REVIEW OF PRELIMINARY RESULTS FROM

GENETIC ANALYSIS OF SKIPJACK BLOOD SAMPLES

COLLECTED BY THE SKIPJACK SURVEY AND ASSESSMENT PROGRAMME

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PREFACE

Agencies involved in the research and management of tunas have a longstanding interest in application and refinement of techniques for subpopulation identification. The South Pacific Commission's Skipjack Programme has been particularly interested in discussing its results from genetic analyses of tuna blood samples against the background of tagging and blood sampling data collected by the Inter-American Tropical Tuna Commission (IATTC). A proposed meeting between the two agencies to review their respective blood sampling programmes proved an opportune time to conduct a statistical review of all preliminary results from the first two years of skipjack blood sampling by the SPC Programme. Therefore, in order to optimize the returns from the visit to Noumea by Dr John Calaprice (IATTC) in late July 1979, we invited Dr Barry Richardson and Mr Tony Lewis of the Research School of Biological Sciences, Australian National University, where the Skipjack Programme's blood samples are analyzed, to participate in a review workshop from 23 to 27 July. Participants in the workshop are listed in Appendix 1.

During the time available, the participants were unable to fully examine the total data set; nor was there time to complete the statistical analyses. Consequently scientists from the Skipjack Programme continued statistical analyses for some time after the workshop. As a result of these later analyses some of the preliminary interpretations reached at the workshop were modified; however, the overall consensus of the workshop remained the same.

Skipjack Programme
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1.0 INTRODUCTION

One of the principal objectives of the Skipjack Survey and Assessment Programme is to determine, if possible, "...the degree to which fisheries in different areas exploit the same stock, and hence interact with each other" (Anon, 1977). Tag recoveries are the Programme's basic source of information on skipjack migrations and population structure. To complement the tagging data in examining population structure, the Programme funded, in accordance with the original proposal (Anon, 1977), genetic analysis of blood samples collected on board the research vessel. These analyses are being carried out at the Department of Population Biology, Research School of Biological Sciences, Australian National University (ANU), Canberra.

On completion of the second year's vessel charter, a review of preliminary results from blood sampling was conducted with the following aims:

(i) To evaluate blood sample data generated to date, and to evaluate results when genetic and tagging data were collected concurrently, i.e. from the same school. Where possible, these data would be related to that available from other sources and areas.

(ii) To use these findings in planning the final year of vessel charter.

The review took the form of a workshop involving participants from ANU, the Inter-American Tropical Tuna Commission (IATTC), and members of the Skipjack Programme. All participants are listed in the Appendix.

2.0 DATA SOURCES

A total of 38 blood samples, each taken from a single school and generally comprising approximately one hundred individual specimens, was collected during 1978 and the first half of 1979 over a wide area of the Pacific. Care was taken to ensure a fairly uniform size distribution within each sample. Where this was not possible, sample size was increased. Blood specimens were collected as described by Fujino (1966) and Sharp (1969) and shipped on dry ice to ANU in Canberra for analysis. Table 1 lists collection details, and Figure 1 shows the location of all blood samples collected by the Skipjack Programme. A total of 19,971 skipjack were tagged from the same schools from which blood samples were taken.

In addition to the above, the workshop was able to draw upon other, mostly unpublished, data made available by the invited participants. Detailed information was thus available on an additional 66 samples, representing over 7,000 individual fish. These data, in conjunction with the accumulating tag returns from the SPC programme (2,588 in total, 854 from blood sample schools, to 1 June 1979) formed the basis of these discussions.

TABLE 1 - SOUTH PACIFIC COMMISSION SKIPJACK BLOOD SAMPLES

Collection details, number of tagged fish released from the same school and the frequency, $\hat{p}$, of the dominant allele of the esterase(E^1_{SJ}) gene.

SAMPLE CODE	AREA	NUMBER OF SPECIMENS	DATE	POSITION	NUMBER OF SKIPJACK TAGS RELEASED	E^1_{SJ} $\hat{p}$
A	New Caledonia	78	8/01/78	21°46'S, 166°42'E	261	.54
B	New Caledonia	116	15/01/78	20°58'S, 164°24'E	842	.61
C	New Hebrides	22	20/01/78	17°36'S, 167°42'E	79	- *
D	New Hebrides	73	21/01/78	16°15'S, 167°51'E	314	.75
E	Fiji	100	31/01/78	18°55'S, 178°24'E	599	.55
F	Fiji	120	10/02/78	17°13'S, 179°17'W	501	.60
T1	Tonga	95	21/04/78	18°19'S, 174°25'W	63	.60
W1	Wallis	114	6/05/78	13°26'S, 176°02'W	599	.50
W2	Wallis	88	15/05/78	13°09'S, 176°22'W	410	.51
W3	Wallis	119	17/05/78	13°29'S, 176°07'W	1,034	.50
W4	Wallis	99	19/05/78	13°30'S, 176°05'W	1,026	.53
W5	Wallis	49	26/05/78	13°19'S, 176°17'W	122	- *
W6	Futuna	145	29/05/78	14°13'S, 178°03'W	38	.59
H	Western Samoa	105	14/06/78	13°42'S, 171°45'W	1,633	.62
J	Tuvalu	158	25/06/78	10°23'S, 178°48'E	486	.60
K	Tuvalu	108	27/06/78	8°40'S, 179°13'E	420	.56
L	Tuvalu	103	1/07/78	8°42'S, 179°10'E	470	.65
M	Kiribati	122	16/07/78	2°57'N, 172°45'E	573	.53
N	Kiribati	126	22/07/78	3°00'N, 172°48'E	844	.49
P	Truk	100	10/08/78	7°42'N, 151°44'E	719	.62
R	Palau	128	20/10/78	7°06'N, 134°54'E	440	.68
T	Penrhyn	81	4/12/78	9°07'S, 157°43'W	32	.46
U	Tuamotu	107	19/12/78	15°38'S, 145°34'W	686	.40
X	Marquesas	102	11/01/79	8°58'S, 140°20'W	182	.38
Y	Marquesas/Tuamotu	176	13/01/79	12°35'S, 143°26'W	256	.47
Z	Tuamotu	99	22/01/79	16°15'S, 145°58'W	384	.43
AA	Tuamotu	140	24/01/79	16°07'S, 146°06'W	299	.44
AB	N.E. New Zealand	106	6/03/79	35°51'S, 175°30'E	692	.54
AC	Bay of Plenty, New Zealand	90	8/03/79	37°41'S, 177°26'E	559	.45
AE	N.E. New Zealand	99	20/03/79	35°47'S, 175°20'E	978	.48
AF	Montagu Island, Australia	87	5/04/79	36°04'S, 150°24'E	218	.66
AG	Jervis Bay, Australia	144	8/04/79	35°06'S, 151°04'E	745	.71
AH	Jervis Bay, Australia	101	9/04/79	34°58'S, 151°05'E	769	.56
AJ	Flinders Reef, Australia	109	1/05/79	17°56'S, 148°22'E	457	.69
AK	Flinders Reef, Australia	98	2/05/79	17°31'S, 148°05'E	123	.66
AL	Willis Islets, Australia	110	3/05/79	16°22'S, 150°12'E	726	.67
AM	Solomon Sea, PNG	107	20/05/79	7°36'S, 149°47'E	487	-**
AN	Cape Lambert, PNG	109	3/06/79	4°04'S, 151°01'E	905	-**

* Omitted from blood genetics analysis due to small sample size.

** Results unavailable.

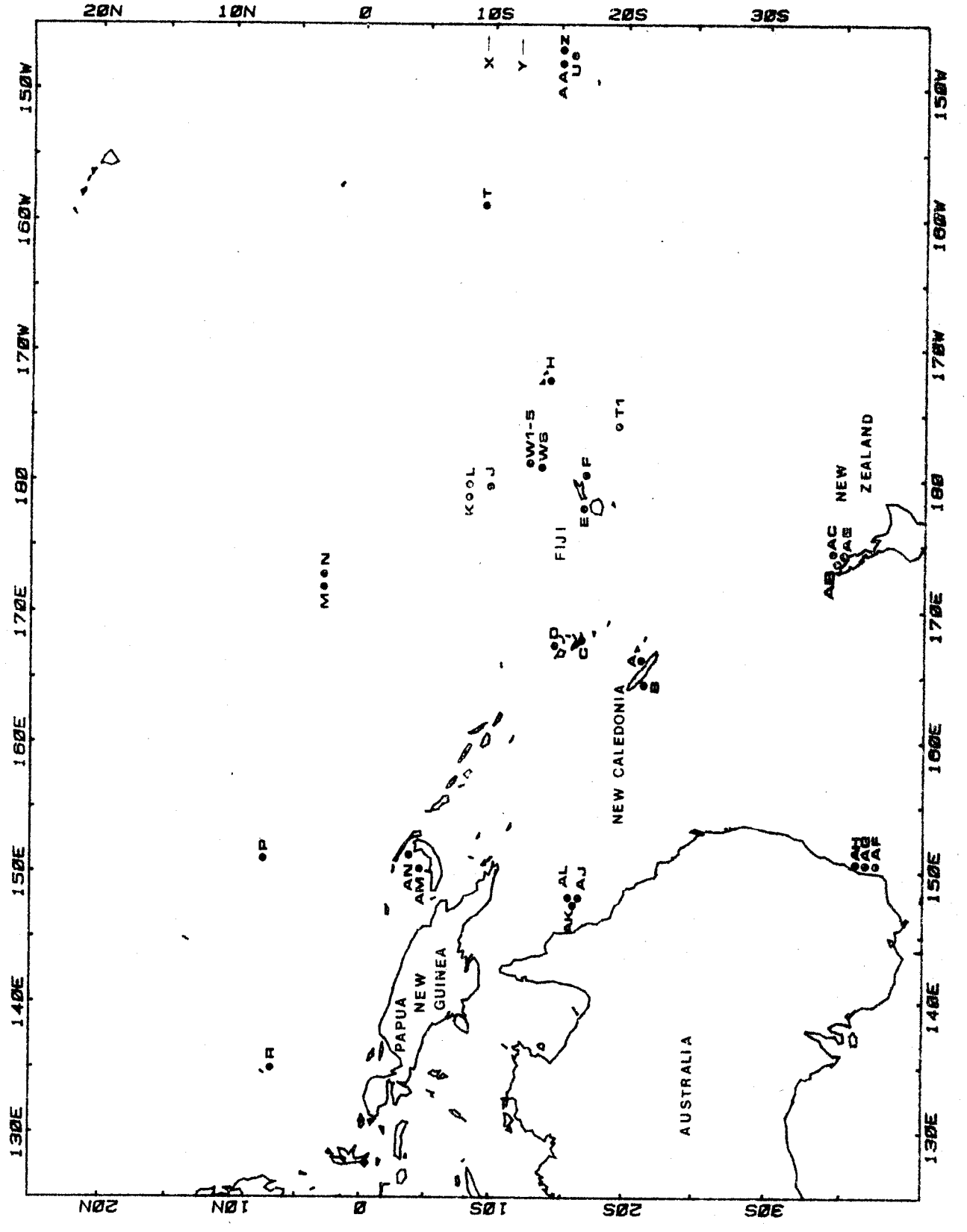

Figure 1. Collection localities for SPC blood sample schools. School codes indicated.

3.0 GENETIC SYSTEMS

The use of electrophoretic techniques to detect geographical variation in enzyme allele frequencies is widespread in the study of animal populations and has previously been specifically applied to skipjack population studies (Fujino 1970a, Sharp 1978). For population analysis, common allele frequencies in the range of 0.1 to 0.9 are desirable, as they confer greater statistical power to moderate sample sizes. The paucity of such polymorphisms in skipjack has somewhat hampered population studies with this species, restricting analyses to serum esterase (E^1_{SJ}) and transferrin (Tf^2_{SJ}) systems.

A good deal of effort was therefore directed by the Australian National University (ANU) at identifying possible useful new systems, and although over 60 enzyme loci have now been screened in skipjack blood, the results are not encouraging. The glucose-phosphate isomerase (GPI) and 6-phosphogluconate dehydrogenase (6-PGD) systems were variable, but at low levels. Two previously unreported systems, adenosine deaminase (ADA) and guamine deaminase (GDA) were also found to be variable. Data on these systems are not yet available for all samples and hence only data on esterase and transferrin were considered in detail at the Workshop.

It should be noted that enzymes from other tissues, e.g. liver, red and white muscle, have not been considered, even though genetic variation is known for certain enzyme loci (A.D. Lewis, pers.comm.), because of practical difficulties which would be involved in sampling such material from commercial catches.

4.0 STATISTICAL METHODS

4.1 Regression Analyses

To investigate whether there was a functional relationship between observed gene frequencies and longitude, a simple linear regression analysis was performed. Sample gene frequencies were regressed against the longitude where the sample was taken. For this purpose east longitude was measured in negative degrees west of 180^o and west longitude was measured in positive degrees east of 180^o.

4.2 Gene Frequency Variability

A computer programme utilized sample gene frequencies from both esterase and transferrin systems, and calculated grand means and deviations of samples from these means. Deviations, divided by the standard deviation of the sample, were plotted on bivariate axes and 60 percent and 95 percent confidence intervals were drawn, based on a standard bivariate normal distribution.

4.3 Homogeneity of Populations

Data analyses proceeded in two parts. First, statistics were calculated on the two genetic systems for various geographic subdivisions of the data as is shown for the E^1_{SJ} system in Table 2. Second, distributions of these statistics were derived by Monte Carlo techniques, i.e. the same statistics were calculated on sets of data drawn from simulated populations having *a priori* known genetic characteristics corresponding to a null and various alternate hypotheses.

These hypotheses corresponded to questions about the heterogeneity of phenotype frequencies among and between sample groups, and whether or not the observed phenotype frequencies corresponded to those expected in a population in Hardy-Weinberg equilibrium. For the Monte Carlo simulations*, stochastic data generation and calculation of the statistics was repeated (generally 1,000 times) in order to generate a distribution function of the sample statistic. Using the distribution functions produced by the simulations, it was possible to determine the power of the test ($1-\beta$ in statistical notation). The power of the test is the probability of accepting the null hypothesis when a specific case of the alternate hypothesis is in fact true. The power of the test is dependent upon, amongst other things, the selected level of α, which is the probability of erroneously rejecting a correct null hypothesis. In this way it was possible to know, whenever a null hypothesis was accepted for the real data, if the statistical tests used were sufficiently powerful to justify rejection of a specific alternate hypothesis. This extra care was taken to avoid confusion in the interpretation of the statistical tests and because of some questions which have arisen concerning appropriate sample sizes for determination of subpopulation gene frequencies in skipjack tuna.

The specific null hypotheses tested and the statistics utilized are described on page 6.

TABLE 2 - SAMPLE GROUPINGS BY AREA FOR PURPOSES OF DATA ANALYSIS

$\hat{p}$ is the serum esterase gene frequency

FILE NAME	NO. OF SAMPLES	$\hat{p}$	COLLECTION AREA
BR TØT	98	.619	All areas
BR NGS	64	.670	Area "A", west of 170°E longitude.
BR BNZ	28	.549	Area "B", 170°E and 160°W longitude
BR ARB	16	.561	Area "B", as above, less samples collected in vicinity of New Zealand, North Island.
BR ARC	6	.428	Area "C", east of 160°W longitude.
BR AAB	92	.630	Area "A" and Area "B".
BR ARW	34	.531	Area "B" and Area "C".
BR AUS	8	.653	East coast Australia.
BR ANZ	12	.539	New Zealand.
BR WAL	5	.533	Wallis Island
BR FIJ	4	.573	Fiji
BR PNG	50	.674	Papua New Guinea and Solomon Islands.
ALL SPC	28	.540	All areas for SPC samples only (excluding Australia).
WST SPC	5	.640	SPC samples west of 170°E longitude, excluding Australia.
CNT SPC	17	.550	SPC samples between 170°E and 160°W longitude.
EST SPC	6	.428	SPC samples east of 160°W longitude.

* The simulation programme was adapted from one written by Pat Tomlinson of the Inter-American Tropical Tuna Commission.

(a) H_o: A group of k samples (size n_i, i = 1, k) were each drawn from a single population in Hardy-Weinberg equilibrium. The statistics utilised tested whether the ratio of phenotypes (Homozygote A : Heterozygote : Homozygote a, in a two allele system) were in the ratio $\hat{p}^2 : 2\hat{p}\hat{q} : \hat{q}^2$ where $\hat{p}$ is an estimate of p, the gene frequency, drawn from the sample itself; where $\hat{p} = (2X+Y)/2N$; where X, Y, and Z sum to sample size N and are the numbers of the three phenotypes in the sample; and where $\hat{q} = (Y+2Z)/2N$. The statistics calculated were Smith's H (Smith, 1970) and a G-statistic for measurement of goodness-of-fit to the expected phenotype distribution calcu-calculated as $\hat{p}^2 : 2\hat{p}\hat{q} : \hat{q}^2$ (Sokal and Rohlf, 1969).

The two alternate hypotheses for which the power of the test was calculated (for both Smith's H and G) were H_1: a subgroup k_1 of the k samples was drawn from one population in Hardy-Weinberg equilibrium with gene frequency $\hat{p}_1$ (calculated from the subgroup) and the remaining $k_2 = k-k_1$ samples from a different population in Hardy-Weinberg equilibrium with gene frequency $\hat{p}_2$; and H_2 : the samples were drawn from a mixed school of fish consisting of half (on the average) from a population with gene frequency $\hat{p}_1$ and half with a gene frequency $\hat{p}_2$. The subgroup of k_1 samples in H_1 was chosen by partitioning the k samples in the populations being modelled into two groups, according to whether the estimated gene frequency for that sample was above or below the average of all gene frequencies in the group.

(b) H_o: A group of k samples (size n_i, i = 1, k) were each drawn from a single population with a gene frequency of $\hat{p}$ (defined as above). The G-statistic used to test this hypothesis was similar to that used in (a) except that there were only two classes corresponding to two allele categories, instead of three classes corresponding to the three common phenotypes in a two allele system. Consequently, the distribution of $\hat{p}$ is tested independently of the distribution of alleles into phenotypes, i.e. whether the populations were in Hardy-Weinberg equilibrium or not.

The two alternate hypotheses (H_1 and H_2) for null hypothesis (b) were parallel to those for null hypothesis (a) except that the assumption of Hardy-Weinberg equilibrium was dropped.

The heterogeneity G-statistic calculated in cases (a) and (b) above is a goodness-of-fit test of the observed phenotype frequencies in (a) (or of the gene frequencies in (b)) to the single estimate of frequency obtained by pooling all the samples. If the group of samples came from fish from more than one population (with different characteristic gene frequencies) then the variance of the observed frequencies from one sample to another should be greater than the variance that would be observed if the samples came from a single population. Simulation studies bore out the truth of this statement.

The mathematical formula for calculation of the G-statistic (Sokal and Rohlf, 1969, page 560) is: $G = 2\Sigma_i f_i \log (f_i/\hat{f}_i)$, where f_i is the observed number in the i^{th} category (phenotype or allele) and $\hat{f}_i$ is the expected number. Adapting this formula for a replicated test, we simply sum over the replicates and estimate the expected number of each replicate from the pooled observed frequency of each category. If f_{ij} is the observed number of animals in the

j^{th} replicate (j = 1, ...m) and the i^{th} category (i = 1, ...3 phenotypes or i = 1, ...2 for two alleles in a two allele system). Then it follows that

$$G = 2\Sigma_j\Sigma_i f_{ij} \log (f_{ij}/\hat{f}_{ij})$$

when $\hat{f}_{ij} = \hat{p}_i n_j = \Sigma_i f_{ij} \Sigma_j f_{ij} / \Sigma_i \Sigma_j f_{ij}$

and when n_j = no. of samples in the j^{th} replicate.

This formula is mathematically identical to the calculation in step 2 of Box 16.4 of Sokal and Rohlf, who call the resulting statistic an "interaction or heterogeneity G-test". Sokal and Rohlf indicate that this G-statistic will have a chi-square distribution with $\{(m-1)(n-1)\}$ degrees of freedom, where n equals number of phenotypes. Simulation studies showed, however, that the G-statistic had a distribution which was approximately chi-square with a mean of $\{m(n-1)-1\}$. Since the mean of the chi-square distribution is equal to its degrees-of-freedom, these latter values were used to test significance of G-statistics presented herein.

4.4 Distribution of Gene Frequencies

In addition to the quantitative statistical tests, a histogram of the gene frequencies in the various samples was drawn. This histogram allows visual comparison of the distribution of frequencies obtained, comparable to Figure 2 of Fujino (1976). Any strong bimodality in distribution of gene frequencies would be readily apparent in such a histogram.

4.5 Sample Size

Binomial sampling theory was applied to the question of how many blood specimens should constitute a sample so that the gene frequency can be estimated for a particular level of precision. The simulation programme was used to estimate the number of samples required to detect whether sampling was from a single population, or from two populations with gene frequencies differing by specified amounts.

5.0 TAG RETURNS FROM BLOOD SAMPLE SCHOOLS

For this analysis, movements of tagged skipjack were compared with esterase gene frequencies. Tag returns from fish that were at large for more than 90 days were plotted, then their recovery location was compared with the gene frequency of the school that they originated from. At the time of the meeting, 102 tag recoveries were available; since the meeting, four more recoveries from blood schools (out for more than ninety days) have been added to the analysis.

6.0 RESULTS FROM STATISTICAL ANALYSES

6.1 Geographic Trends in Gene Frequency

The results of the regression analysis for esterase (E^1_{SJ}) are shown in Figure 2, which graphs the observed esterase gene frequencies against the longitude where the sample was taken. The regression accounted for 61% (r = 0.78) of the mean squared deviations from the mean gene frequency. The regression equation is predicted gene frequency = .5576 - .0035 (longitude of sample). The marginal lines in Figure 2 are 95% prediction limits. An F test for difference of the regression coefficient from zero was significant at the 99% level.

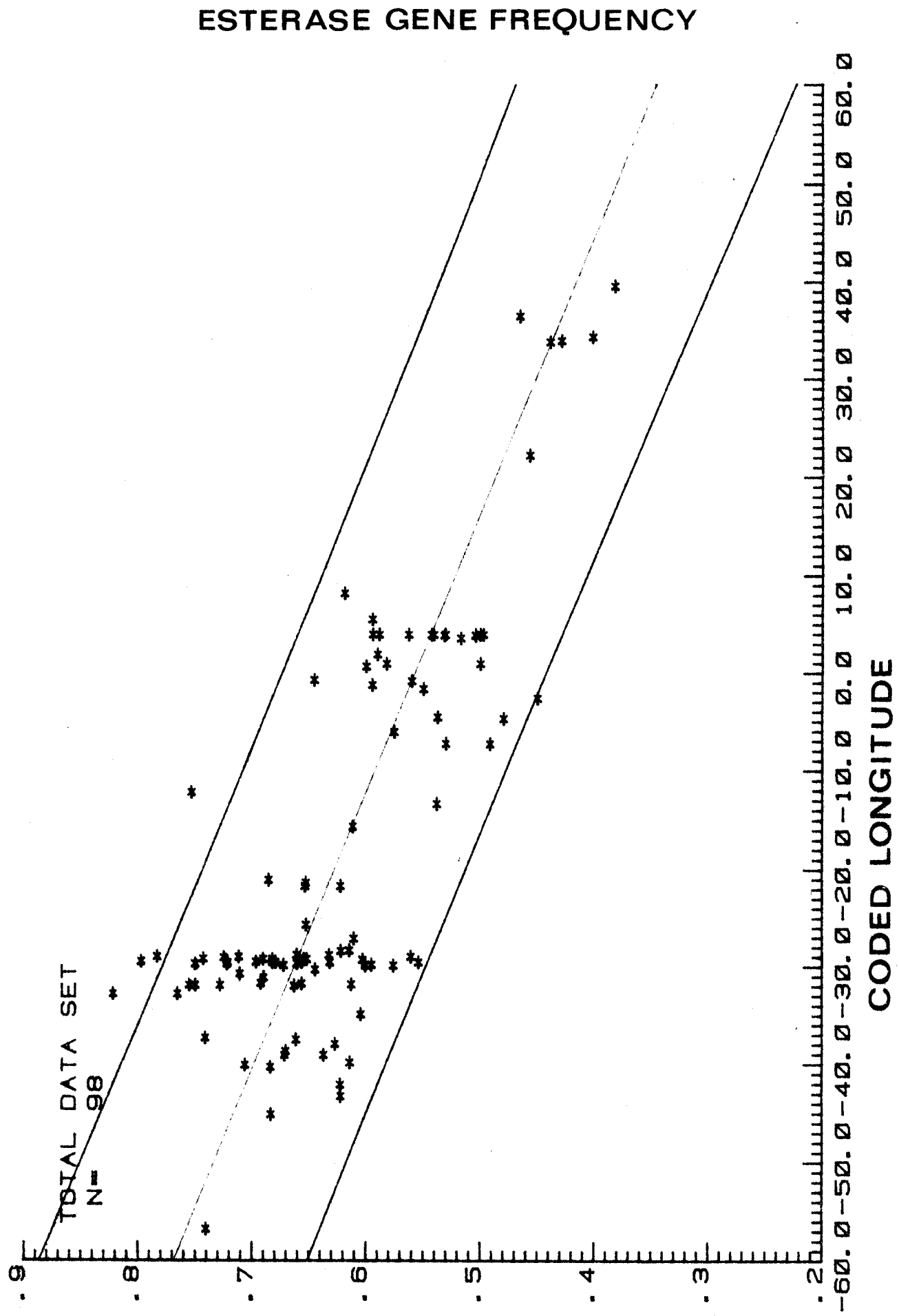

Figure 2. Regression of esterase sample gene frequency against longitude where the sample was obtained. Regression line and 95% prediction limits indicated.

Figure 3 shows the observed transferrin gene frequencies graphed against longitude. Clearly these gene frequencies show no trend with respect to longitude. The regression accounted for only one percent ($r = 0.12$) of the mean squared deviations from the mean gene frequency, and, of course, the regression coefficient was not significantly different from zero ($P>0.05\%$).

6.2 Heterogeneity of Phenotype Frequencies

The bivariate plot (Figure 4) of standardized deviations for esterase and transferrin indicated that esterase gene frequencies varied independently of transferrin gene frequencies, and suggested that there was considerable heterogeneity within the esterase gene frequencies. In other words, the esterase gene frequencies most likely represent more than one gene frequency distribution. The lack of longitudinal variation for the transferrin data set discouraged further statistical analyses of these samples at this time.

Table 3 shows the results of heterogeneity G-statistics and Smith's H-statistic calculated for the 16 sample groups of Table 2. For each sample, the estimated frequency, $\hat{p}$, of the dominant allele of the esterase system (E^1_{SJ} of Fujino, 1970a) and the number of samples in the group are given.

As previously described, a G-statistic to test the null hypothesis that the group of samples came from a single population with a binomially distributed estimate of gene frequency was calculated. This was done both for the (a) hypothesis (assuming population in Hardy-Weinberg equilibrium, G_{HW}) and for the (b) hypothesis (no assumption about genetic assortment, G_H) of the statistical methods section. Table 3 reports the probability ($P(G_H)$ or $P(G_{HW})$) that a lower value of G_H or G_{HW} would be drawn from a chi-square distribution (with n-1 or 2n-1 degrees of freedom). The chi-square is the theoretical distribution of the G-statistic under the null hypotheses; this was verified by the simulation studies. A value of P(G) of 95% or greater indicates significant deviation from the null hypothesis at the $\alpha = 5\%$ level.

Smith's H-statistic was also calculated for each sample group. A combined statistic for all samples in the group was calculated, under the null hypothesis that each group was taken from a single population. Smith's H-statistic is distributed approximately normally and is a measure of deficiency (positive H) or excess (negative H) of animals in the sample with the heterozygote phenotype. A deficiency of heterozygote phenotypes is to be expected when samples are taken from a mixture of populations with different gene frequencies (Wahlund effect) and phenotype ratios are calculated as if the samples were drawn from a single population. However, other population processes, eg. heterozygote advantage, geographical selection for particular alleles, school integrity, may also contribute to either an excess or a deficiency of heterozygotes, either compensating for or supplementing the Wahlund effect. The value P(H) reported in Table 3 is the probability of getting a smaller value than the H calculated for the sample, if the sample was drawn from a normal distribution with mean zero and variance equal to the appropriate sample variance $\hat{p}^2\hat{q}^2/(\Sigma_i n_i - \frac{1}{2})$. A value of P(H) greater than 97.5% or less than 2.5% indicates significant deviation from the Hardy-Weinberg expected proportion of phenotypes at the $\alpha = 5\%$ level (two-tailed test).

For those cases in which the value of H was not significant, Table 3 presents the power of the test statistic to guard against failure to detect a false null hypothesis, relative to the two alternate hypotheses, which are described in the methods section. For example, a value of P(H) of 60% is not significant; however, if the value of $1-\beta_{H2}$ were, say 20%, then the test had only one chance in five of demonstrating significance even if the null hypothesis

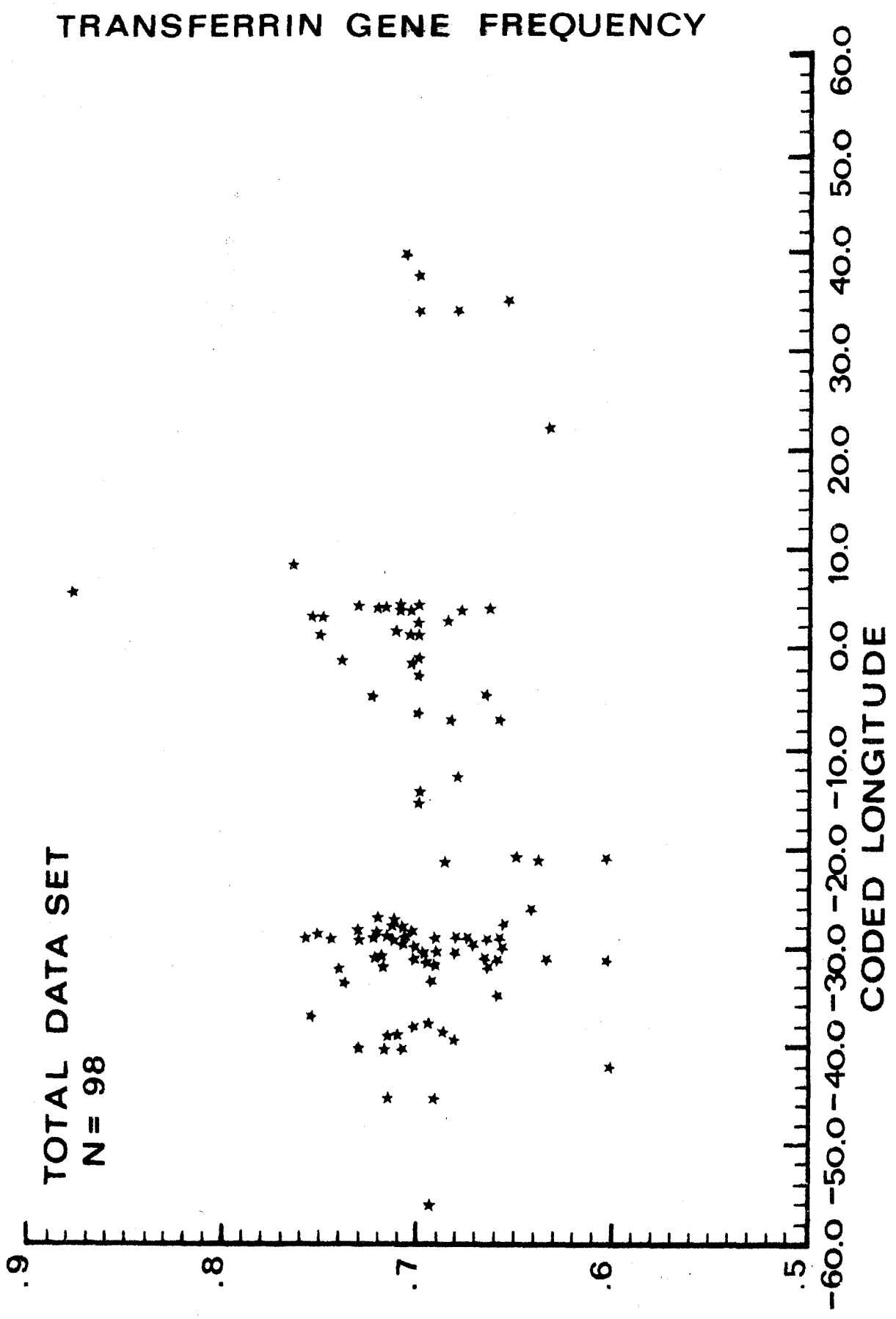

Figure 3. Graph of transferrin sample gene frequency plotted against longitude where the sample was obtained.

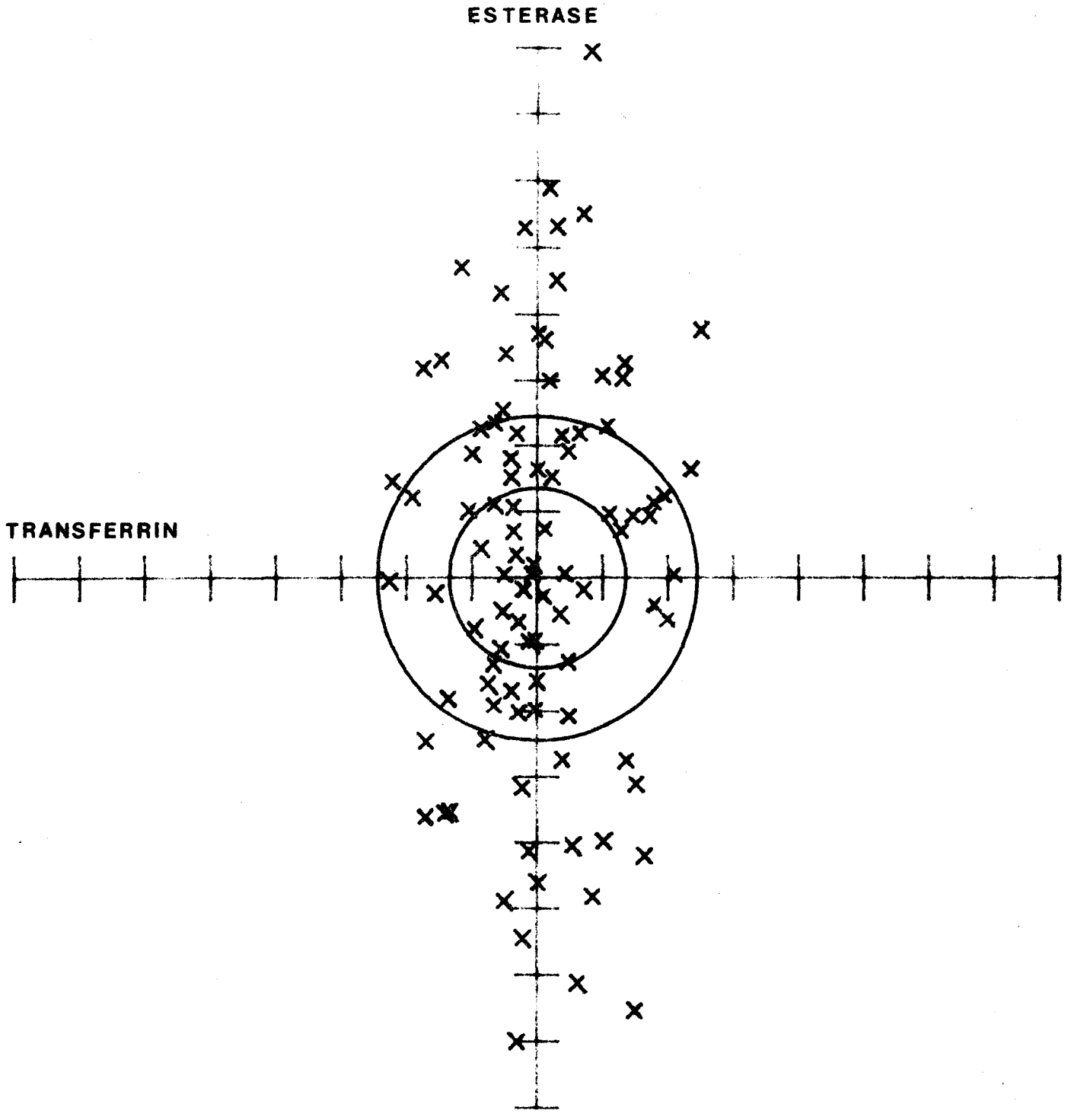

Figure 4. Bivariate plot of standardized deviations for esterase and transferrin gene frequencies from their grand means. Inner circle represents the 60% confidence interval, outer circle represents the 95% confidence interval.

TABLE 3 - RESULTS OF STATISTICAL ANALYSES FOR 16 SAMPLE GROUPS, WHERE: $\hat{p}$ IS THE FREQUENCY OF THE MOST COMMON ALLELE OF THE ESTERASE ENZYME SYSTEM; n IS THE NUMBER OF SAMPLES IN THE GROUP; G_{HW} IS THE G-STATISTIC CALCULATED FOR THE PHENOTYPE RATIOS, WHICH WERE ASSUMED TO BE IN HARDY-WEINBERG EQUILIBRIUM; $P(G_{HW})$ IS THE PROBABILITY (%) OF GETTING A LOWER VALUE OF G_{HW} DRAWN FROM A χ^2 DISTRIBUTION (WITH 2n-1 DEGREES OF FREEDOM) THAN THE VALUE OF G_{HW} CALCULATED FOR THE OBSERVED PHENOTYPE RATIOS; G_H IS THE G-STATISTIC CALCULATED FOR THE GENE FREQUENCIES; $P(G_H)$ IS THE PROBABILITY OF GETTING A LOWER VALUE OF G_H DRAWN FROM A χ^2 DISTRIBUTION (WITH n-1 DEGRESS OF FREEDOM) THAN THE VALUE OF G_H CALCULATED FOR THE OBSERVED GENE FREQUENCIES; $1-\beta_{G1}$ IS THE POWER OF THE TEST (%) FOR G_H (NULL HYPOTHESIS b); H IS SMITH'S H-STATISTIC; P(H) IS THE PROBABILITY OF GETTING A LOWER VALUE OF H DRAWN FROM A NORMAL DISTRIBUTION, WITH MEAN ZERO AND VARIANCE EQUAL TO $\hat{p}^2\hat{q}^2/(\Sigma_i n_i - 0.5)$, THAN THE VALUE OF H FOR THE OBSERVED PHENOTYPE RATIO OF EACH SAMPLE GROUP; AND $1-\beta_{H1}$ AND $1-\beta_{H2}$ ARE BOTH POWER OF THE TEST VALUES FOR THE TWO ALTERNATE SMITH'S HYPOTHESES.

SAMPLE GROUP	FILE NAME	$\hat{p}$	n	G_{HW}	$P(G_{HW})$ %	G_H	$P(G_H)$ %	$1-\beta_{G1}$ %	H	P(H) %	$1-\beta_{H1}$ %	$1-\beta_{H2}$ %
1	BR TOT	.6191	98	902.7	>99.9	780.5	100.0	>95.0	.0008	63.7	>95.0	>95.0
2	BR NGS	.6696	64	297.1	>99.9	222.8	100.0	>95.0	.0023	72.0	>95.0	>95.0
3	BR BNZ	.5488	28	93.5	>99.9	57.0	99.9	>95.0	-.0030	73.9	58.0	81.0
4	BR ARB	.5608	16	52.2	99.3	28.5	98.1	>95.0	-.0021	36.7	19.0	46.0
5	BR ARC	.4279	6	15.4	87.5	4.1	46.1	>95.0	.0053	70.2	< 7.0	22.0
6	BR AAB	.6304	92	692.3	>99.9	581.5	100.0	>95.0	.0005	58.2	>95.0	>95.0
7	BR ARW	.5313	34	170.9	>99.9	122.9	100.0	>95.0	-.0018	33.7	77.0	>95.0
8	BR AUS	.6533	8	21.5	>99.9	16.6	98.0	>95.0	-.0007	46.5	2.0	26.0
9	BR ANZ	.5386	12	37.7	>99.9	24.9	99.0	>95.0	-.0038	29.8	8.0	36.0
10	BR WAL	.5331	5	14.4	92.6	5.5	76.4	>95.0	-.0035	37.7	< 7.0	27.0
11	BR FIJ	.5732	4	4.9	20.4	2.1	44.9	>95.0	.0194	94.4	< 7.0	20.0
12	BR PNG	.6744	50	244.3	>99.9	180.2	100.0	>95.0	-.0020	74.3	>95.0	>95.0
13	ALL SPC	.5396	28	218.0	>99.9	170.1	100.0	>95.0	.0093	97.6	58.0	84.0
14	WST SPC	.6404	5	26.9	98.7	19.9	99.9	>95.0	.0257	99.4	< 7.0	21.0
15	CNT SPC	.5497	17	67.0	100.0	40.9	99.9	>95.0	.0036	72.6	22.0	49.0
16	EST SPC	.4279	6	19.3	87.5	4.6	46.7	>95.0	.0135	70.2	< 7.0	22.0

were false and alternate hypothesis (H_2) were true. In this case, the power of the statistical test with the data available is insufficient to draw any conclusion. Continuing with Table 3, $1-\beta_{H2}$ is the power of Smith's H-statistic to distinguish the alternate hypothesis H_2 when the gene frequencies for the two populations are assumed to be 0.668 $\hat{p}_1$ and 0.482 $\hat{p}_2$ (from Fujino, 1976); $1-\beta_{H1}$ is the same for hypothesis H_1; and $1-\beta_{G1}$ is the power of the heterogeneity G-statistic (without assuming Hardy-Weinberg equilibrium) to detect that samples in a group came from a single population, against alternate hypothesis H_1 with gene frequency as above ($1-\beta_{G1}$ exceeded 95% in all sample groups). The G_H-statistic was unable to distinguish alternate hypothesis H_2 since only phenotype ratios would be affected, so $1-\beta_{G2}$ equals zero.

The tests of heterogeneity revealed that every subset of the samples displayed significant heterogeneity if the number of samples included was over six. This is true for both of the G-statistics, i.e. whether or not Hardy-Weinberg equilibrium is assumed for the population sampled. Smith's H-statistic failed to show significant deviation from the distribution of phenotype ratios expected from a population in Hardy-Weinberg equilibrium in nearly every sample group. In many cases, the power of this test was too small for conclusive results; however, in the case of samples from Area "A" and from Areas "A" and "B" combined, power of the test values ($1-\beta$) exceeded 95% and there was no significant deviation from the Hardy-Weinberg expected phenotype distribution.

6.3 Distribution of Gene Frequencies

Figure 5 shows the histogram for observed esterase gene frequencies. There is no striking bimodality in this data; however, the breadth of the distribution is much greater than would be expected from a single binomially distributed population with sample sizes of the order of 100.

6.4 Sample Size

There are several questions concerning the sample size necessary to estimate a population gene frequency (given a single population); and to detect whether samples are from multiple populations, when this condition exists.

For the first case above, estimation of gene frequency with a given precision, assuming a sample from a single population, a sample of m fish (specimens) is used to calculate an estimate, $\hat{p} = n_A/2m$, where n_A is the number of alleles having the particular characteristics of interest. Since n_A is a binomially distributed random variable with parameters m and $\hat{p}$ (the frequency of the allele in the population), a $1-\alpha$ confidence interval about $\hat{p}$ can be easily calculated using the Normal distribution as an approximation to the binomial. Table 4 gives the results of such calculations. Tabulated values, w, are the total width of a confidence interval about the estimate of $\hat{p}$, therefore the precision of estimates may be expressed as $\hat{p} \pm \frac{1}{2}w$ for a given level of α and sample size m. Samples used in the following analyses are generally of about 100 fish, therefore $\hat{p}$ has a precision (for single samples) of about $\pm.07$ at the 95% confidence level

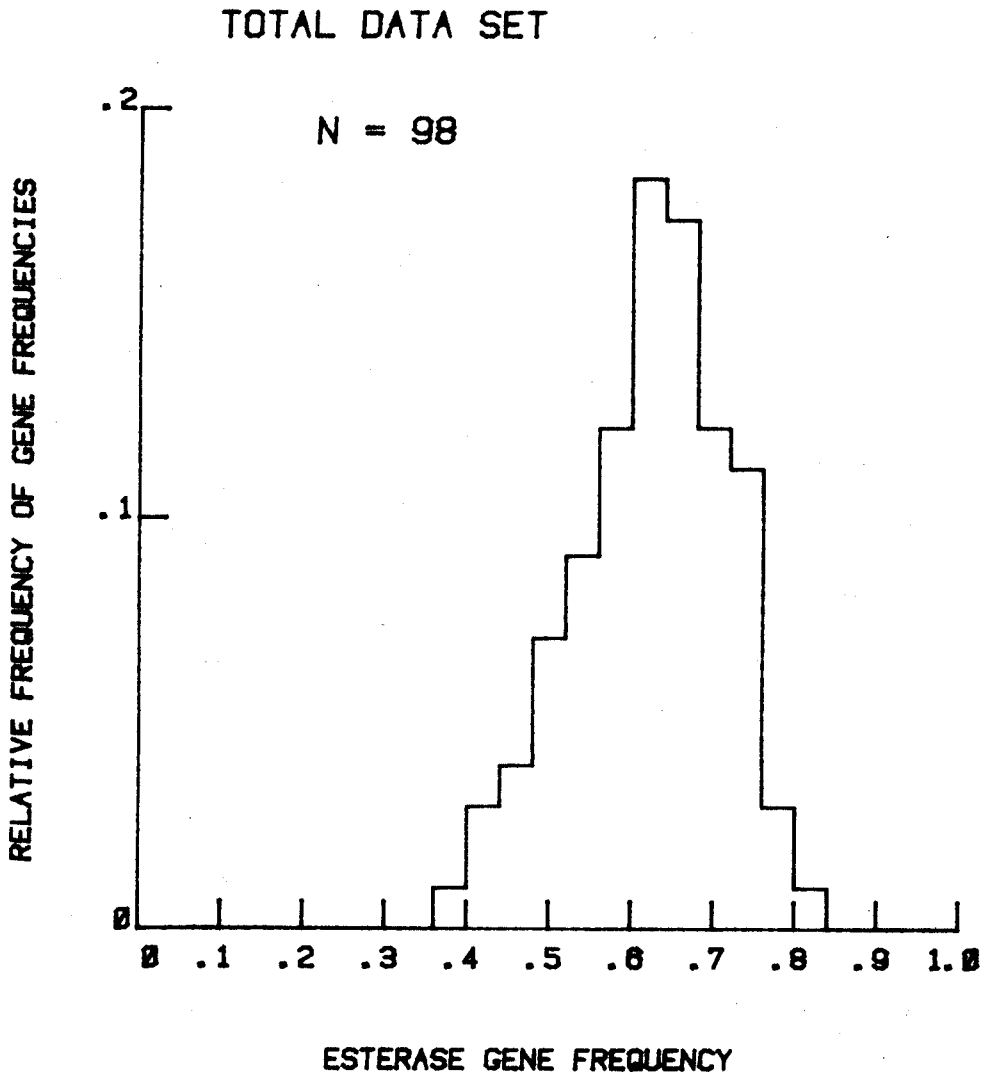

Figure 5. Histogram showing frequency of occurrence of observed gene frequencies for the 98 samples. Step size, $\Delta\hat{p}$, is .04.

TABLE 4. TABLE OF 1-α SYMMETRIC CONFIDENCE INTERVAL WIDTHS ABOUT $\hat{p}$ FOR VARIOUS α LEVELS, SAMPLE SIZES (m) AND POPULATION GENE FREQUENCIES (p).

α	p	m					
		10	20	40	80	100	160
1%	.5	.576	.407	.288	.204	.182	.144
	.75	.499	.349	.250	.176	.158	.125
5%	.5	.438	.310	.219	.155	.139	.110
	.75	.380	.269	.190	.134	.120	.095
10%	.5	.368	.261	.184	.130	.117	.092
	.75	.319	.226	.159	.112	.100	.080

For groups of samples each having about 100 fish, the precision can be calculated by dividing .07 by the square root of the number of samples. Thus a group of 28 samples of 100 fish each would estimate $\hat{p}$ with a precision of $\frac{1}{2}w \simeq .07/\sqrt{28} = .013$.

When samples are not taken from a single homogeneous population, the number of samples necessary to detect this fact is determined by $(1-\beta)$, the power of the statistical test being used to test the null hypothesis. Conversely, if a given power is required, say 90%, then the sample size can be determined using simulation techniques to numerically calculate the distribution of the test statistic. For this simulation, samples are repeatedly taken from two populations with a given difference, Δp, between their allele frequencies and the G-statistic, described above, is calculated. The critical value is taken from the distribution of G opposite the $1-\alpha$ cumulative percentage point when $\Delta p = 0$. Then the value of β can be read from the cumulative distribution of G opposite the critical value determined above and $1-\beta$ can be calculated. This procedure was followed repeatedly for different values of Δp, and different numbers of samples, n. The results are given in Figure 6. In each case the value of p for $\Delta p = 0$ was .5. When Δp was greater than zero, half of the n samples were assumed to be taken from each of two populations with p values symmetric about p = .5. For example, if $\Delta p = .10$ the two populations had gene frequencies of $p_1 = .45$ and $p_2 = .55$. One thousand simulations were used for each data point plotted.

The scatter in Figure 6 represents normal statistical variation in the procedure. This variability causes some difficulty in determination of the appropriate value of n, but an approximate value of n = 25 seems an appropriate minimum to distinguish two populations having a Δp greater than or equal to .1, with a power of 90% certainty.

7.0 RESULTS FROM TAG ANALYSIS

The regression analysis showed a gradual but significant decrease in esterase gene frequency, $\hat{p}$, from west to east across the study area. One interpretation of this trend is that it represents some form of longitudinal population structuring (see Section 8.0). It was on this basis (also considering tagging location) that tag recoveries from blood schools were analyzed. For instance, skipjack from Papua New Guinea schools with $\hat{p}$ of ≥ 0.65, might be expected to be recovered west of an area bounded longitudinally by, say 170°E longitude; and schools with similar $\hat{p}$ values but found in, for example, Wallis and Futuna Islands, should produce tag recoveries only as far eastwards as 160°W longitude; and finally schools with $\hat{p}$ of ≤ 0.55, and from Wallis and Futuna Islands, should only produce tag recoveries to the east of 170°E longitude and perhaps as far east as French Polynesia. The above reference longitudes are those that bound geographical Areas A, B and C, first described in Table 2, Section 4.3 and illustrated in Figure 7.

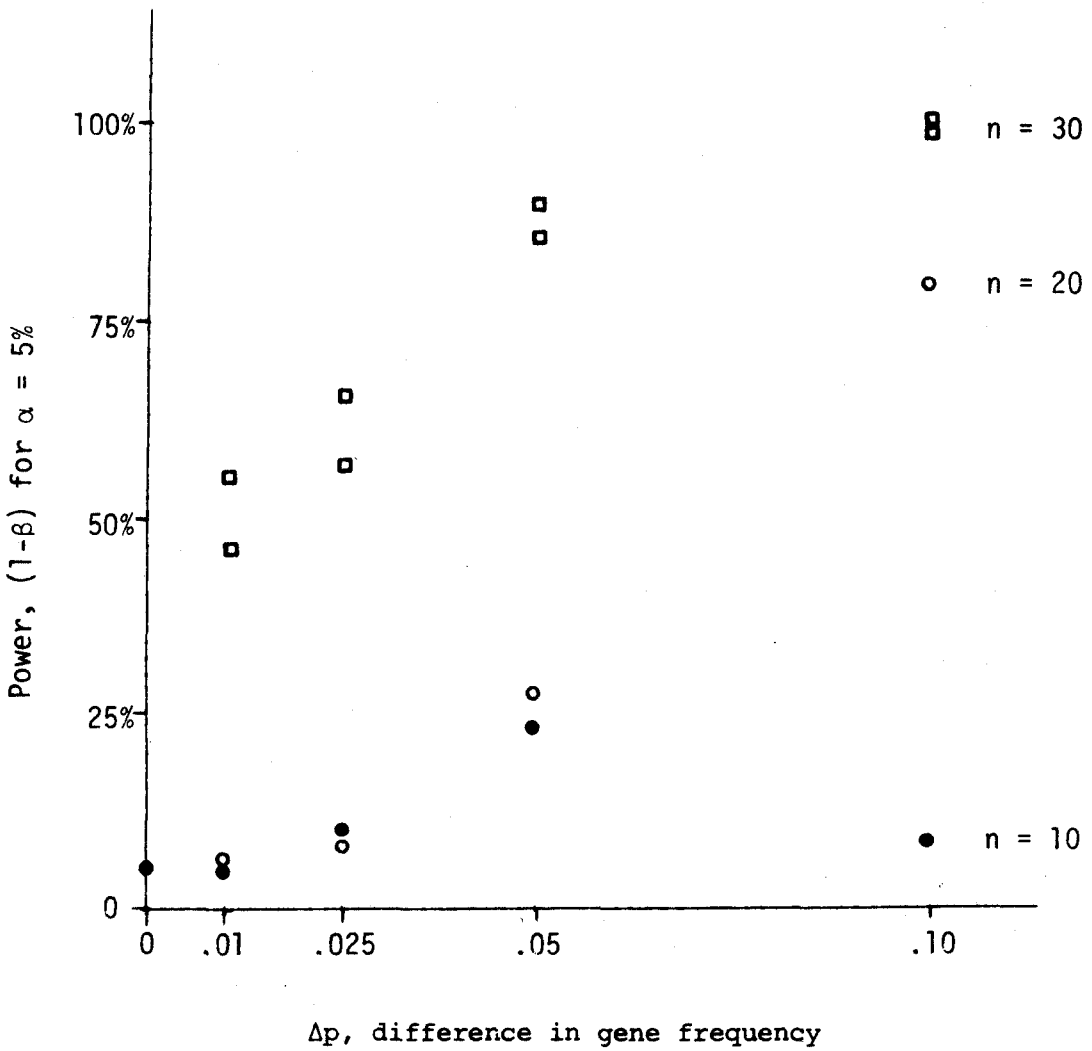

Figure 6. Power function for distinguishing between two populations with Δp difference in gene frequencies. Based on p values symmetrically spaced about p = .5; n refers to total number of samples of 100 animals each with ½n coming from each of the two populations. Each sample is assumed to contain animals from only one of the two populations.

Analysis of tag recoveries from blood schools proceeded by predicting in which of the above three areas tagged skipjack would be recovered. Table 5 presents results for 106 tag recoveries from fish that were at large for at least 90 days; Figure 7 maps those recoveries that moved more than 60 nautical miles.

Only one skipjack, from school M in Kiribati, was recovered in an area other than the predicted area. Some fish from schools with high gene frequencies moved towards the 170°E longitude boundary but did not cross it, for example see the Truk school (P) in Figure 7. Fish from the New Caledonia (B) and New Hebrides (D) schools moved well to the west of 170°E longitude, as expected from their high gene frequencies. Unfortunately skipjack from schools in the eastern region, Area C, have not been at large for enough time to result in useful tag recoveries. It was encouraging, however, that only two of the 106 tag recoveries crossed 170°E longitude, and one of these recoveries from school M in Kiribati appeared to have moved just 3° to the west. Within Areas A and B, tag recoveries suggest considerable intermingling of skipjack, particularly in a north-south direction.

Examination of approximately 200 long distance skipjack tag recoveries from Skipjack Programme tagging showed that only a modest number of these fish crossed 170°E longitude, and the vast majority of the crossings were localized between 0° and 10°N latitude. Further analysis of tag recoveries will consider tagging and recovery seasons, as well as tag recovery effort (fishing effort).

8.0 DISCUSSION

Analyses in this document are admittedly complex, because they are in large measure of a statistical nature. However, blood genetics of skipjack is a complex, technical subject, requiring such treatment in order to develop meaningful results for application to fisheries management. In this case, we are confident that the combination of tagging data and genetic analysis of blood samples will enable us to greatly improve our understanding of the interaction between fisheries in the Skipjack Programme area, to the benefit of the countries and territories that are concerned with the skipjack resource.

The results of the statistical tests of heterogeneity suggest that only the small sample groups from Wallis (BR WAL, n = 5), Fiji (BR FIJ, n = 4) and French Polynesia (BR ARC, n = 6) could each have come from a single population. In all of the others, the statistical tests are inconsistent with the hypothesis that all of the samples were drawn from the same population; notably the sets of samples from Area "A" and Area "B" show distinctly more heterogeneity than can be statistically accounted for by a hypothesis of a single population in each of these areas.

The analysis using Smith's H-statistic showed that it is possible to have an apparent agreement with the phenotype distribution expected under Hardy-Weinberg equilibrium, even when sampling from a heterogenous population (e.g. Area "A" samples).

The regression analysis result is more revealing in that a gradual but continual cline of esterase gene frequencies is clearly observable across the western and central Pacific Ocean. These results together are not inconsistent with a stock picture consisting of a number of geographically localized subpopulations whose individuals have a high probability of breeding only with each other, and a low but not insignificant probability of breeding with neighbouring subpopulations. At this time if lines are to be drawn between genetically isolated subpopulations, they would have to be drawn in arbitrary places.

TABLE 5 - NUMBERS OF SKIPJACK TAG RECOVERIES FROM SCHOOLS WHICH WERE SAMPLED FOR BLOOD GENETIC ANALYSIS

SCHOOL CODE	GENE FREQUENCY $\hat{p}$	TAGGING AREA	OBSERVED RECOVERY AREA			EXPECTED RECOVERY AREA
			A	B	C	
B	.61	A	3	0	0	A
D	.75	A	2	0	0	A
E	.55	B	0	3	0	BC
F	.60	B	0	4	0	AB
W3	.50	B	0	5	0	BC
W1	.50	B	0	2	0	BC
W2	.51	B	0	2	0	BC
W4	.53	B	0	12	0	BC
H	.62	B	0	6	0	AB
R	.68	A	3	0	0	A
K	.56	B	0	1	0	AB
L	.65	B	0	2	0	AB
M	.53	B	1	35	0	BC
P	.62	A	22	0	0	A
J	.60	B	1	0	0	AB
AH	.57	A	1	0	0	AB
TAG TOTALS			33	73	0	106

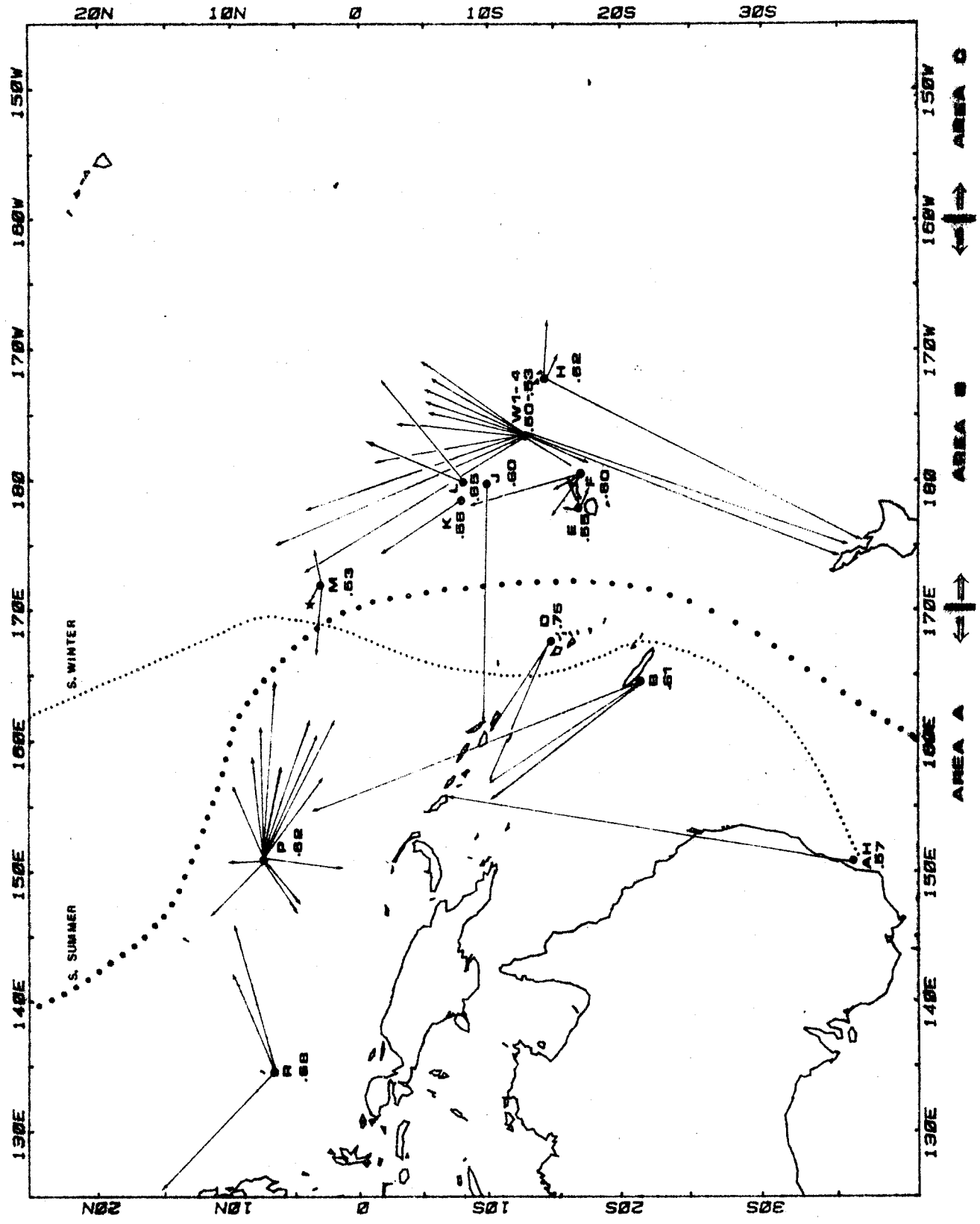

Figure 7. Tag recoveries from blood sample schools. School codes and $\hat{p}$ indicated. The two curved lines show southern summer and southern winter boundaries proposed by Fujino (1970b) for the skipjack tuna subpopulation in the western Pacific Ocean. The star at the end of the tag recovery line for school M indicates that 33 recoveries were made at or nearby this location.

The tagging data suggest that skipjack intermingling is greater within the bounds of 140°E and 170°E longitude, and 170°E and 160°W longitude, than between the two areas. This implies some form of partial isolation, such as Fujino (1970b) proposed with his definition of a skipjack tuna sub-population in the western Pacific Ocean (see Figure 7). Further tag returns, supplemented with specific blood sample collections, may clarify this situation; however, for the time being both tagging and blood sampling results imply that there is little short-term mixing of skipjack between longitudinal extremes in the study area.

In the future there is a need to consider seasonal and annual variability in gene frequencies. In doing so, it is important to remember that in the Pacific as a whole, north-south variations in gene frequency need to be considered.

There was only limited discussion of other polymorphic loci (eg. 6-PGD, GPI, ADA, GDA), since the larger sample sizes needed for proper evaluation were not generally obtainable, and preliminary indications are that geographical variation is similar to that found for transferrin, and therefore not useful for our purposes. There is only a slight possibility that useful new genetic systems will be discovered for skipjack in the near future.

The analyses did not take into account size-related (or life-history related) effects, although it was recognized that these could be important. There is some evidence that skipjack mobility changes with age, for example pre-spawners and pre-recruits may be more mobile, hence more widely distributed than spawners. Thus it is possible that population genetics based on adults and spawners may not be as representative as that for sub-adults. Genetic analysis of eggs and larvae, for comparison with genetic analysis of adult blood samples from the same areas and times, would be useful, but practical difficulties are obviously considerable.

9.0 RECOMMENDATIONS

(a) The blood sampling data collected to date has been useful in an empirical way; however, further opportunistic sampling is not likely to significantly improve the analyses.

(b) An important question for management is whether the suggested relationship between esterase gene frequency and longitude represents a cline, or a series of "steps" (partial isolation). Some further useful information on the above would result if certain geographical gaps in sampling were filled. Specifically, samples from the following areas are suggested:

(i) Santa Cruz (Fujino line)
(ii) 5°N Micronesia (Equatorial Counter Current)
(iii) Marshall Islands (Fujino line)
(iv) Cook Islands (only one sample to date)

(c) Sequential sampling of fisheries in Fiji and French Polynesia would clarify whether schools with esterase gene frequencies near .4, typical of the eastern Pacific, and schools with gene frequencies near .5, typical of Fiji area, moved between the two locations.

(d) Results of the genetic studies should be fully written up so that unanswered lines of research may be pursued by appropriate institutions.

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APPENDIX 1

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