.019 | 162 |
| Phoenix | AS | 0.125 | 0.767 | 0.092 | 0.016 | 0.036* | 120 |
| Penrhyn Is | T | 0.081 | 0.887 | 0.025 | 0 | 0 | 160 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| T..amotu Gp | U | 0.101 | 0.813 | 0.086 | 0 | -0.006 | 198 |
| " | Z | 0.094 | 0.772 | 0.128 | 0.006 | 0.005 | 180 |
| " | AA | 0.111 | 0.794 | 0.095 | 0 | 0.027 | 262 |
| " | AZ | 0.122 | 0.797 | 0.067 | 0.014 | -0.006 | 148 |
| Marquesas | X | 0.125 | 0.764 | 0.106 | 0.005 | 0.013 | 208 |
| " | Y | 0.131 | 0.798 | 0.071 | 0 | -0.004 | 168 |
| " | AV | 0.091 | 0.823 | 0.085 | 0 | -0.008 | 164 |
| " | AW | 0.136 | 0.743 | 0.107 | 0.014 | -0.018 | 140 |
| " | AX | 0.097 | 0.769 | 0.134 | 0 | 0.000 | 216 |
| Gambier Is | AY | 0.125 | 0.798 | 0.077 | 0 | 0.005 | 104 |
| New Zealand | AB | 0.086 | 0.805 | 0.100 | 0.005 | 0.008 | 220 |
| " | AC | 0.107 | 0.815 | 0.073 | 0.006 | -0.022 | 178 |
| " | AE | 0.142 | 0.721 | 0.127 | 0.010 | -0.008 | 204 |
| Norfolk Is | BC | 0.144 | 0.781 | 0.075 | 0 | 0.021 | 146 |
| S.E. Australia | AG | 0.132 | 0.786 | 0.068 | 0.013 | 0.009 | 234 |
| " | AF | 0.171 | 0.726 | 0.104 | 0 | -0.016 | 164 |
| " | AH | 0.120 | 0.766 | 0.105 | 0.010 | -0.007 | 192 |

Table 4. Results for Adenosine deaminase and glucose phosphate isomerase. A number of rare alleles has been combined for GP1.

| Country and Station | | Locus | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ADA | | | | | GPI | | | | | |
| | | 1 | 2 | 4 | $H_2$ | 2N | 2/8 | 3 | 4/5 | 6/7 | 1/9 | 2N |
| Palau | R | 0.004 | 0.976 | 0.008 | 0.008 | 250 | 0 | 0.977 | 0.016 | 0 | 0.008 | 258 |
| Papua NG | MAS-A | 0.005 | 0.962 | 0.033 | -0.001 | 210 | 0 | 0.990 | 0.005 | 0 | 0.005 | 210 |
| " | KVP | 0.019 | 0.976 | 0.005 | 0 | 210 | 0 | 0.976 | 0.010 | 0 | 0.014 | 210 |
| " | KVQ | 0.005 | 0.981 | 0.014 | 0 | 208 | 0 | 0.995 | 0.005 | 0 | 0 | 206 |
| " | KVT | 0.005 | 0.956 | 0.024 | -0.002 | 206 | 0 | 0.985 | 0.005 | 0 | 0.010 | 206 |
| " | SW | 0.016 | 0.984 | 0 | 0 | 124 | | - | | | | |
| " | SC | 0.011 | 0.956 | 0.011 | -0.002 | 180 | | - | | | | |
| Solomon Is | D | 0.015 | 0.985 | 0 | 0 | 134 | | - | | | | |
| " | E | 0.010 | 0.985 | 0 | 0 | 194 | | - | | | | |
| Tonga | T1 | 0 | 0.989 | 0 | 0 | 182 | | - | | | | |
| Wallis Is | W1 | 0.027 | 0.965 | 0.004 | -0.001 | 226 | | - | | | | |
| " | W2 | 0 | 0.960 | 0.011 | 0.010 | 174 | | - | | | | |
| Samoa | H | 0.029 | 0.962 | 0.010 | -0.001 | 208 | | - | | | | |
| Penrhyn Is | T | 0.012 | 0.951 | 0.019 | -0.002 | 162 | 0.006 | 0.981 | 0.012 | 0 | 0 | 162 |
| Tuamotu Gp | U | 0.019 | 0.962 | 0.010 | -0.001 | 208 | 0.014 | 0.981 | 0.005 | 0 | 0 | 210 |
| " | Z | 0 | 0.973 | 0.021 | -0.001 | 188 | 0.005 | 0.975 | 0.005 | 0 | 0.015 | 198 |
| " | AA | 0.011 | 0.967 | 0.015 | -0.001 | 270 | 0.007 | 0.961 | 0.025 | 0 | 0.007 | 280 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Marquesas | X | 0.005 | 0.980 | 0.005 | 0 | 200 | 0 | 0.981 | 0.014 | 0 | 0.005 | 214 |
| " | Y | 0.011 | 0.960 | 0.023 | -0.001 | 176 | 0 | 0.980 | 0.020 | 0 | 0 | 196 |
| New Zealand | AB | 0.013 | 0.965 | 0.004 | -0.001 | 228 | 0.009 | 0.978 | 0.009 | 0 | 0.004 | 230 |
| " | AC | 0.005 | 0.968 | 0.005 | 0.010 | 186 | 0 | 0.968 | 0.016 | 0.010 | 0.005 | 186 |
| " | AE | 0.009 | 0.977 | 0.009 | 0 | 214 | 0 | 0.981 | 0.014 | 0 | 0.005 | 214 |

Tab 5. Summary of Area Results giving the gene frequency (P) homogeneity tests, the unweighted variance ($\sigma$) of the gene frequency and Smith's H value. *Significance level $p < .05$.

| | Es[1] | Tf[2] | ADA[3] | GPI[2] | GDA[1] |
|---|---|---|---|---|---|
| **Total Area** | | | | | |
| P | 0.589 | 0.704 | 0.970 | 0.979 | 0.128 |
| No. Schools | 70 | 70 | 22 | 14 | 45 |
| Homogeneity $\chi^2$ (df) | 781.1(69)* | 75.8(69) | 14.0(18) | - | 93.2(45)* |
| 2N | 14,440 | 13,336 | 4,338 | 2,980 | 8,490 |
| $\bar{H}$ | 0.0075* | 0.0018 | 0.0006 | - | 0.0074* |
| **Area A** | | | | | |
| P | 0.673 | 0.696 | 0.973 | 0.985 | 0.153 |
| No. Schools | 32 | 32 | 9 | 5 | 17 |
| Homogeneity $\chi^2$ (df) | 107.4(31)* | 30.6(31) | 6.3(8) | - | 37.8(16)* |
| 2N | 6,498 | 5,836 | 1,716 | 1,090 | 3,276 |
| $\bar{H}$ | 0.0120* | 0.0026 | 0.0006 | - | 0.0101* |
| **Area B** | | | | | |
| P | 0.547 | 0.720 | 0.969 | - | 0.107 |
| No. Schools | 20 | 20 | 4 | - | 11 |
| Homogeneity $\chi^2$ (df) | 46.5(19)* | 23.3(19) | 3.2(3) | | 6.9(10) |
| 2N | 4,534 | 4,080 | 790 | | 1,930 |
| $\bar{H}$ | 0.0071 | 0.0056 | 0.0016 | | 0.0064* |
| **Area C** | | | | | |
| P | 0.427 | 0.689 | 0.966 | 0.975 | 0.109 |
| No. Schools | 11 | 11 | 6 | 6 | 11 |
| Homogeneity $\chi^2$ (df) | 10.2(10) | 10.2(10) | 3.0(5) | - | 5.5(10) |
| 2N | 2,022 | 1,998 | 1,204 | 1,260 | 1,948 |
| $\bar{H}$ | 0.0039 | 0.0023 | -0.0010 | - | 0.0045 |
| **Temperate Area** | | | | | |
| P | 0.569 | 0.703 | 0.970 | 0.976 | 0.127 |
| No. Schools | 7 | 7 | 3 | 3 | 7 |
| Homogeneity $\chi^2$ (df) | 52.2(6)* | 3.0(6) | - | - | 7.6(6) |
| 2N | 1,386 | 1,422 | 628 | 630 | 1,336 |
| $\bar{H}$ | -0.0074 | -0.0134 | 0.0026* | - | 0.0063 |

Appendix 1. The median size (LCF) and number of phenotypes obtained at the esterase, transferrin and guanine deaminease loci for each sample set. The figures in brackets give the phenotype and number for very rare types.

| Station | LCF | Esterase 11 | 12 | 13 | 22 | 23 | 33 | 14 | 24 | Transferrin 22 | 23 | 33 | 34 | 13 | 12 | 11 | GDA 12 | 22 | 23 | 11 | 33 | 24 | 25 | 13 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AREA A | | | | | | | | | | | | | | | | | | | | | | | | | |
| R | 57 | 62 | 49 | 1 | 14 | 0 | 0 | 1 | 1 | 59 | 49 | 13 | 0 | 0 | 1 | 0 | 22 | 74 | 12 | 7 | 2 | 0 | 0 | 4 | 0 |
| BL | 37 | 51 | 43 | 2 | 18 | 1 | 0 | 0 | 0 | 62 | 40 | 7 | 0 | 0 | 3 | 0 | 22 | 57 | 19 | 6 | 0 | 1 | 0 | 3 | [14-1] |
| BM | 38 | 56 | 47 | 0 | 16 | 0 | 0 | 0 | 0 | 43 | 40 | 10 | 0 | 0 | 1 | 0 | 32 | 58 | 16 | 6 | 1 | 1 | 0 | 7 | [14-1] |
| BK | 33 | 49 | 29 | 1 | 3 | 1 | 0 | 0 | 0 | 46 | 29 | 4 | 0 | 0 | 1 | 0 | 18 | 33 | 10 | 5 | 1 | 0 | 0 | 6 | 0 |
| P | 50 | 38 | 45 | 2 | 14 | 1 | 0 | 0 | 0 | 48 | 34 | 9 | 0 | 1 | 0 | 0 | | | | | | | | | |
| AO | 51 | 24 | 32 | 0 | 15 | 0 | 0 | 1 | 1 | 40 | 20 | 11 | 0 | 1 | 1 | 0 | 14 | 46 | 5 | 1 | 2 | 0 | 0 | 4 | 0 |
| AP | 33 | 20 | 30 | 1 | 12 | 0 | 0 | 0 | 0 | 36 | 18 | 7 | 0 | 0 | 0 | 0 | 14 | 34 | 9 | 1 | 1 | 1 | 0 | 2 | 0 |
| AQ | 33 | 32 | 29 | 2 | 13 | 0 | 0 | 0 | 0 | 35 | 33 | 6 | 0 | 1 | 0 | 0 | 10 | 44 | 15 | 1 | 1 | 0 | 0 | 0 | [34-1] |
| AR | 30 | 67 | 72 | 2 | 22 | 2 | 0 | 0 | 0 | 72 | 53 | 17 | 0 | 0 | 6 | 0 | 35 | 101 | 11 | 1 | 2 | 1 | 0 | 3 | 0 |
| BH | 62 | 59 | 56 | 1 | 11 | 1 | 0 | 0 | 0 | 75 | 41 | 11 | 0 | 0 | 0 | 0 | 18 | 78 | 18 | 5 | 1 | 0 | 0 | 1 | [34-1] |
| BJ | 49 | 29 | 31 | 1 | 18 | 0 | 0 | 0 | 1 | 36 | 33 | 10 | 0 | 0 | 0 | 0 | 9 | 60 | 4 | 1 | 1 | 0 | 0 | 2 | 0 |
| Red A | 49 | 77 | 60 | 3 | 16 | 1 | 0 | 0 | 2 | 39 | 37 | 4 | 0 | 0 | 1 | 0 | | | | | | | | | |
| Unlab | 54 | 61 | 42 | 0 | 12 | 0 | 0 | 1 | 0 | 28 | 26 | 6 | 0 | 0 | 1 | 2 | | | | | | | | | |
| Mas A | 52 | 44 | 48 | 1 | 8 | 1 | 0 | 0 | 3 | 57 | 36 | 11 | 0 | 1 | 0 | 0 | 20 | 61 | 16 | 3 | 0 | 1 | 0 | 2 | 0 |
| KVP | 58 | 54 | 36 | 4 | 8 | 0 | 0 | 1 | 1 | 46 | 47 | 5 | 0 | 0 | 6 | 0 | 24 | 52 | 12 | 8 | 0 | 2 | 1 | 1 | 0 |
| KVQ | 58 | 60 | 34 | 2 | 6 | 1 | 0 | 1 | 0 | 45 | 50 | 7 | 0 | 2 | 0 | 0 | 23 | 51 | 15 | 2 | 2 | 0 | 0 | 5 | [15-1] |
| KVT | 55 | 61 | 32 | 0 | 7 | 0 | 0 | 1 | 0 | 50 | 45 | 5 | 0 | 1 | 1 | 0 | 21 | 61 | 14 | 2 | 1 | 0 | 0 | 2 | 0 |
| SW | 49 | 43 | 16 | 0 | 2 | 1 | 0 | 0 | 0 | 31 | 21 | 8 | 0 | 0 | 0 | 0 | | | | | | | | | |
| SC | 49 | 54 | 34 | 2 | 3 | 1 | 0 | 0 | 0 | 21 | 14 | 3 | 0 | 0 | 0 | 0 | 18 | 39 | 12 | 1 | 0 | 0 | 0 | 1 | 0 |
| AM | 51 | 45 | 51 | 2 | 8 | 0 | 0 | 1 | 0 | 61 | 38 | 7 | 0 | 0 | 1 | 0 | | | | | | | | | |
| AN | 54 | 55 | 43 | 1 | 11 | 0 | 0 | 0 | 0 | 52 | 47 | 10 | 0 | 0 | 0 | 0 | | | | | | | | | |
| AJ | 64 | 53 | 43 | 2 | 10 | 0 | 0 | 0 | 1 | 49 | 46 | 12 | 0 | 1 | 1 | 0 | | | | | | | | | |

| | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AK | 48 | 42 | 46 | 0 | 10 | 0 | 0 | 0 | 0 | 41 | 42 | 15 | 0 | 0 | 0 | 0 | | | | | | | | | |
| AL | 48 | 53 | 40 | 2 | 13 | 2 | 0 | 0 | 0 | 51 | 48 | 8 | 0 | 0 | 1 | 0 | | | | | | | | | |
| D | 37 | 42 | 42 | 2 | 12 | 0 | 0 | 0 | 0 | 25 | 27 | 7 | 0 | 0 | 1 | 0 | | | | | | | | | |
| H | 51 | 40 | 36 | 2 | 12 | 0 | 0 | 1 | 0 | 35 | 34 | 12 | 0 | 0 | 2 | 0 | | | | | | | | | |
| E | 49 | 49 | 37 | 2 | 20 | 1 | 0 | 0 | 1 | 53 | 41 | 9 | 0 | 2 | 2 | 0 | | | | | | | | | |
| G | 65 | 29 | 29 | 0 | 3 | 0 | 0 | 0 | 0 | 13 | 27 | 4 | 0 | 0 | 0 | 0 | | | | | | | | | |
| BG | 44 | 56 | 47 | 0 | 6 | 1 | 0 | 2 | 1 | 50 | 51 | 8 | 0 | 0 | 2 | 0 | 25 | 59 | 18 | 6 | 2 | 0 | 0 | 2 | [34-1] |
| A | 44 | 25 | 32 | 2 | 18 | 1 | 0 | 0 | 0 | 38 | 27 | 9 | 0 | 1 | 2 | 0 | 13 | 37 | 14 | 0 | 0 | 1 | 0 | 2 | 0 |
| B | 51 | 43 | 53 | 2 | 15 | 1 | 0 | 1 | 1 | 53 | 50 | 8 | 1 | 0 | 0 | 0 | | | | | | | | | |
| D | 49 | 43 | 23 | 0 | 6 | 0 | 0 | 1 | 0 | 31 | 39 | 1 | 0 | 1 | 1 | 0 | | | | | | | | | |
| AREA B | | | | | | | | | | | | | | | | | | | | | | | | | |
| E | 49 | 34 | 42 | 0 | 24 | 0 | 0 | 0 | 0 | 37 | 38 | 2 | 0 | 1 | 0 | 1 | | | | | | | | | |
| F | 45 | 44 | 53 | 2 | 19 | 0 | 0 | 1 | 1 | 58 | 51 | 8 | 0 | 1 | 0 | 0 | 13 | 71 | 10 | 1 | 0 | 0 | 0 | 1 | 0 |
| B | 49 | 26 | 33 | 0 | 13 | 1 | 0 | 0 | 0 | 23 | 16 | 2 | 0 | 3 | 0 | 0 | | | | | | | | | |
| A | 47 | 65 | 83 | 2 | 40 | 0 | 0 | 0 | 0 | 81 | 49 | 11 | 0 | 0 | 3 | 0 | | | | | | | | | |
| BD | 51 | 32 | 44 | 0 | 27 | 0 | 0 | 0 | 0 | 52 | 44 | 7 | 0 | 0 | 0 | 0 | 23 | 58 | 15 | 2 | 2 | 0 | 0 | 1 | [34-1] |
| T1 | 49 | 29 | 53 | 2 | 10 | 1 | 0 | 0 | 0 | 55 | 25 | 3 | 0 | 0 | 1 | 0 | | | | | | | | | |
| W1 | 51 | 25 | 63 | 2 | 23 | 0 | 1 | 0 | 0 | 58 | 42 | 7 | 0 | 0 | 0 | 0 | | | | | | | | | |
| W2 | 59 | 24 | 39 | 2 | 18 | 1 | 0 | 1 | 2 | 44 | 30 | 12 | 0 | 0 | 0 | 0 | 8 | 45 | 15 | 1 | 1 | 0 | 0 | 0 | 0 |
| W3 | 51 | 35 | 47 | 2 | 32 | 1 | 0 | 1 | 1 | 53 | 29 | 7 | 0 | 1 | 1 | 0 | | | | | | | | | |
| W4 | 51 | 28 | 44 | 1 | 26 | 0 | 0 | 0 | 0 | 53 | 37 | 5 | 0 | 0 | 0 | 0 | 18 | 56 | 15 | 2 | 1 | 0 | 0 | 1 | 0 |
| W6 | 60 | 45 | 79 | 1 | 16 | 2 | 0 | 0 | 1 | 67 | 43 | 14 | 0 | 0 | 0 | 0 | 14 | 42 | 13 | 1 | 3 | 1 | 0 | 0 | 0 |
| BE | 45 | 38 | 49 | 3 | 30 | 0 | 0 | 1 | 0 | 70 | 39 | 12 | 0 | 0 | 2 | 0 | 23 | 76 | 12 | 1 | 1 | 0 | 1 | 2 | [34-1] |
| H | 48 | 35 | 59 | 1 | 9 | 1 | 0 | 0 | 0 | 56 | 35 | 4 | 0 | 1 | 0 | 0 | 9 | 52 | 9 | 2 | 0 | 1 | 0 | 0 | 0 |
| BB | 50 | 30 | 48 | 1 | 37 | 1 | 0 | 0 | 0 | 59 | 45 | 8 | 0 | 0 | 2 | 0 | 18 | 75 | 16 | 1 | 2 | 1 | 0 | 3 | [34-1] |
| J | 51 | 57 | 71 | 3 | 27 | 0 | 0 | 0 | 0 | 73 | 59 | 10 | 0 | 1 | 5 | 2 | | | | | | | | | |
| K | 51 | 34 | 51 | 2 | 20 | 1 | 0 | 0 | 0 | 53 | 32 | 8 | 0 | 0 | 1 | 0 | | | | | | | | | |
| L | 52 | 43 | 45 | 1 | 12 | 1 | 0 | 1 | 0 | 42 | 43 | 5 | 1 | 0 | 1 | 0 | 14 | 50 | 14 | 1 | 0 | 0 | 1 | 1 | 0 |

| | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M | 47 | 33 | 52 | 2 | 33 | 1 | 0 | 0 | 1 | 56 | 51 | 11 | 0 | 1 | 1 | 0 | | | | | | | | | |
| N | 47 | 29 | 63 | 0 | 34 | 0 | 0 | 1 | 0 | 47 | 56 | 9 | 0 | 2 | 1 | 0 | 15 | 55 | 10 | 2 | 0 | 0 | 0 | 0 | 0 |
| AS | 39 | 15 | 26 | 1 | 19 | 0 | 0 | 0 | 0 | 31 | 17 | 12 | 0 | 0 | 1 | 0 | 9 | 37 | 8 | 3 | 1 | 0 | 1 | 0 | [35-1] |
| AREA C | | | | | | | | | | | | | | | | | | | | | | | | | |
| T | 50 | 13 | 47 | 0 | 19 | 1 | 0 | 1 | 0 | 31 | 38 | 7 | 0 | 2 | 1 | 0 | 11 | 63 | 4 | 1 | 0 | 0 | 1 | 0 | 0 |
| U | 50 | 23 | 37 | 3 | 41 | 0 | 0 | 0 | 3 | 45 | 41 | 13 | 0 | 1 | 3 | 0 | 14 | 66 | 15 | 3 | 1 | 0 | 0 | 0 | 0 |
| Z | 52 | 18 | 48 | 1 | 31 | 0 | 0 | 0 | 1 | 50 | 35 | 10 | 0 | 0 | 2 | 0 | 10 | 54 | 20 | 2 | 0 | 1 | 0 | 3 | 0 |
| AA | 53 | 29 | 61 | 1 | 45 | 1 | 0 | 3 | 0 | 62 | 67 | 11 | 0 | 0 | 0 | 0 | 19 | 86 | 17 | 4 | 3 | 0 | 0 | 2 | 0 |
| AZ | 44 | 19 | 36 | 0 | 19 | 0 | 0 | 1 | 0 | 31 | 34 | 6 | 0 | 0 | 2 | 0 | 14 | 47 | 8 | 1 | 0 | 2 | 0 | 2 | 0 |
| X | 46 | 17 | 42 | 2 | 39 | 1 | 0 | 0 | 1 | 51 | 44 | 8 | 0 | 0 | 0 | 0 | 21 | 62 | 13 | 1 | 3 | 1 | 0 | 3 | 0 |
| Y | 46 | 16 | 49 | 1 | 21 | 0 | 0 | 0 | 1 | 41 | 38 | 5 | 1 | 0 | 1 | 0 | 18 | 53 | 10 | 2 | 1 | 0 | 0 | 0 | 0 |
| AV | 51 | 14 | 41 | 2 | 24 | 1 | 0 | 0 | 1 | 44 | 30 | 8 | 0 | 0 | 0 | 0 | 15 | 53 | 14 | 0 | 0 | 0 | 0 | 0 | 0 |
| AW | 46 | 12 | 35 | 1 | 20 | 1 | 0 | 0 | 2 | 32 | 29 | 10 | 0 | 0 | 0 | 0 | 18 | 36 | 12 | 0 | 1 | 2 | 0 | 1 | 0 |
| AX | 42 | 20 | 50 | 2 | 40 | 0 | 0 | 0 | 0 | 55 | 43 | 11 | 0 | 0 | 2 | 0 | 17 | 62 | 25 | 1 | 1 | 0 | 0 | 2 | 0 |
| AY | 45 | 7 | 22 | 0 | 21 | 1 | 1 | 1 | 0 | 33 | 15 | 3 | 0 | 0 | 2 | 0 | 11 | 33 | 6 | 1 | 1 | 0 | 0 | 0 | 0 |
| TEMPERATE | | | | | | | | | | | | | | | | | | | | | | | | | |
| AB | 49 | 28 | 58 | 0 | 19 | 1 | 0 | 0 | 0 | 59 | 47 | 8 | 0 | 0 | 0 | 0 | 11 | 72 | 20 | 4 | 1 | 1 | 1 | 0 | 0 |
| AC | 54 | 16 | 47 | 1 | 23 | 2 | 0 | 1 | 0 | 43 | 43 | 6 | 0 | 0 | 1 | 0 | 17 | 57 | 13 | 1 | 0 | 0 | 0 | 0 | 0 |
| AE | 46 | 23 | 48 | 1 | 25 | 2 | 0 | 0 | 0 | 47 | 47 | 11 | 0 | 1 | 0 | 0 | 22 | 52 | 19 | 1 | 1 | 2 | 0 | 5 | 0 |
| BC | 61 | 13 | 37 | 0 | 14 | 0 | 0 | 0 | 1 [44-1] | 34 | 29 | 9 | 0 | 0 | 0 | 0 | 15 | 44 | 11 | 3 | 0 | 0 | 0 | 0 | 0 |
| AF | 45 | 38 | 39 | 0 | 8 | 1 | 0 | 0 | 1 | 43 | 41 | 4 | 0 | 0 | 0 | 0 | 22 | 43 | 11 | 1 | 1 | 0 | 0 | 4 | 0 |
| AG | 46 | 74 | 52 | 0 | 13 | 0 | 0 | 0 | 0 | 70 | 57 | 11 | 0 | 0 | 1 | 0 | 23 | 72 | 14 | 3 | 0 | 2 | 1 | 2 | 0 |
| AH | 46 | 33 | 49 | 1 | 16 | 0 | 0 | 0 | 2 | 45 | 51 | 3 | 0 | 0 | 0 | 0 | 18 | 55 | 18 | 2 | 1 | 0 | 1 | 1 | 0 |

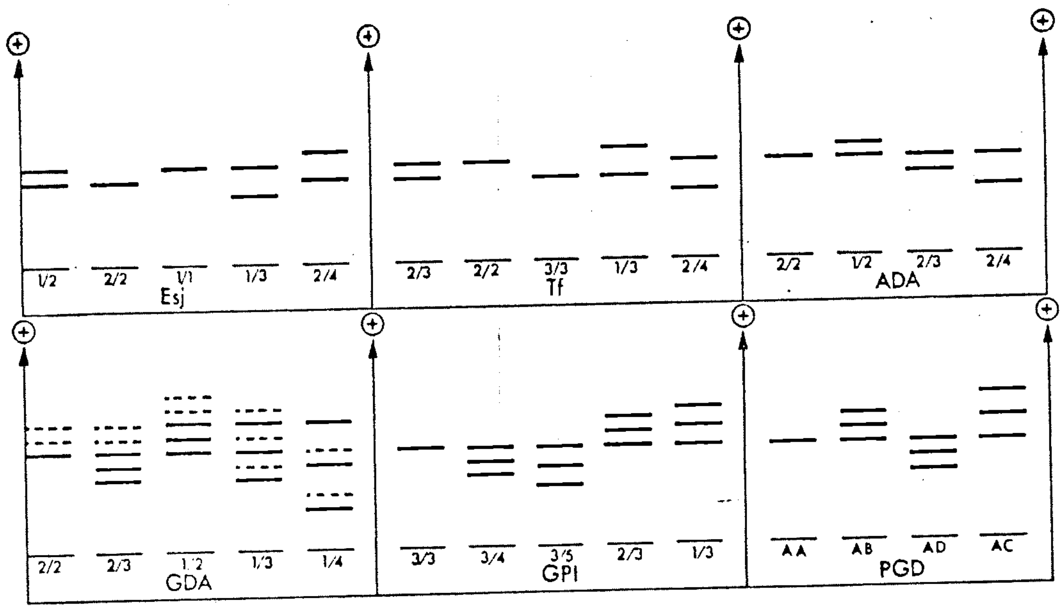


Figure 1. Diagramatic representation of the common electrophoretic pattern observed at each locus. GPI and ADA alleles are numbered in a continuous series from fastest to slowest electrophoretic mobility except that the extremely rare $GPI^9$ and $GPI^8$ are fastest and second fastest alleles respectively.

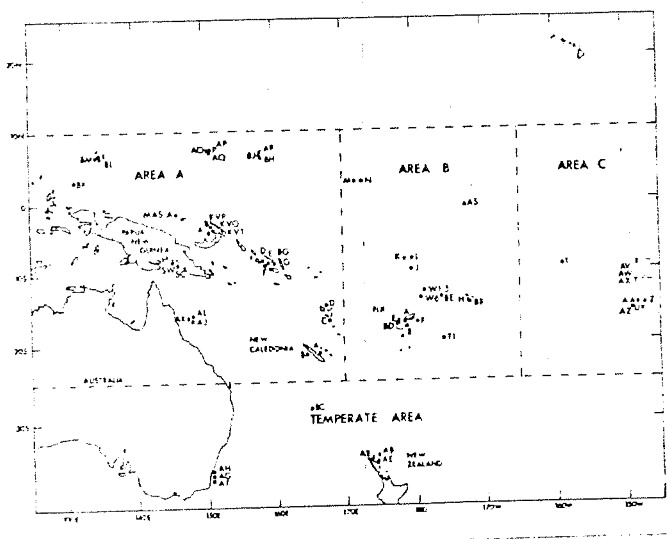


Figure 2. Map showing the sampling stations and area boundaries used in this study.

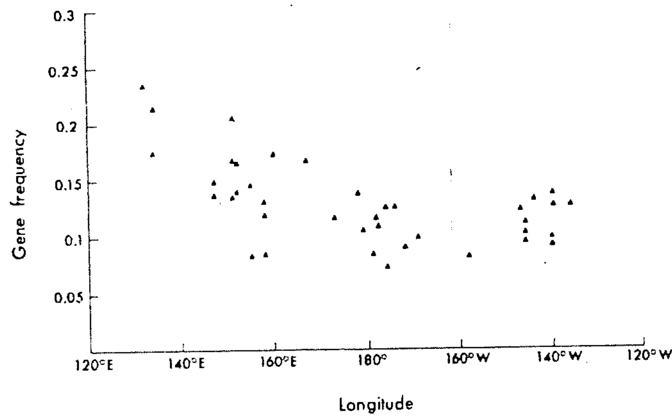


Figure 3. Plot of GDA gene frequencies against longitude.

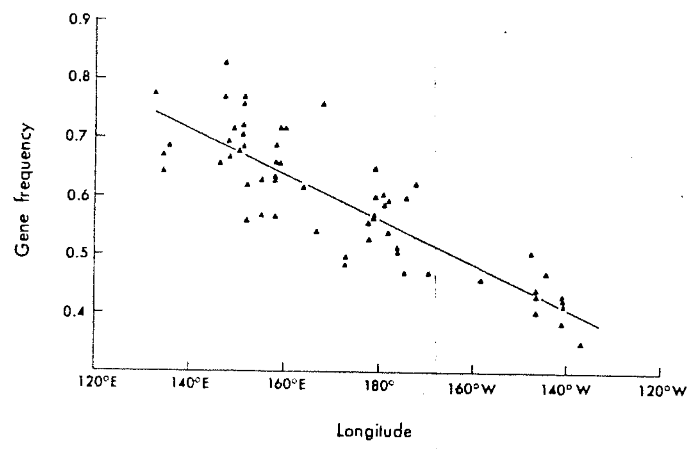


Figure 4. Plot of $Es^1$ gene frequency from tropical areas against longitude.

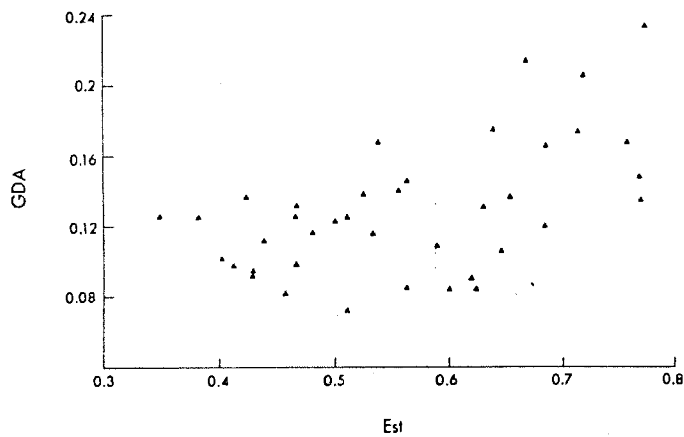


Figure 5. Scattergram of Esterase gene frequency against GDA gene frequency for sample sets from the tropical Pacific.