REPORT OF THE SECOND SKIPJACK SURVEY AND ASSESSMENT PROGRAMME WORKSHOP TO REVIEW RESULTS FROM GENETIC ANALYSIS OF SKIPJACK BLOOD SAMPLES

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PREFACE

The Skipjack Survey and Assessment Programme is an externally funded part of the work programme of the South Pacific Commission. Governments which have provided funding for the Programme are Australia, France, Japan, New Zealand, United Kingdom and the United States of America.

The staff of the Programme at the time of preparation of this report comprised the Programme Co-ordinator, R.E. Kearney, Research Scientists, P.M. Kleiber, A.W. Argue, C.P. Ellway, R.D. Gillett, J.P. Hallier, T.A. Lawson and C.A. Maynard; Research Assistants, Susan Van Lopik, Veronica van Kouwen and Louise El Kik; and Programme Secretary, Carol Moulin.

This report of the second Skipjack Programme Workshop to review results from genetic analysis of skipjack blood samples follows closely from daily meeting summaries prepared by the workshop rapporteurs. It has been compiled and edited by a senior member of the Skipjack Programme, A.W. Argue, who was also a workshop participant. Preparation of the report was considerably assisted by the editorial comments of all workshop participants.

The report does not rigidly adhere to the usual format for a workshop report in that to give full coverage to different points of view, certain data and analytical results are included. The report is generally structured to present these different opinions on analysis and interpretation of the data.

Skipjack Programme
South Pacific Commission

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REPORT OF THE SECOND SKIPJACK SURVEY AND ASSESSMENT PROGRAMME WORKSHOP TO REVIEW RESULTS FROM GENETIC ANALYSIS OF SKIPJACK BLOOD SAMPLES

1.0 INTRODUCTION

The Skipjack Survey and Assessment Programme has collected a sizeable data base from which to assess skipjack population structuring and the impact of population structuring on fisheries for skipjack in the region. These data, collected between October 1977 and August 1980 over a wide area of the central and western Pacific Ocean (Figure 1), include electrophoretic analyses of skipjack blood samples from 58 separate schools and results from the release of more than 140,000 tagged skipjack, 32,000 of these in schools from which blood samples were taken.

A workshop to review preliminary results from the Skipjack Programme's first two years' collection of skipjack blood samples was held in July 1979 (Anon, 1980). Subsequently, the Programme completed its third and final survey year, which included further limited collections of skipjack blood samples. Once samples from the third cruise had been analyzed by the Department of Population Biology, Research School of Biological Sciences, Australian National University (ANU), a second workshop was convened in Noumea (29 September to 3 October 1980). This report summarizes deliberations from the 1980 workshop.

The goal of this workshop was to establish to what extent interpretation of the available blood genetic data could be used in formulating fisheries policy for exploitation of skipjack resources in the South Pacific Commission (SPC) area (Figure 2). The workshop addressed the general question of whether there was evidence of population structuring in the resource that could affect yield from the resource, either overall or in small areas, now or in the future. In particular, the workshop considered to what extent the available genetics data could be used in determining degree, timing and geographical extent of skipjack intermingling.

Since the analysis and interpretation of tuna blood genetics data is a complex technical subject, experts in the fields of population genetics and fishery population biology met with the Programme's scientists to jointly consider the data, statistical analyses and resulting preliminary interpretations. Participants at this second workshop included two population geneticists, one from the New Zealand National Research Advisory Council and one from the Department of Animal Science, University of New England, Australia, as well as all participants from the first workshop (see Appendix A).

It was planned that workshop participants would review results from as complete a set of Pacific Ocean skipjack blood samples as possible. Participants from the Inter-American Tropical Tuna Commission (IATTC) and ANU kindly provided additional unpublished data and analyses for consideration. Unfortunately, some data were not in a form amenable to detailed consideration at the meeting, and this included a large set of published and unpublished Japanese data for the central and western Pacific.

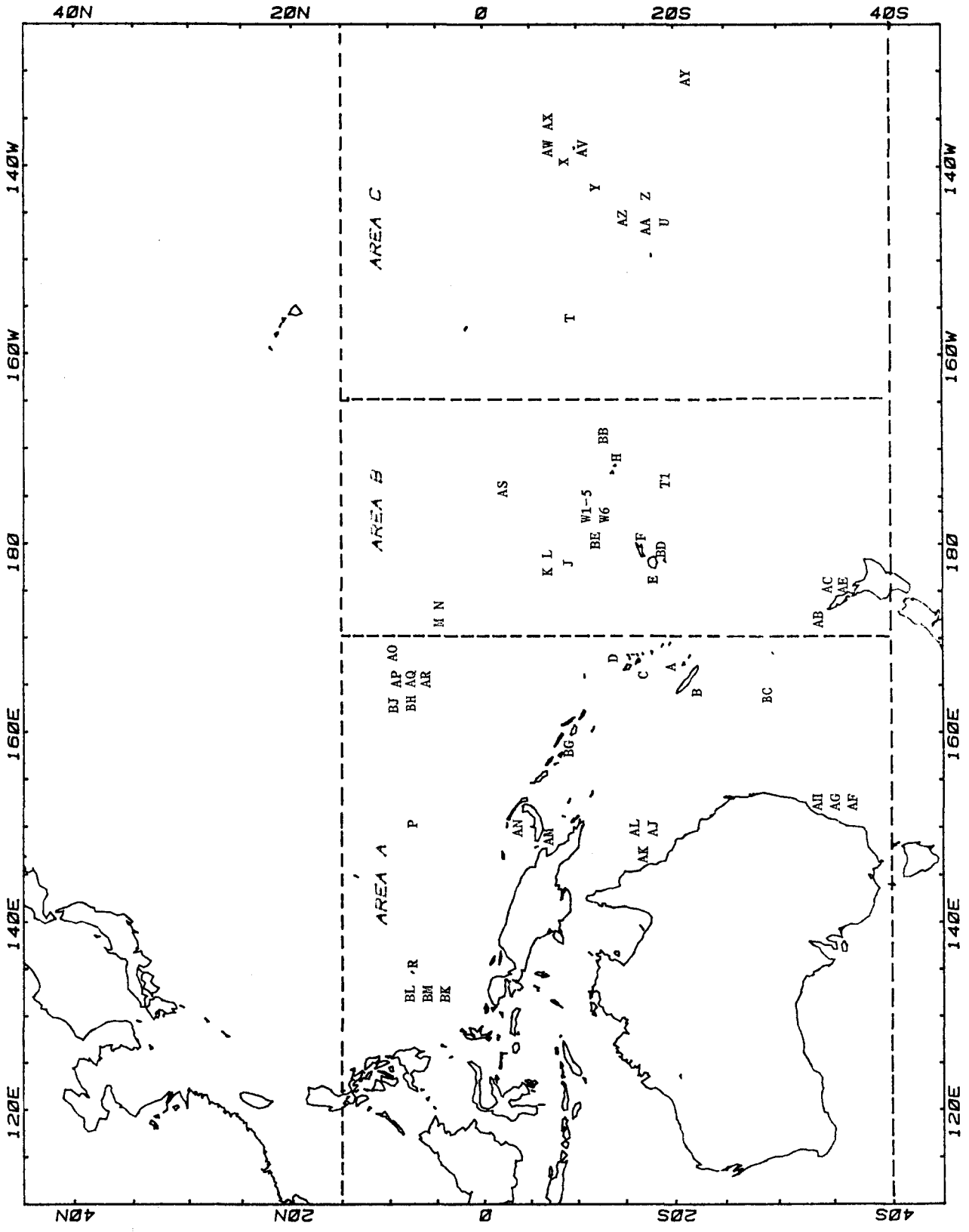


Figure 1 - Collection localities for SPC blood sample schools. School codes are indicated (see Appendix B). The dashed lines represent boundaries for Areas A, B and C (see text, section 5.2)

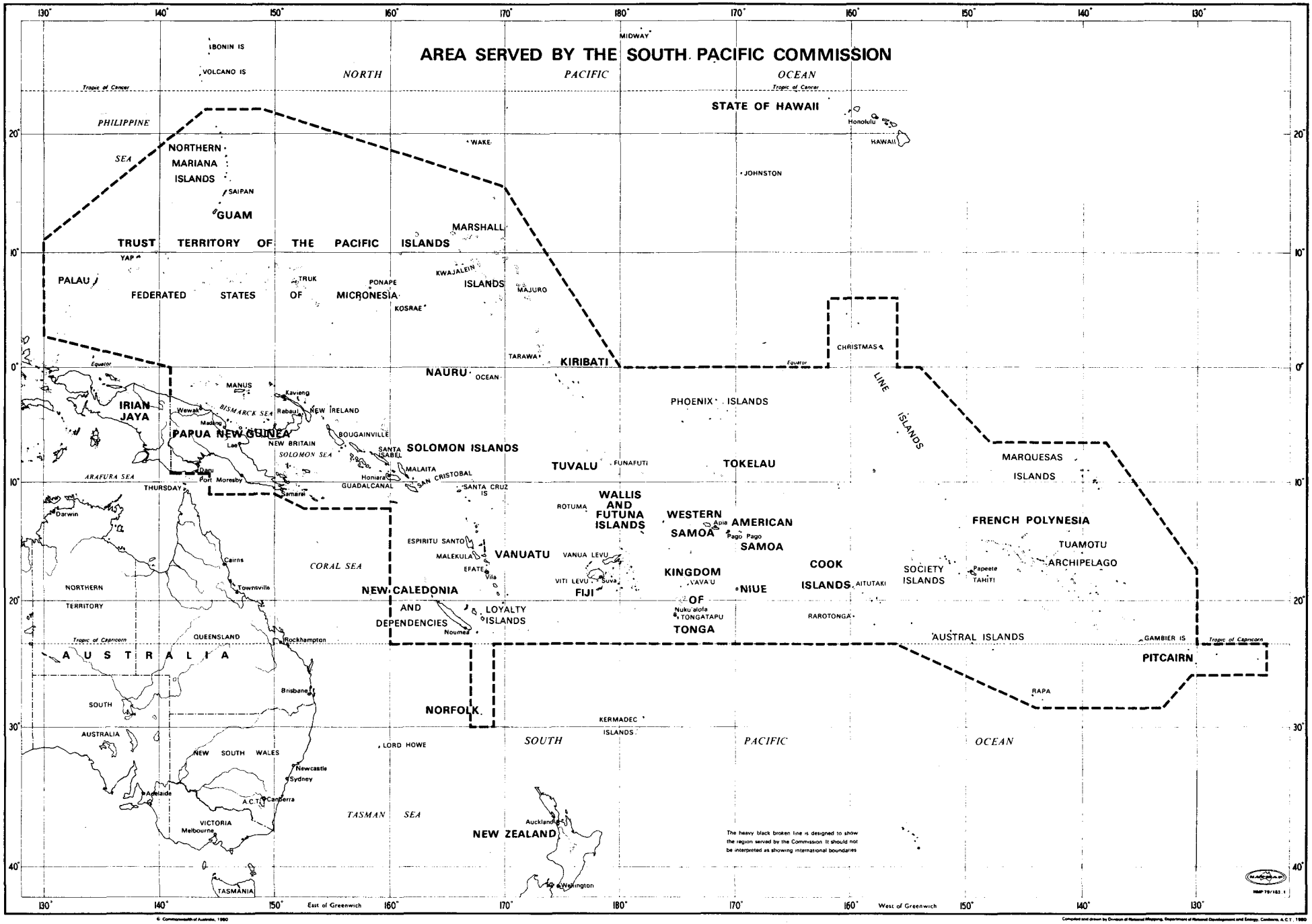


Figure 2 - Area of the South Pacific Commission

In contrast to the previous workshop, this year's was primarily one of interpretation of analyses prepared before the meeting, in concert with various relevant publications. Methods used for most of these analyses are documented in the report of the first workshop (Anon, 1980). This was followed by definition and discussion of population models which accommodate the data at hand, and discussion of possible management implications consonant with each model. While unanimous agreement on interpretation of the blood genetics data was considered desirable, it was recognised that the meeting might generate several interpretations. Whenever possible, the participants highlighted key tests for discriminating between alternate population hypotheses. Workshop conclusions and research recommendations end the report.

The structure of this report is basically a narrative exposition of the meeting's discussions, divided into sections that are consistent with the workshop agenda, although in some cases the order was changed to improve presentation. As such, this paper draws heavily on the daily reports of the meeting prepared by the rapporteurs.

2.0 DISCUSSION OF TERMINOLOGY

After a free-ranging discussion, it was agreed that there was little need to establish explicit definitions of population biology or genetics terms. However, the term "population" - all individuals of a species which inhabit a specified region (e.g. all Pacific Ocean skipjack), and the term "stock" - the exploitable group of fish existing in a particular area at a particular time, as defined by the December 1976 Ad Hoc Meeting of Scientists to Discuss Skipjack Fisheries Developments and Research Requirements (Anon, 1976), were accepted as working definitions.

It was pointed out that fisheries population biologists use terms such as "subpopulation" in a different context from geneticists. To a fisheries person a subpopulation is often defined spatially, temporally, and in terms of its basic demographic processes (natality, mortality, emigration, immigration, and growth), without the constraint that it be a self-sustaining genetic unit, which implies a high degree of biological uniformity. Such a broad definition allows those in fisheries to cope with uncertainties such as reproductive structuring, emigration and immigration. The workshop agreed that the term "subgroup" would best be substituted for "subpopulation" and would be used in its common heuristic sense throughout the workshop unless a more precise definition was required.

3.0 IMPLICIT ASSUMPTIONS UNDERLYING USE OF BIOCHEMICAL ENZYME SYSTEMS AS GENETIC MARKERS

3.1 Simple Mendelian Segregation of Co-dominant Autosomal Alleles

From the available SPC, IATTC and ANU data the second workshop considered results for three polymorphic loci in detail: serum napthyl esterase (EST), serum transferrin (TF), and erythrocyte guanine deaminase (GDA). It was recognized that the interpretation of the phenotype patterns for these three loci as representing the Mendelian segregation of co-dominant autosomal alleles was made without reference to a breeding experiment. Nonetheless, it was reasonable to assume on the basis of experience and the

implicit assumption is that the polymorphs represent co-dominant alleles (cannot test this)

simple electrophoretic patterns involved that this interpretation could be accepted as a working hypothesis. It was recognized, however, that there may be multi-locus or hidden allele effects concealed in the electrophoretic patterns. Although this was not tested, there was no evidence of such effects in the available data. As well, it was noted that in some fish species occurrence of transferrin phenotypes appeared to be correlated with presence of certain diseases.

3.2 Storage Effects

It was recognized that two of the loci involved, GDA and EST, were sensitive to storage deterioration that could, under some conditions, result in degenerate electrophoretic patterns. However, for the data presented here, sufficient laboratory controls (retyping of random samples) were maintained to detect such anomalies, and anomalies that would significantly alter interpretation of electrophoretic patterns were not detected.

3.3 Post-translational Modification

Systematic, or genetically programmed, post-translational modification of proteins, as an explanation for observed variation, has not been described in the literature for these three enzymes, nor was there any evidence of this from the electrophoretic patterns observed in the analysis of Skipjack Programme samples.

3.4 Environmental Effects

Environmental effects that cause switching on or off of gene expression for these three loci is a theoretical possibility. This possibility has not been tested; however, there was no evidence available from the very large number of electrophoresis "runs" studied that would indicate that this environmental effect had occurred.

Environmental effects that cause changes in specific activity (staining intensity) of these three loci may occasionally occur. In particular, a second locus, for serum α-napthyl esterase (B.J. Richardson, unpublished data), appears to be sensitive to such effects resulting in low staining intensities and consequent ambiguities in the interpretation of heterozygote genotypes. Environmental effects were not considered to significantly bias the results examined at the meeting.

In summary, the three loci for EST, TF and GDA were, in the absence of verification by breeding and other experiments, conditionally accepted as representing the Mendelian segregation of co-dominant autosomal alleles.

4.0 BLOOD GENETIC DATA AVAILABLE TO THE WORKSHOP

Table 1 summarizes the source, number and general collection locations for all blood samples that were available to the workshop participants. A portion of these data were from uncompleted theses and manuscripts. Data supplied by the IATTC from the northern and southern fishing areas of the eastern tropical Pacific Ocean, and all the Japanese data, were not in a form that allowed ready analysis of phenotype frequencies. EST and TF gene frequencies were provided separately for the IATTC samples. Data for the GDA

TABLE 1

Blood Sample Data Available at the Second Skipjack Blood Genetics Workshop

SOURCE	NUMBER OF SAMPLES	GENERAL SAMPLING LOCATION
South Pacific Commission	18	Western Pacific
South Pacific Commission	30	Central Pacific
South Pacific Commission	7	East Coast of Australia
South Pacific Commission	3	New Zealand
Australian National University	17	East Coast of Australia
Australian National University	2	West Coast of Australia
Australian National University	7	Indonesia
Australian National University	105	Western Pacific
Inter-American Tropical Tuna Commission	18	Eastern Pacific
Inter-American Tropical Tuna Commission	15	Western Pacific
Inter-American Tropical Tuna Commission	12	New Zealand
Data collected by Japanese scientists and sent to the SPC, by the IATTC, just prior to the second Workshop	270	Western Pacific, Central Pacific, Hawaii
TOTAL	504	

locus were only available for 43 samples, 38 of which were SPC samples.

Appendix B presents complete collection details, gene frequencies and summarizes biological and tagging data for each of the 58 SPC samples. Each SPC sample consisted of approximately 100 specimens from the dominant size class in a single skipjack school. ANU and IATTC samples, usually of 100 to 200 specimens, were also collected from a single size class in a single skipjack school.

Forty-two loci have been surveyed from SPC samples for electrophoretically detectable polymorphisms. Twenty-seven loci were monomorphic, nine showed variation that could be ascribed to post-transcriptional changes, three showed low-frequency heritable variation, and only three (EST,TF,GDA) showed polymorphisms that were suitable for traditional population analysis (Richardson, unpublished MS).

5.0 REVIEW OF RESULTS FROM STATISTICAL ANALYSES

5.1 Gene Frequency Versus Longitude

Figures 3 and 4 present, respectively, EST and TF gene frequencies plotted against longitude at which the samples were taken. Samples from Japan, the west coast of Australia, Sumatra, Java, and Ambon in the waters of Indonesia, and some of the sequential samples taken at similar locations in Papua New Guinea have not been included in these Figures (some Papua New Guinea samples were not available in time to be used for the second workshop). Figure 5 presents the same relationship for GDA gene frequencies. Gene frequencies for EST, TSF and GDA represent, respectively, frequencies for the E^{1}_{SJ}, Tf^{2}_{SJ}, and GDA_{1} alleles.

The regression slope for the EST-longitude relationship was significantly different from zero at the 99 percent level; the slope of the GDA-longitude relationship was less than for the EST relationship and was statistically different from zero at the 95 percent level. The slope for the TF gene frequency versus longitude relationship was not significantly different from zero. The esterase regression reduced the sum of squares about the mean esterase gene frequency by 65.8 percent. All regressions exclude samples east of 130°W longitude (dotted line on the graphs).

These relationships provoked considerable discussion. For example, when esterase data from the eastern Pacific Ocean are included (samples to the right of the dotted line in Figure 3), the overall esterase-longitude relationship becomes non-linear. A mean EST gene frequency of approximately 0.45 characterizes eastern Pacific samples and no longitudinal gradient is apparent. From 130°W to the western limit of samples, the esterase gradient does appear linear. Yet if one looks only at subsets of EST gene frequencies, for example between 130°E and 170°E, and between 170°E and 170°W, one could say that within each of these bounded areas the regression is not significant, but the average values for these subsets differ considerably. The gradient in EST gene frequency may flatten out to the west of 130°E; this should be verified by obtaining more samples from this area.

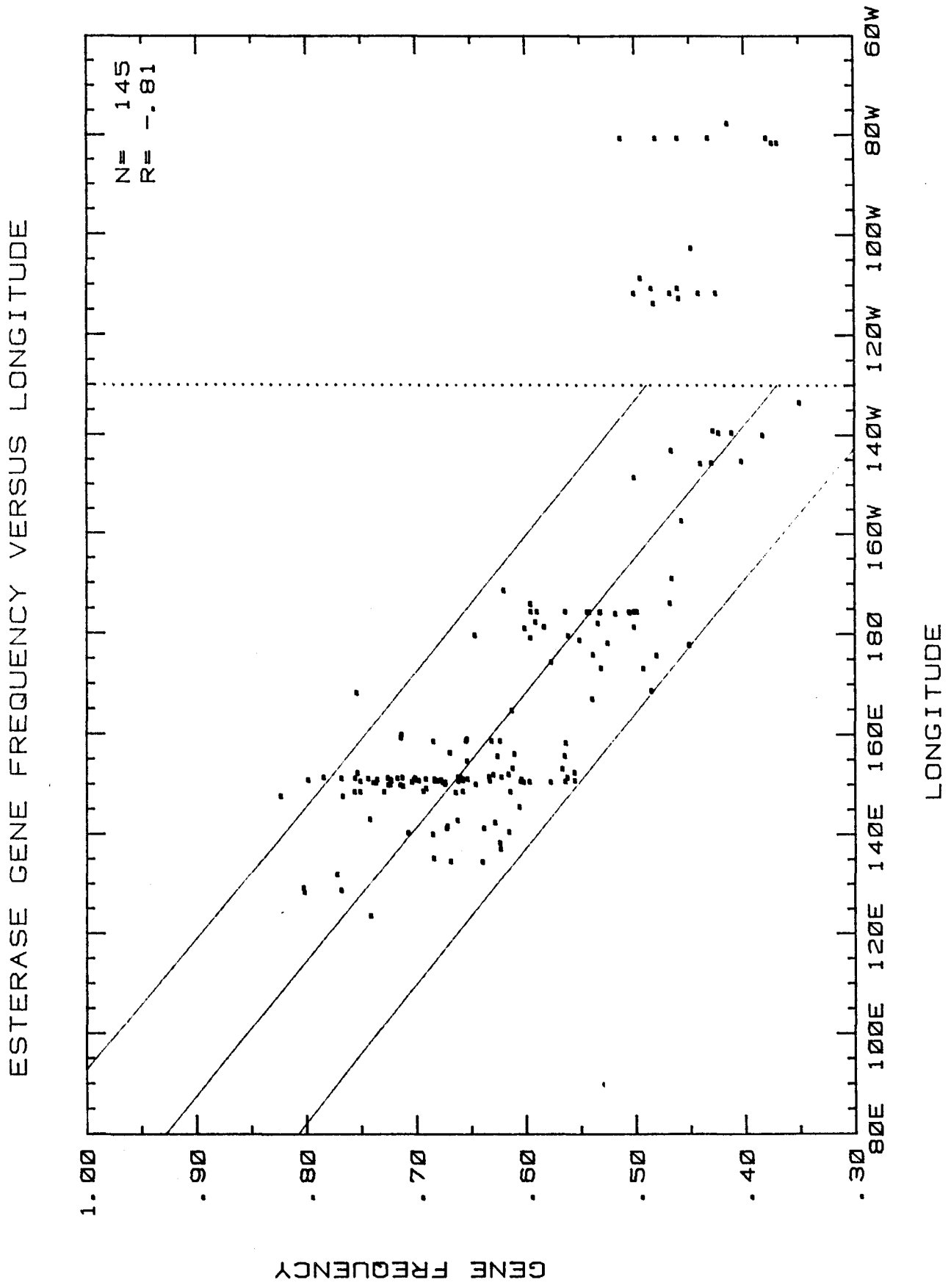


Figure 3 - Graph of esterase sample gene frequency plotted against longitude where the sample was taken. Regression line and 95 percent prediction limits indicated on the graph. The regression excludes samples from east of the SPC area (east of dotted line). N is the number of samples used for the regression and R is the correlation coefficient. Gene frequencies from a total of 163 samples are plotted.

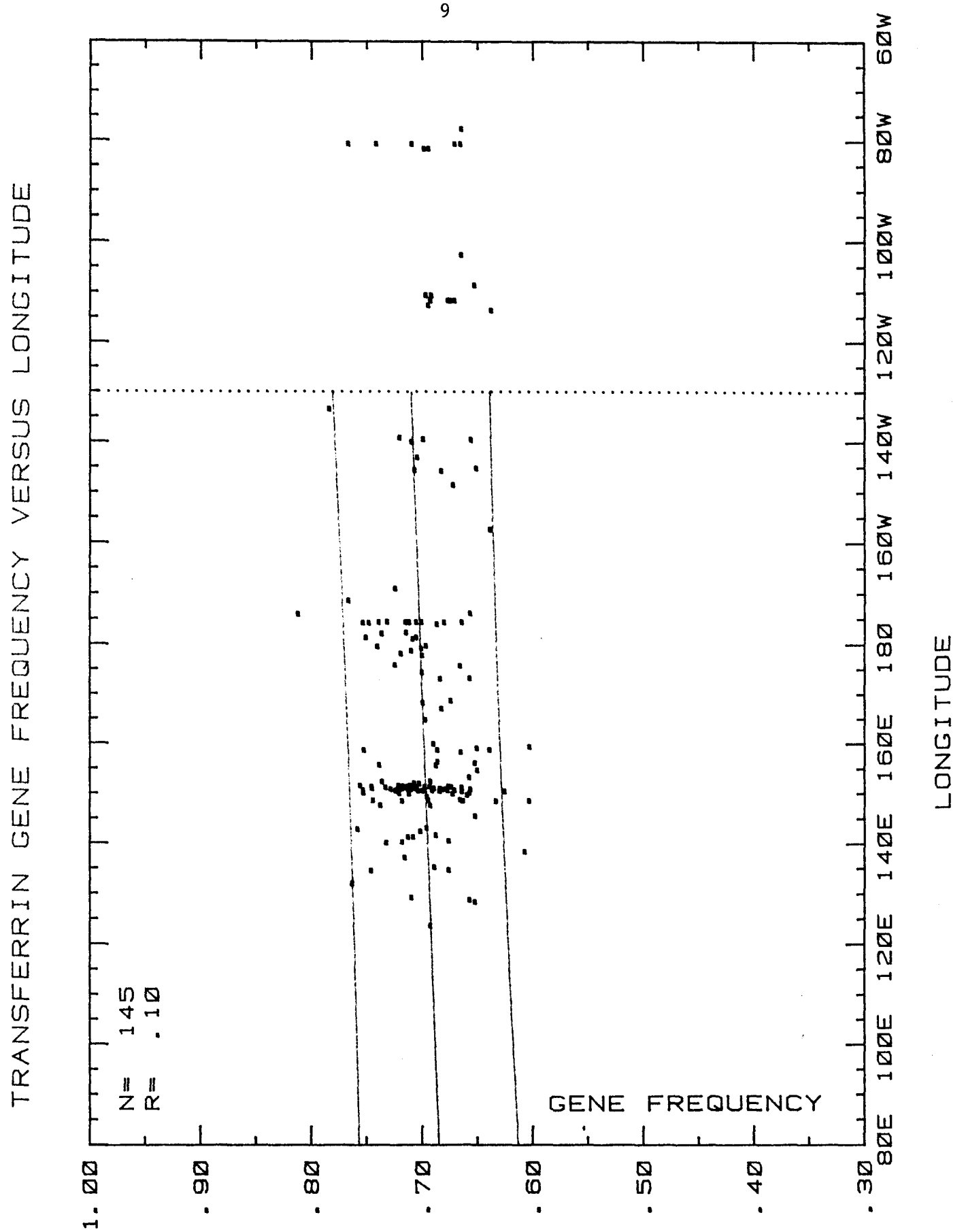


Figure 4 - Graph of transferrin sample gene frequency plotted against longitude where the sample was taken. Regression line and 95 percent prediction limits indicated on the graph. The regression excludes samples from east of the SPC area (east of dotted line). N is the number of samples used for the regression and R is the correlation coefficient. Gene frequencies from a total of 163 samples are plotted.

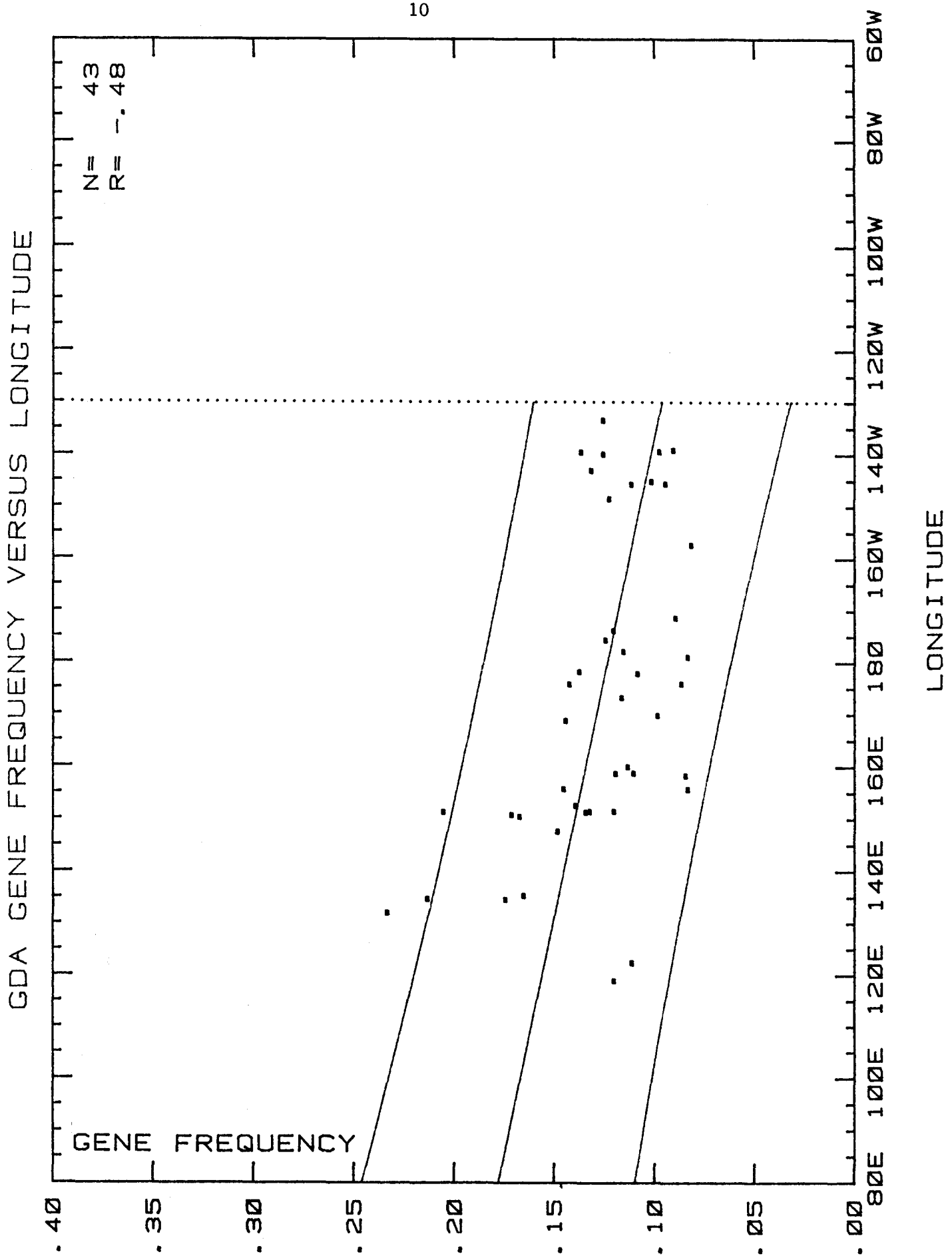


Figure 5 - Graph of guanine deaminase sample gene frequency plotted against longitude where the sample was taken. Regression line and 95 percent prediction limits indicated on the graph. The regression excludes samples from east of the SPC area (east of dotted line). N is the number of samples used for the regression and R is the correlation coefficient. Gene frequencies from a total of 43 samples are plotted.

It was agreed that between 130°E and 130°W the esterase gene frequencies suggested a clinal relationship, and that variance about this cline was not uniform with longitude and appeared to decrease from west to east.

Esterase gene frequency was plotted against other variables such as month of collection, average fork length of blood sample schools, and sea surface temperature at time of blood sample collection. Most of these plots used SPC samples only. These individual plots showed no obvious relationships. There was some concern that adult skipjack in SPC samples, particularly those from French Polynesia, might not be representative of the size composition of adult skipjack present in the area, i.e. that large skipjack might be under-represented in SPC samples. It was noted that further multivariate analysis of the apparent cline in EST gene frequency could prove useful.

Analysis of esterase gene frequency versus longitude for north and south latitudinal groupings (and for different EST gene frequency classes: 0.35-0.44, 0.45-0.54, 0.55-0.64, 0.65-0.75) showed a slight tendency towards higher gene frequencies northwards at longitudes west of 130°W longitude (Table 2). However, in a multiple correlation between EST gene frequency, longitude and latitude at which the samples were collected, the addition of latitude only slightly increased the R^2 value, from .658 to .660. Thus there appears to be little latitudinal spread in gene frequency for the EST data set considered by the workshop.

5.2 Statistical Analyses for Heterogeneity and Hardy-Weinberg Equilibrium

In the previous workshop, the study area had been divided into three Areas, A,B,C, as shown in Figure 1. The division between Areas A and B roughly paralleled the line of demarcation between Fujino's proposed western and eastern Pacific skipjack subpopulations (Fujino, 1972 and 1976; see Figure 9 in this report). Esterase phenotype frequencies, and gene frequencies, for each sample (excluding eastern Pacific samples) were analyzed for each of these areas and modifications of these areas (e.g. Areas A and B combined, Area B less New Zealand samples, Wallis samples alone, etc.). G-statistics and Smith's H-statistics were calculated for each of 16 area groupings of blood sample data. Results for these groupings were presented in Table 2 of the previous report (Anon 1980). The G-statistics tested whether samples in each of the groups were from a single, (assumed) binomial population, which in one case was assumed to be in Hardy-Weinberg equilibrium, and in a second case this assumption was dropped. The Smith's H-statistic is similar: it measures whether the numbers of phenotypes in a sample are in the ratios one would expect from simple Mendelian assortment of alleles (i.e. in Hardy-Weinberg equilibrium). For groups of samples, the Smith's H-statistic was calculated under the null hypothesis that each group of samples was from a single genetic subgroup under which circumstances Smith's H would not differ significantly from zero. An explanation of a statistically significant positive value for Smith's H (i.e. the Wahlund effect) is that the group of samples represents a mixture of genetic subgroups, each with different gene frequencies. Of course, other genetic mechanisms such as inbreeding and asymmetrical selection could give rise to positive Smith's H values. There is considerable discussion of the above statistics in the report of the previous workshop (Anon, 1980); however, there was concern at this workshop that results from these tests can and have been misinterpreted, which in part relates to their

TABLE 2

Average Esterase gene frequency (p) for ten degree latitude/longitude cells. Data are from the esterase gene frequency versus longitude regression, excluding samples east of 130°00'W longitude. n is the number of samples.

LONGITUDE / LATITUDE		120°01'E to 130°00'E	130°01'E to 140°00'E	140°01'E to 150°00'E	150°01'E to 160°00'E	160°01'E to 170°00'E	170°01'E to 180°00'	180°01'W to 170°00'W	170°01'W to 160°00'W	160°01'W to 150°00'W	150°01'W to 140°00'W	140°01'W to 130°00'W
10°00'N to 0°	p	.7407	.6693	.6910	.6112		.5559					
	n	1	8	11	6		3					
0°01'S to 10°00'S	p	.7900		.6485	.6798		.5602	.4672		.4568	.3824	.4166
	n	3		4	49		1	1		1	1	2
10°01'S to 20°00'S	p		.6134	.7179	.6895	.7534	.5451	.5525	.4608		.4473	.4277
	n		1	5	3	1	3	11	1		5	1
20°01'S to 30°00'S	p					.5451						.3491
	n					4						1
30°01'S to 40°00'S	p			.6134	.6247		.5109	.5422				
	n			1	7		4	8				

statistical power, and in part to the several genetic processes that can give rise to similar results.

Prior to the second workshop, SPC scientists prepared an updated set of G-statistics and Smith's H-statistics (Table 3), for the same groupings presented in Anon (1980), but with the addition of 22 new SPC samples (numbers 37-58, Appendix B) assigned to their respective groups based on location of collection.

The workshop also considered a second analysis for a subset of the data from Areas A, B and C (Richardson, unpublished MS), where chi-square statistics were used to measure the statistical significance of differences in gene frequency between samples within a group, and Smith's H-statistics were used to measure deviations from Hardy-Weinberg equilibrium.

Both analyses showed significant esterase gene frequency heterogeneity within various major groupings of blood samples (i.e. Area A, Area B), from which it was inferred that fish of different genetic origins were present in each area. This is consistent with the presence of a cline, or the presence of different genetic subgroups. Both analyses showed heterogeneity in esterase gene frequency to be insignificant in Area C, but this lack of heterogeneity may be an artifact of the small sample size, limited longitudinal range for the samples used, and/or the limited sampling period. Inclusion of eastern Pacific samples in this grouping might well change this result.

In the SPC analysis, Smith's H did not differ significantly from zero for all groups with more than 12 blood samples. The second analysis, using a subset of the data for Area A, showed a significant positive Smith's H value, as would be expected for a mixed population. In the SPC analysis, most groups with more than 12 samples, except the Area B, and Area B plus C groups, had a slight but not statistically significant excess of homozygotes (positive Smith's H). This was noted to be consistent with a stepped cline, that is a situation where there are small groups, somewhat distinct genetically and with slightly different gene frequencies and some measure of interbreeding. The fact that the Smith's H-statistic was significantly positive for the subset of Area A was ascribed to samples in this subset not being truly representative for the area as a whole.

Statistical tests used to detect deviations from Hardy-Weinberg equilibrium are notoriously weak, particularly with respect to detecting small deviations from equilibrium with sample sizes less than 1,000. There was considerable discussion of this problem.

It was noted that there was heterogeneity in the TF gene frequencies in the eastern Pacific, and in some, but not all, groupings of samples within the SPC area. Chi-square analyses of the limited numbers of GDA gene frequencies showed significant heterogeneity within Area A.

During the course of discussions on the above analyses, it was often commented that drawing inferences from subsets, or incomplete sets of data (e.g. excluding eastern Pacific and Japanese samples) was undesirable.

TABLE 3

Updated results of statistical analyses for the 16 blood sample groupings described in Anon (1980), where: p is the frequency of the most common allele of the esyterase enzyme system; N is the number of samples in the group; GHW is the G-statistic calculated from the phenotype ratios, which were assumed to be in Hardy-Weinberg equilibrium; GH is the G-statistic calculated from the gene frequencies; and H is Smith's H-statistic

COLLECTION AREA	FILE NAME	P	N	GHW	GH	H
All areas	BR TOT	.6127	120	1154.2 **	1013.2 **	.0026 NS
Areas A (1)	BR NGS	.6677	76	344.6 **	261.1 **	.0036 NS
Area B	BR BNS	.5441	32	187.9 **	98.5 **	-.0055 NS
Area B, excluding New Zealand samples	BR ARB	.5493	20	72.2 **	40.9 **	.0051 NS
Area C	BR ARC	.4268	11	22.3 NS	10.3 NS	.0039 NS
Areas A and B	BR AAB	.6298	107	782.7 **	658.0 **	.0024 NS
Areas B and C	BR ARW	.5206	43	305.0 **	205.4 **	-.0036 NS
East Coast Australia	BR AUS	.6408	9	37.0 *	30.9 **	-.0031 NS
New Zealand (2)	BR ANZ	.5386	12	115.6 **	57.6 **	-.0167 *
Wallis Island	BR WAL	.5331	6	16.1 NS	5.5 NS	.0027 NS
Fiji	BR FIJ	.5612	5	8.5 NS	3.6 NS	.0238 *
Papua New Guinea and Solomon Islands	BR PNG	.6759	53	249.0 **	182.4 **	.0016 NS
SPC samples for all areas, excluding Australia	ALL SPC	.5449	50	446.6 **	385.4 *	.0052 NS
SPC samples for Area A, excluding Australia	WST SPC	.6445	18	86.1 **	69.2 **	.0101 NS
SPC samples for Area B	CNT SPC	.5412	21	82.6 **	49.7 **	.0022 NS
SPC samples for Area C	EST SPC	.4268	11	22.3 NS	10.3 NS	.0039 NS

(1) See Figure 1 for boundaries to Areas A, B and C.

(2) Results differ from those in Anon (1980), Table 3, for the same number of samples, due to correction of a phenotype frequency error in Anon (1980).

* Significant at $p < 0.05$

** Significant at $p < 0.01$

NS Not statistically signficant

5.3 Numerical Simulation of Dispersal and Selection Effects

Results from simulations with six populations in a linear array, (2,000 animals in each) to examine the effect of varying dispersal rates and selection levels on maintenance of the observed esterase cline (Richardson, unpublished data) were reviewed. Ten samples of 100 fish were taken at random after 50 generations. Results were as follows: (i) given six percent selection, nearly 50 percent dispersal between areas was required to eliminate a cline; (ii) steps in the cline were difficult to induce with selection alone; (iii) similarly, reducing dispersal between particular areas induced little stepping; and (iv) in none of the simulations did the heterogeneity produced (longitudinal variability in esterase gene frequency) match that observed in the genetic data, nor was there any divergence from Hardy-Weinberg equilibrium, as measured by Smith's H. Other combinations (stronger selection at both ends; fish moving as schools; "leakage" or diffusion of fish of a different gene frequency into the population from one end) also did not produce results corresponding with the observed situation. Previous attempts by Richardson to incorporate environmental patchiness were not successful.

The participants noted that a cline could be maintained in the face of quite extensive mixing and that the high levels of within-area heterogeneity still required satisfactory explanation.

The importance of selective forces drew considerable discussion. It was noted that the clinal effect appeared to be specific to an esterase allele and therefore probably had a selective component. The simulations did not permit differentiation between a model based on a number of discrete groups with little mixing, from one incorporating a few groups with considerable mixing.

5.4 Time Period Considerations

In earlier discussions, the participants raised the question of what balance between selection and dispersal might maintain a cline such as observed for the EST locus in skipjack. This led to a consideration of whether there were any examples in the literature of significant between-cohort or within-cohort variation in gene frequency that could be due to environmental selection.

The workshop then considered some New Zealand data for a demersal fish species (i.e. esterase for snapper, Chrysophrys auratus). Smith (1979) suggested that variation in the environment in the year of spawning (in this case water temperature) could lead to brood-year variation in snapper gene frequencies in one area as great as that found between different areas of New Zealand. The point was made that selection of the magnitude necessary to produce this phenomenon in skipjack, could produce significant between-year variation in gene frequency which could mask other sources of variation. There were differences of opinion as to the likelihood of such a process in skipjack populations. It was suggested that such between-cohort variability due to selection, if present in skipjack, would affect the interpretations one would make on area of origin of schools with a specific gene frequency, found in a particular area. It was suggested that the effect of time of year and fish size on gene frequency should be thoroughly checked using time series samples. Most of the data available to the workshop were inadequate for this purpose.

There was a short discussion of some time series data from Kavieng, Papua New Guinea, which is soon to be released (A.D. Lewis, thesis in preparation). These data suggested the same seasonal changes in esterase gene frequency in two consecutive years. Alternative explanations of these data were discussed, in terms of size of recruits to the fishery, varying environmental conditions and immigration and emigration of different groups of fish.

5.5 Measures of Genetic Distance - Kinship Coefficients

Measures of genetic distance, such as kinship coefficients, are interpreted as a measure of relatedness between groups of organisms. This measure was proposed for determination of appropriate geographic management horizons, since it was held that results could be translated into a measure of the geographical range of closely related and presumably interbreeding individuals. The kinship coefficients are estimated from allele frequencies at all variable loci, and the base-line or reference measure is the average coefficient among samples collected at approximately the same place at about the same time. It was noted that sampling effort should be evenly distributed over the geographic range under consideration.

This particular use of kinship coefficients assumes an isolation-by-distance component in the population structuring (see section 6.2). Doubts were expressed as to whether the isolation-by-distance component accounted for a significant proportion of the observable variance in gene frequency at different longitudes.

With this use of kinship coefficients it is also assumed that selection does not occur anywhere in the "population" at one or more of the variable loci, after dispersal from spawning or nursery areas. If such selection were present then one could not distinguish whether the gene frequency for a sample (school) is an indication of the area from which it has come, or whether these fish have come from anywhere in the Pacific, with selection within that school (within a cohort) changing the frequency towards the average for the area near where the school was sampled. As well, the acceptance of kinship coefficients assumes school integrity from a very early age, and most certainly for the period after selection. If membership within schools is a function of previous contact between post recruitment schools, and these schools originated from different portions of a cline, then when a mixed school is sampled, say outside the breeding area, its gene frequency will reflect the weighted average from its component schools, rather than the gene frequency value for a particular portion of the cline.

Participants were concerned that the assumptions underlying this use of kinship coefficients were suspect, hence, any calculation of distance between closely related individuals (i.e. steps in the cline, see section 6.3) could be misleading.

5.6 Tag Recoveries - General

Figure 6 presents straight line migrations of SPC tagged skipjack at large for 30 or more days. A maximum of two movements were plotted within any single 5 degree square or between each ordered pair of 5 degree squares. Figure 7 presents histograms of numbers of skipjack tag recoveries in terms of straight line distance moved, and time at large. The workshop also considered

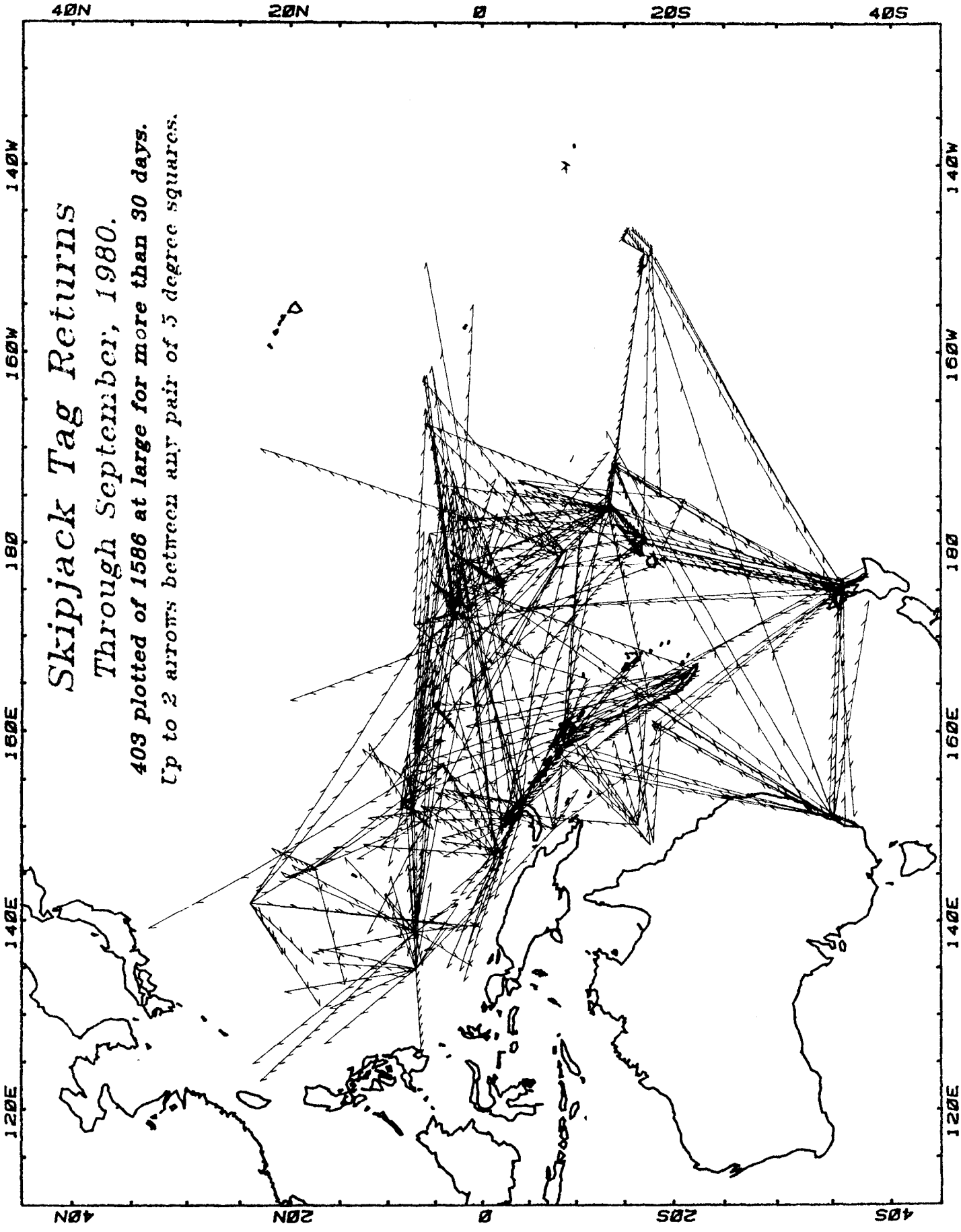
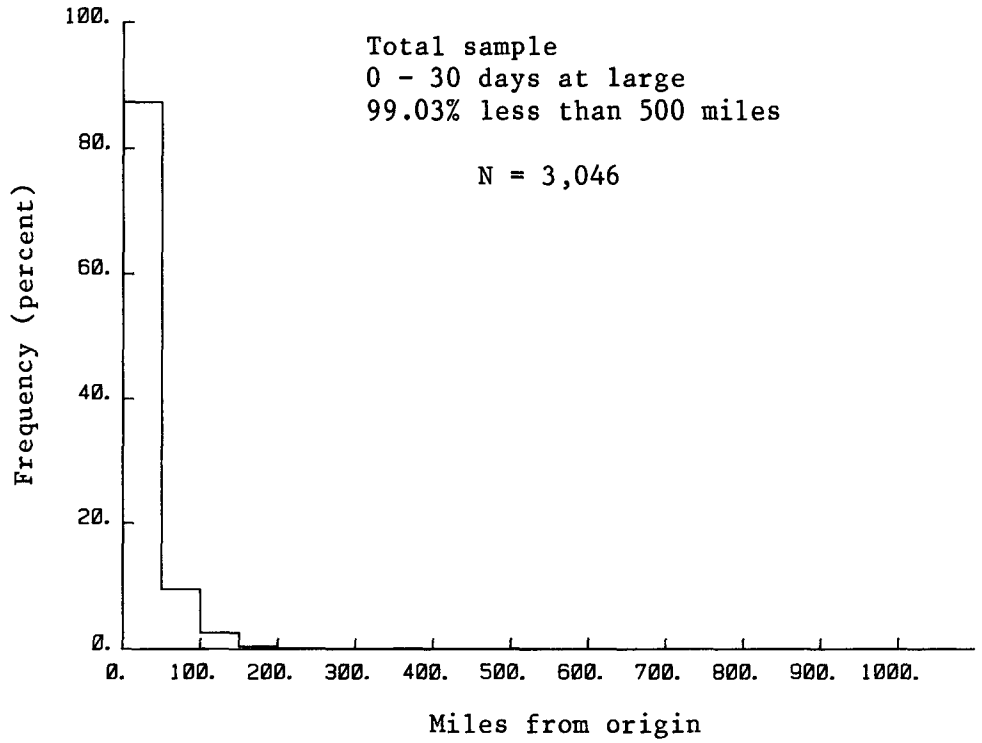


Figure 6 - A selection of skipjack tag recoveries plotted as direct line trajectories from the point of release to the point of recapture. Tick marks on the lines indicate the direction and time of movement, one tick being printed for every 30 days that a fish is at large. A maximum of two moves were plotted within each 5° square or between each ordered pair of 5° squares.



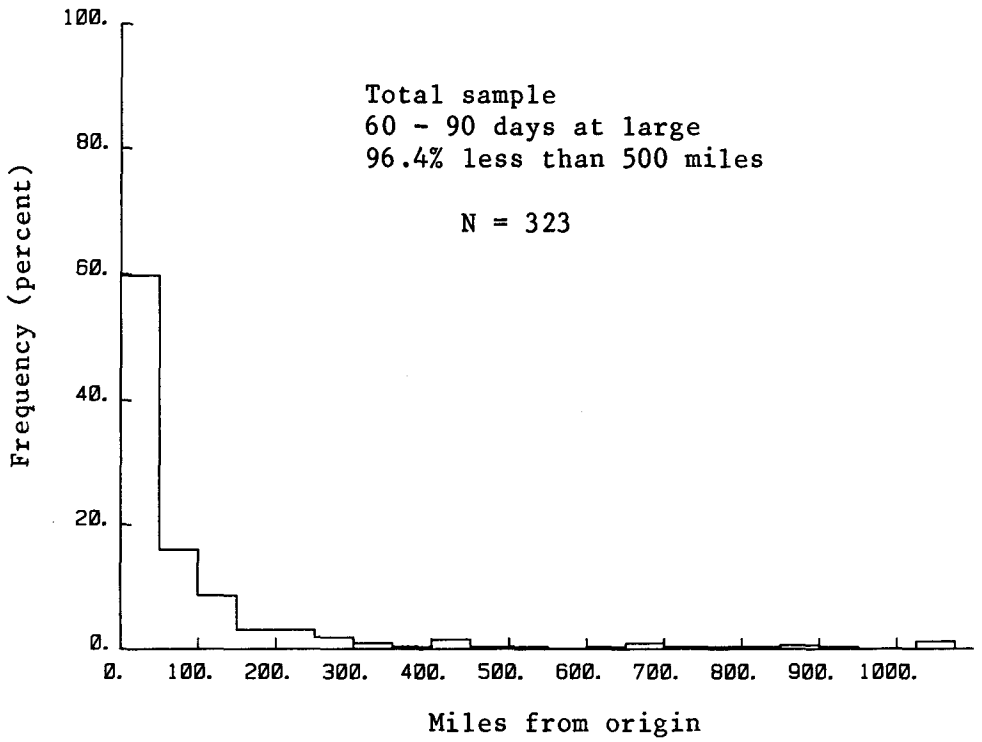
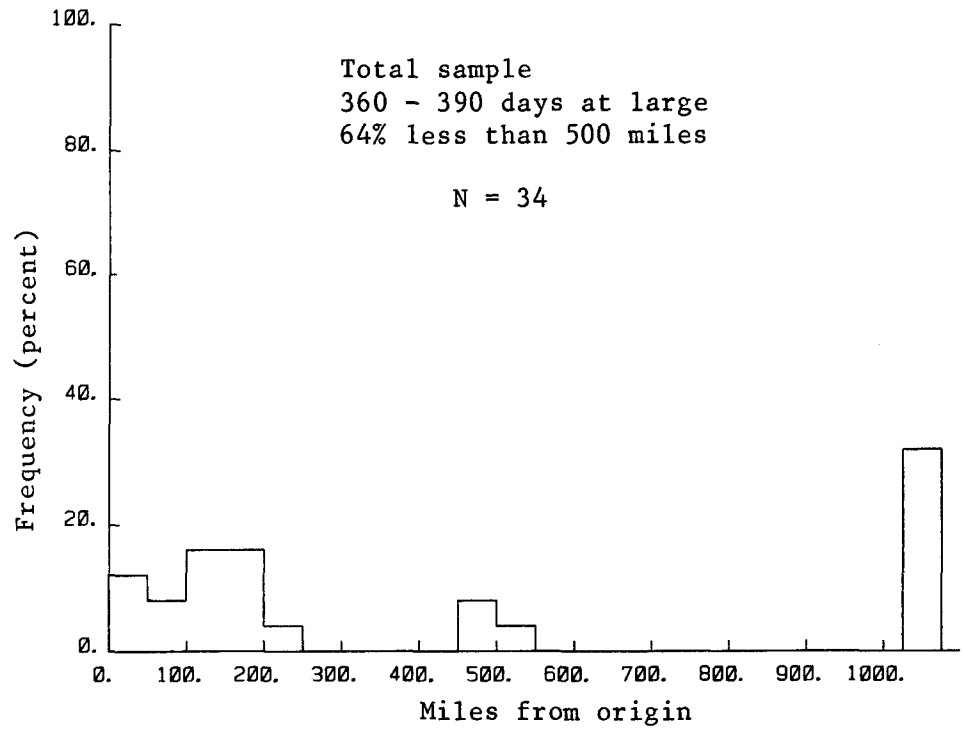


Figure 7 - Movement distributions for all skipjack tag recoveries at large during four different time periods.



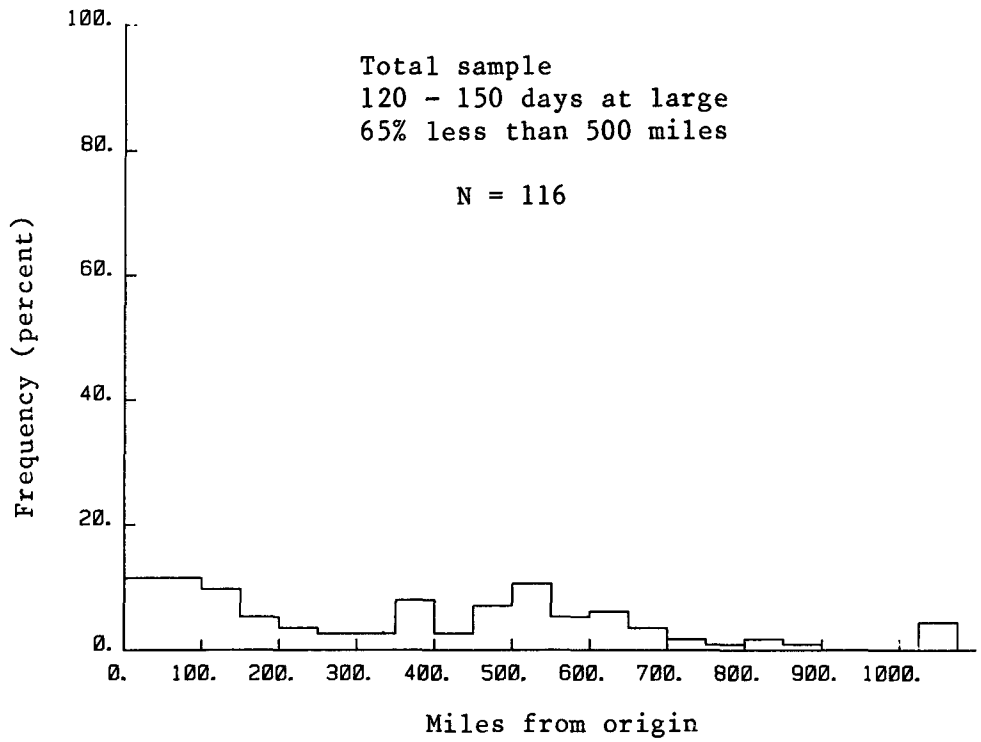


Figure 7 - Movement distributions for all skipjack tag recoveries at large (cont.) during four different time periods.

maps of straight line tag migrations for individual countries visited by the Skipjack Programme survey vessel (e.g. Figure 8 for Kiribati).

Examination of tag recovery maps for countries between the equator and 10°N latitude suggested a net movement of tagged fish to the east, particularly near the southern boundary of the North Equatorial Counter Current. It was noted that this directional movement would likely be more pronounced when tag recoveries were adjusted for fishing effort; seasonal components to this movement pattern may exist, and are being examined.

The absence of tag recoveries outside French Polynesia, from large numbers of tag releases (30,000) in French Polynesia, particularly in the Marquesas Islands, was noted.[1] As well, to date there has been little northward movement of fish tagged within and immediately below the North Equatorial Counter Current (i.e. 4°N to 10°N).

The tag recapture data in Figure 7 indicated little movement for the average tagged skipjack, but with some fish moving long distances. If the raw data were adjusted for fishing effort, the average distance moved may be much greater. However, a major problem is that tag recovery data gives no direct information on gene flow or population structure - it merely indicates that gene flow between different areas is possible. For instance, it is not possible to ascertain with any confidence whether fish spawn continuously or periodically during their wanderings, or whether they tend to wander widely and then "home" prior to spawning.

Integrity of schools or aggregations, once their individual members reach recruitment size, is an implicit assumption underlying some postulated uses of blood genetics sampling data for skipjack. The workshop briefly considered tag recovery data from individual schools in light of this assumption, and noted that this assumption may well frequently be violated. For example, there are numerous instances where two or more fish tagged in one school have been recovered several months later, on the same day, but in different schools that were many hundreds of nautical miles apart. Analyses of school integrity are continuing.

5.7 Tag Recoveries from Blood Sample Schools

Tag recoveries from blood sample schools, when plotted as in Figure 9, show no discernable pattern with respect to gene frequency and direction of movement. However, this simple presentation may mask an underlying movement pattern which could be related to gene frequency. The workshop reviewed results from an analysis of net west/east movement for all tag recoveries from each blood school, compared with the difference between actual EST gene frequency and predicted EST gene frequency (from the EST versus longitude regression) for that school. A significant positive relationship would imply that skipjack tend to return to an area that, on the average, has a gene frequency that is close to the gene frequency of the school at time of

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[1] After the workshop, the Skipjack Programme received three tags recovered by Japanese distant-water pole-and-line vessels from skipjack tagged in the Marquesas Islands. One was recovered in the Phoenix Islands and two were recovered just east of the Line Islands.

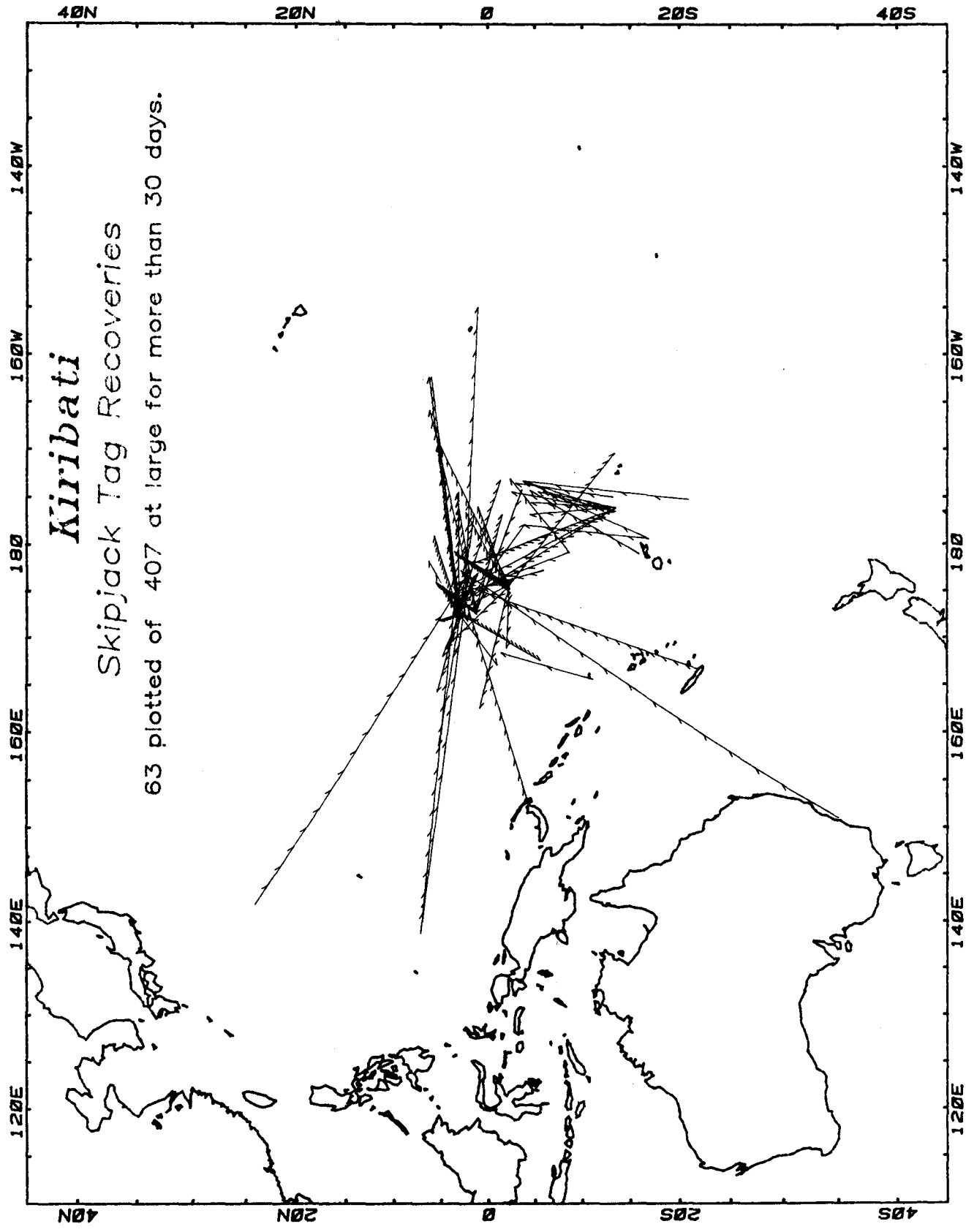

Figure 8 - A selection of skipjack tag recoveries entering and leaving the waters of Kiribati plotted as direct line trajectories from point of release to the point of recapture. Tick marks on the lines indicate the direction and time of movement, one tick being printed for every 30 days that a fish is at large. A maximum of two moves were plotted within each 5° square or between each ordered pair of 5° squares.

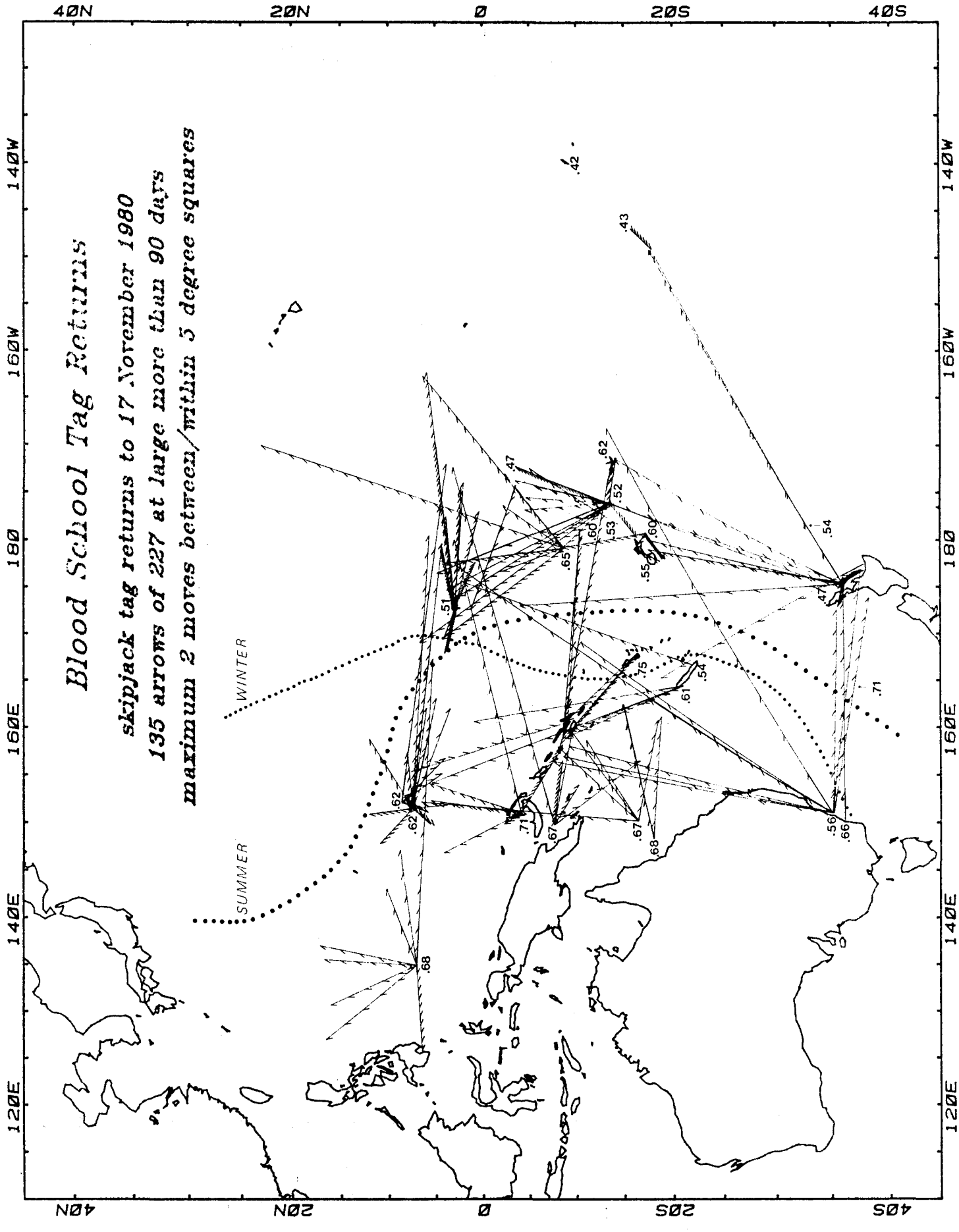

Figure 9 - Tag recoveries from blood sample schools at large for more than 90 days. Esterase gene frequencies are indicated for most schools. The two curved lines show southern summer and southern winter boundaries proposed by Fujino (1972) for the skipjack tuna subpopulation in the western Pacific Ocean.

sampling. The relationship between EST and net west/east movement was not statistically significant (Figure 10). Note that negative movement values imply net movement to the east.

At the time of the workshop the analysis of tag recoveries from blood schools, and for that matter analysis of all tag recoveries was, at a preliminary stage. Large numbers of recoveries were received after the workshop, and recoveries continue to be sent in. The participants noted that full analysis of tagging data, weighted for tag recovery effort and speed of migration, would be necessary before specific migration models, incorporating estimates of mixing rates between spatial units, could be defined from the tagging data.

5.8 Distribution of Spawners

At various times the participants discussed available evidence for distinguishing where skipjack breeding is concentrated. Larval skipjack occurrence is generally limited to waters where the sea surface temperatures average at least 24°C. Even though water of this temperature is present east of 130°W longitude, albeit in a relatively narrow band and primarily north of the equator, larval density east of this longitude is very low. These data, corroborated by maturity data, suggest that there is little skipjack spawning in the eastern Pacific. Moving westward from 130°W longitude, the north-south boundary for the 24° isotherm widens, as does the distribution of larval skipjack. Occurrence of pre-spawning adult skipjack based on gonad development indices, juvenile skipjack as determined by stomach analysis of predators such as tuna and billfish, and larval skipjack from plankton samples, suggest that spawning skipjack are present between 10°N and 10°S all year, and are seasonally present at latitudinal extremes such as Hawaii, the south coast of Japan, and northern New South Wales, Australia. The available data are not sufficient to define, with any degree of confidence, areas of concentrated spawning within this broad region.

5.9 Summary

There was general agreement on the following points: i) that the available data suggest a cline in esterase gene frequency within the SPC area, with flattening of the cline to the east of French Polynesia; ii) that variability in EST gene frequency decreased from west to east in the SPC area; iii) that when sampling from groups which demonstrated significant heterogeneity, the apparent statistical agreement with phenotype distributions expected under Hardy-Weinberg equilibrium could well be an artifact of the lack of power of the test to distinguish small deviations from Hardy-Weinberg equilibrium (e.g. a slight Wahlund effect, or a slight excess of heterozygotes); iv) that over part of the study area large numbers of skipjack may show net directional migrations (e.g. west to east), and that most skipjack may move only a short distance after tagging; however, further analyses of tagging data are required, in particular taking into account tag recovery effort, before explicit migration models can be hypothesized; v) that there is little skipjack spawning in the eastern Pacific Ocean; and vi) that it may be profitable to continue analysis of the blood genetic data, a) using all available samples from all sources, rather than subsets of the data, b) applying multivariate and other statistical analyses, including, where appropriate, data from all polymorphic loci, and c) developing and testing simulation models.

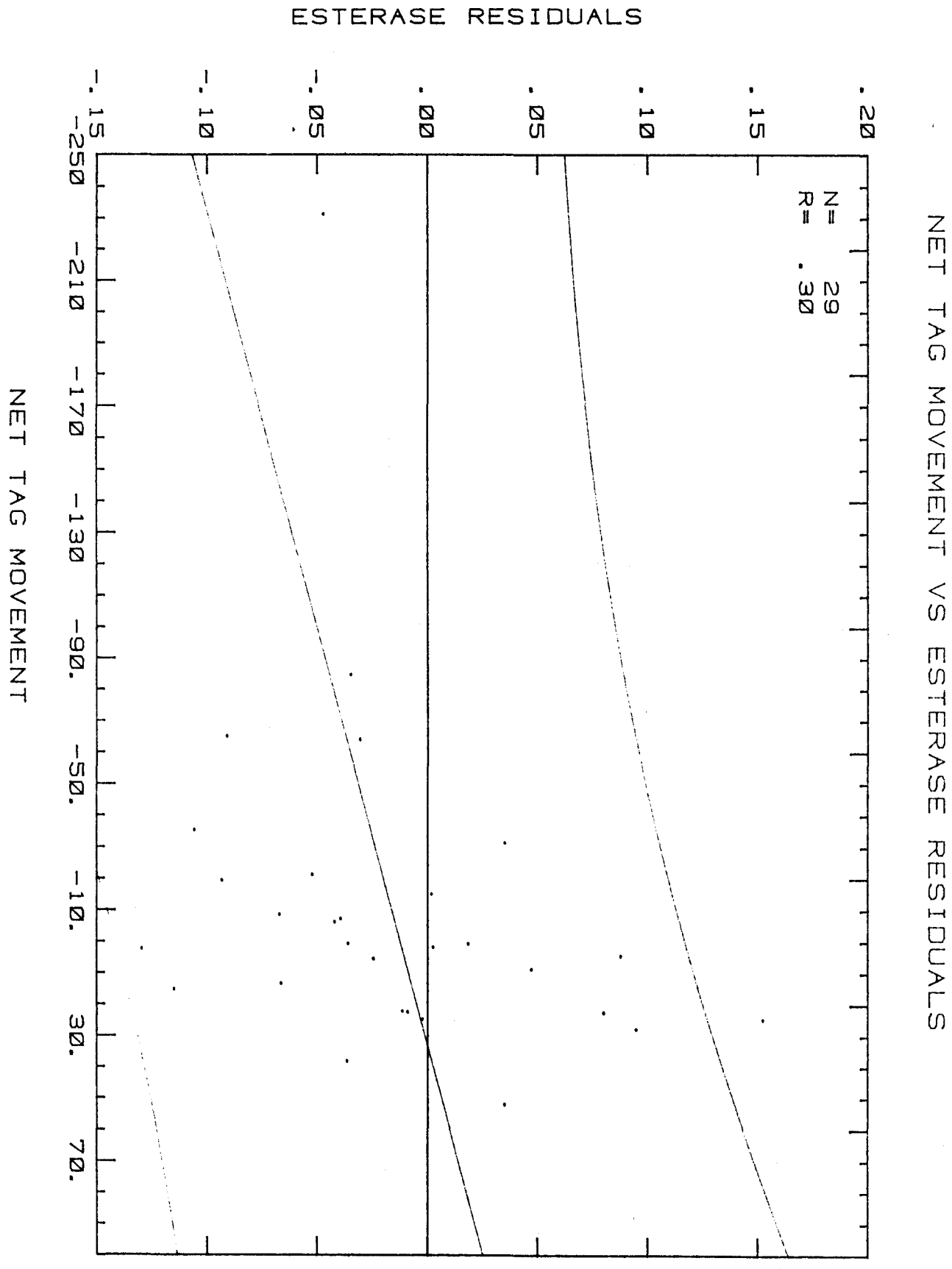

Figure 10 - Net movement to east (negative) or west (positive) by all tag recoveries from individual blood sample schools versus the difference between the school's esterase gene frequency and the esterase gene frequency predicted by the regression in Figure 3. N is the number of samples used for the regression and R is the correlation coefficient.

6.0 POSSIBLE MODELS OF POPULATION STRUCTURE

There was discussion of the propriety of using only esterase data to define possible population structure models. It was noted that most loci examined in skipjack were either monomorphic (i.e. gene frequency of 1.0) or exhibited low and non-systematic genetic variability. This could be interpreted to show a high degree of genetic similarity between skipjack groups across the western and central Pacific. While clearly important, the question "Why are most tuna genetically very similar?" is quite different from the question "Why are tuna genetically variable for some loci?" It was noted that in terms of the second question, genetic variability at a few loci (in particular the EST locus) could be used to infer possible population structures.

6.1 Single "Panmictic" Population

This model assumes that the total tuna population in the Pacific Ocean is a single random mating population such that all adults have an equal probability of mating with each other within one generation and across the species' full geographical range. Breeding of individuals may occur anywhere in a breeding area, which could be a subset or subsets of the full geographical range of the population, but there cannot be homing to specific sites within the breeding area.

There was some question as to the advisability of adhering to the rigid condition of panmixis since schooling results in a non-random aggregation of fish and any tendency for mating within schools would result in a form of positive assortive mating. Thus the term "panmixis" was placed in quotations to allow for schooling and secondly to allow for what may be referred to as reduced mixing. That is to say, fish or schools need only mix enough to ensure uniform gene frequency in the absence of selection.

Using this model, one could explain a cline, such as observed for esterase in post-recruitment skipjack, on the basis that there was strong differential selection gradient across the Pacific affecting relatively non-migrant, pre-breeding skipjack. Prior to breeding the fish would have to switch to vigorous dispersal behaviour in order to achieve "panmixia". The sampling of blood sample schools would have to favour the pre-dispersal stage in order for the esterase gradient to emerge. Considering tag recovery, sexual maturity and size frequency data for skipjack from blood schools, this latter assertion was clearly wrong.

This hypothesis was rejected by the workshop participants.

6.2 Isolation-by-Distance : A Continuous Cline

Under this model there should be a continuous shift in gene frequency from one end of the area of concern or breeding range to the opposite end. There should be no restrictions to movement, and selection pressure, if present, should also be continuous and evenly distributed. It is assumed that the probability of two fish mating is a decreasing function of the distance between their birth places, and that there are no severe restrictions to gene flow at any point across the range.

The cline in esterase gene frequency could be either a slowly eroding relic of past genetic drift or selection, or it could be actively maintained by geographic variations in currently acting selective forces. How the cline is maintained is particularly relevant to management considerations (see section 7.3). It was noted that environmental variables such as surface temperature and salinity show longitudinal gradients across the study area, and this variability might be directly or indirectly responsible for the observed cline.

If the cline is simply a relic of past genetic divergence, and at present selection is negligible along the cline, then the model would predict negligible net movement of breeding skipjack along the cline (i.e. if skipjack migrate widely prior to spawning, they must then home to the general location at which they were spawned). In contrast, if significant selection is present along the cline, then an interplay of selection on pre-recruits and dispersal could maintain the cline, i.e. high dispersal would require high selection. Further predictions are as follows: i) variance amongst samples along the cline should be relatively constant[2] compared to that expected under the model of section 6.4; ii) the difference in gene frequency between two samples from along the cline should be positively correlated with the longitudinal distance between the locations where the samples were collected; iii) the cline should be stable in time, unless there was wide time-period variation in the environmental vector causing selection; and iv) if there was significant net directional dispersal of mature adults, there would have to be opposite net directional movement of pre-spawners.

With this model, local fisheries would have the greatest impact within the area of the fishery, and for a limited distance outside this area, depending on the degree to which the fish migrated between neighbouring areas, or dispersed prior to spawning. Over longer periods of time, localized fisheries would impact over a wider area, depending on the rate of interbreeding between areas, measured over successive generations. Unless rates of spawner dispersal and hence selection are extremely high, one would expect that fishery impacts between geographical extremes of the cline would be minimal. However, without knowledge of the degree and type of movement (pre-spawning, spawning; random, directional), it is impossible to quantify the degree and geographical extent of interaction between neighbouring fisheries.

Under the assumption that the cline arose by past genetic divergence (hence skipjack must exhibit either negligible within generation dispersal of spawners, or else they may migrate as pre-spawners, subsequently homing to their natal area), then one would expect that most fishery effects would be limited in geographical scope, at most to the limit of significant pre-spawning migration.

This hypothesis was retained for further consideration.

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[2] Under this isolation-by-distance model there could be some difference in variance amongst samples along the cline if, for example, year to year or latitudinal variation in the selective factor(s) fluctuated along the cline.

6.3 Isolation-by-Distance : A Stepped Cline

This model is very similar to the previous continuous isolation-by-distance model, except that the geographic change in gene frequency is stepped, due to a partial restriction of gene flow along portions of the clinal gradient; or there could be a number of patches along the gradient where intense selection takes place. Both situations should result in a discontinuous distribution of gene frequencies. It was suggested that no more than five patches or steps be considered; this was the number of "clusters" which were postulated from the figure of esterase gene frequency plotted against longitude (section 5.1). More, or less, steps could of course be considered; and the effect of longitudinal clustering of sampling effort should not be overlooked.

Predictions following from this model were : i) discontinuities in gene frequency at each locus should occur at the same barrier, if the steps are the result of barriers to gene flow and the alleles are responding to the same selection factors; and ii) the relationship between ΔP, the difference in gene frequency between two samples, and the geographic distance between the samples should differ from that expected in the previous model.

There was a suggestion that there would be different management considerations between the case where there was significant movement of individuals between steps and the case where the steps were more fully isolated. In general, this model would predict broader fishing effects than the previous model, that is within the steps which, in the case of five equidistant steps, cover one-fifth of the distance between the clinal extremes.

The workshop participants agreed to retain this hypothesis for further consideration.

6.4 Two Breeding Subgroups at the East and West Extremes of the Study Area

This model is described as a cline generated by the contact of two previously isolated subgroups, or the point of contact of two large subgroups that differ because of unlike selection coefficients. The assumptions are: i) there are two, or perhaps more, different breeding groups at either end of the cline; and ii) the zone of mixing is a zone of weak hybridization, and may be a region of lowered breeding success (a genetic "sink").

The predictions are as follows: i) there should be a region of constant gene frequency followed by a cline and a second plateau in gene frequency; ii) the variances in gene frequencies should be much greater among samples in the area of mixing than at the extremes; and iii) a Wahlund effect should be measurable in the area of mixing, given a large enough sample size.

Under this model, fishing the mixing zone should have little or no impact on the breeding subgroups at the extremes (assuming negligible pre-spawning migration into the mixing zone by members of the breeding subgroups). Similarly, fishing one of the two hypothesized breeding subgroups should have negligible impact on the other, but could impact in the zone of mixing. The degree of impact in the middle, from fishing at the extremes, would depend on the rate of movement into this middle zone, the relative

contribution to this zone by the subgroup that was under exploitation, and of course, level of exploitation for the subgroup being fished.

The participants agreed to retain this model for further consideration.

6.5 Fishery Migration Model

This model was presented as a counterpoint to the other models which are constrained by genetic or breeding structure assumptions. This model considers that movement of fish within and between locations, which could be defined as areas of concentrated fishing effort, is a function of migration, natural mortality, and fishing mortality. The migration coefficients could be estimated for each area from the tagging data. It would be necessary to assume initial population sizes within areas, and these could be stratified by size (as a proxy for age). Allowances could be made for growth.

Predictions of fishery impacts between locations would be for single cohorts. Model validation could be assessed with tag and recovery data as well as with catch statistics.

Concern was expressed that knowledge was inadequate to make reasonable estimates of levels of abundance, or mortality, catchability and migration parameters. Consequently output from such a model could be misleading. It was noted, however, that even though a model such as this would represent a simplification of the actual situation, it would be improved as more data came to hand; and different parameter values could be tested to determine system sensitivity. As such it could be a valuable tool for quantifying short-term fishery impacts between areas.

7.0 SUMMARY OF INTERPRETATIONS

At this stage the workshop participants reconsidered the objectives of the meeting (see INTRODUCTION). In this context, two questions emerged: i) will fishing in one area have an impact on fishing (or potential fishing) in other areas, and ii) can the time/area horizon for management decisions be defined, that is, given some time scale, how big is the geographic area that has to be taken into account? A summary of views and interpretations of the genetic data were sought from the participants in light of these kinds of questions, and in terms of the previously discussed models. These interpretations are based on individual presentations by workshop participants. They are presented in detail so as to give as complete coverage as possible to the different points of view of the meeting participants.

7.1 Interpretation One

If one assumes that the EST cline is continuous, is stable over time, is caused by differential selection of esterase genotypes in longitudinally defined areas; and that within the breeding area, EST gene frequency in adults sampled represents the gene frequency of spawning adults in the area, then it follows that: i) when larvae, juveniles and sub-adults do not disperse over long distances (say less than 5°), then selection during these stages must operate to produce gene frequencies in progeny similar to that of their parents, and ii) when larvae, etc., do disperse over long distances, then

selection during these stages must operate to produce gene frequencies in progeny similar to that of the region where they are caught as young adults. In either case, movement of adults from areas with different selection coefficients would lead to heterogeneity of gene frequencies amongst samples of adults caught in the same area.

Taking available analyses of tagging data into account, there appears to be a net dispersal of adults to the east (between 10°N and 10°S). If this is correct, then it suggests a net dispersal of larvae, etc. to the west.

Thus one would postulate that: (i) dispersal of young and adult stages over long distances is possible and likely; and (ii) selection operates throughout life favouring the dominant EST gene towards the west and against it to the east.

The above implies that over more than one generation, there could be interaction among all components of the skipjack fishery. The magnitude and time scale of the interaction would depend on average dispersal rates and distances moved by immature stages and adults.

This interpretation means that final definition of management areas would depend on investigation and knowledge of dispersal and selection at all life cycle stages. The completed analysis of the tagging data will provide information on adult movement, but it still must be recognized that adult movement measurements from tagging data do not necessarily measure gene flow, particularly if homing is operative. The primary deficiency in trying to understand the structure of skipjack populations is the lack of information on the breeding behaviour and early life history of the species.

It was noted, in the discussion of the above interpretation, that the critical question of whether adults that were spawned in a particular area, return to that same area to spawn, had not been addressed. However, this question simply relates to a balance of selection and dispersal. For instance, if selection is very weak, then adults must return to the same area within narrow limits. On the other hand, if selection is strong in immature stages, spawning area fidelity need not be so pronounced, and strong adult dispersal would give rise to high variance in gene frequency at any location (as compared with weak adult dispersal).

The tagging data, corrected for recovery effort and speed of migration, will allow some quantification of the amount of adult movement. There was disagreement as to whether the tagging results available at the workshop suggested net adult dispersal to the east. This is being examined.

It was suggested that the increased EST gene frequency variance observed in the west indicates that fish in the west originated from a wider range of breeding areas with different environmentally influenced selection coefficients.

Clearly, under this view, the geographical and temporal potential for fisheries interactions will depend on the degree to which skipjack home to breeding areas, the degree to which they disperse, and whether dispersal is directional.

7.2 Interpretation Two

Postulate the following: that selection (if present) occurs before recruitment to the fishery; that diffusive movement occurs in all directions from the point of recruitment; that some isolation-by-distance occurs; that pre-recruits do not move much or their movement is random; that skipjack aggregations are the result of mixing of different spawning groups (originally from a single area of recruitment); that there is aggregation integrity after recruitment; and that gene frequency measured at or after recruitment (within the breeding area, 15°N to 15°S) is a measure of "true" gene frequency at that point in the longitudinal cline.

Then, under conditions of strong selection, the gene frequency of an aggregation at or after recruitment should reflect and identify the area of origin (based on the "average" value from the esterase gene frequency - longitude regression), and groups found outside the "breeding zone" or later in their life cycle in the tropics can be located to an area of origin along the cline by their gene frequency.

Under conditions of gentle selection (or neutral selection), the gene frequency of an aggregation at recruitment reflects the effects of selection, the parental gene frequency and mixing effects. Over all, these effects will lead to the recruitment gene frequency approximating that of the average for the point of recruitment, but with a greater variance than in the strong selection example. Groups found outside the "breeding zone" and later in the life cycle can be located to an area of origin but with less confidence than the above.

In neither of the two previous examples can the future movement of fish be predicted from the gene frequency at time of sampling (unless homing is assumed).

Under this interpretation one would expect that aggregations of large fish would show greater variance in gene frequency in an area than would aggregations of smaller fish, since the large fish have undergone more mixing. As well, the variance of the gene frequency of a sample should be able to be used to estimate the longitudinal range of origin of the fish.

In the ensuing discussion of this interpretation, it was again emphasized that the presence or absence of homing behaviour has important implications for management. Analyses of tag recovery patterns may shed light on whether adults undergo directional dispersal and/or home for breeding. Previously in the workshop (section 5.5) concern had been expressed over assigning a specific geographical "origin" or range of geographical origin to post-recruit fish, based on their gene frequency. Concern centred on the validity of assumptions relating to school (aggregation) integrity, selection, use of average gene frequencies for post-recruit skipjack in the breeding area to estimate the "true" gene frequency in the "area of origin", and so on.

7.3 Interpretation Three

Accept that the cline exists over part of the skipjack range in the western and central Pacific, and that this in turn implies some form of population structuring. Accept that the cline appears linear over the range of spawning for skipjack, thus suggesting some selective effect on juveniles,

but is flat in the eastern Pacific where spawning is thought to be negligible. Accept that the cline is in some dynamic equilibrium, but recognize that the data does not allow us to say much about how long this cline can be expected to persist. The cline in EST could have arisen by different evolutionary mechanisms and could be maintained by a different combination of factors, which available data do not allow us to discriminate.

For erecting what can be referred to as "optimum" management strategies it is very important to understand how the cline is maintained. However, at this stage, neither the electrophoretic data nor the tagging data analyses have proceeded far enough to allow one to discriminate between the different hypotheses: if dispersal is random; if there is weak selection - weak dispersal; if there is strong selection - strong dispersal; if the cline is continuous, stepped or just the central zone of contact between two (or more) relatively homogeneous breeding units. While gains might be made from additional analysis of the "genetic" data, without a great deal more new data, not much light will be shed on the subject of management implications. This relates to the inherent lack of power of the genetics methodology in this situation.

It is tempting to draw inferences about the presence of steps and the homogeneity of units, yet there is little objective basis for doing so, i.e. the presence of more heterogeneity in one area than another. As well, it is tempting to use genetic distance (for example with kinship coefficients) as a meaningful number for management. While perhaps analytically correct, at this time it would be premature and even misleading to do so; gaps in the data can provide numerical artifacts and lead to erroneous inferences.

It is important to recognize that questions relating to homing, extent and location of prime breeding and feeding areas, and degree of selection are critical to improving our understanding of skipjack population structuring. For now, we could probably accept that parts of the SPC area are the breeding grounds for "migratory types" from the eastern Pacific or elsewhere, and as such, fishing in these areas could impact the resource over a much larger area. For further refinement, it was suggested that all available electrophoretic data be re-examined, compared and contrasted with more detailed analyses of tagging and other ancillary data, then if necessary, depending on the relevant management questions, a new field programme or programmes of limited scope and tight experimental design could be proposed.

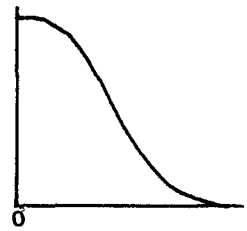
Comments after this interpretation were somewhat contradictory. One view, supported by the above interpretation, was that on the basis of blood genetics data all one could say was that skipjack in the Pacific have some form of population structuring, and that re-analysis of the existing data, in concert with tagging and other ancillary data, will allow little further refinement of the most likely form that this structuring takes. A second view was that qualified advice could be given on certain broad management questions concerning potential fishing effects over short time periods, but this advice would rely most heavily on tagging measures of movement, using genetic data as background for contrast with movement hypotheses arising from tagging data.

7.4 Interpretation Four

Under this interpretation one accepts that the data demonstrate an east-west gradient in EST gene frequency, that the variance of the gradient is about ten times greater than that expected from binomial sampling, and that variance at the west end of the gradient is about four times greater than variance at the east end.

Consistent with these observations is a situation where the greater than expected variance reflects significant migration of adults over their post-recruitment lifetime, and after selection has occurred. Two possible situations regarding localization of breeding are: i) a strong selection gradient operating on pre-recruits resulting from breeding in areas with a high probability of adults returning to the same general area, but low probability of returning to exactly the same place.

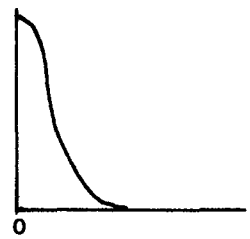
Chance of breeding between two individuals with origins at a given distance apart



distance between birth-places

and ii) a weak (or zero) selection gradient operating on pre-recruits which return to a very narrow distance range for breeding.

Chance of breeding between two individuals with origins at a given distance apart



distance between birth-places

The increased variance in the west could be due to increased mixing as adults (testable with tag data, but not evident so far). One could compare width of the variance increment (expressed as geographical distance), at various longitudes on the gradient, with mean adult mixing distance from tag data. It may also be possible to distinguish between (i) and (ii) by looking at gene frequencies resulting from a model incorporating different selective mortalities. The model suggested in (ii) could be used to predict widening of variance due to different mixing widths. And finally, it would be valuable to examine more samples from longitudes west of the present data.

In general, this interpretation implied non-directional dispersal and homing, to a greater or lesser degree, to a natal area. These assumptions are difficult to support with the currently available data. Post-recruitment school integrity, if demonstrated, would support this interpretation but is not a necessary condition. Again, fisheries interactions would depend to a large extent on the degree of dispersal and migration/homing.

During discussion of this interpretation, it was noted that the workshop now generally accepted that the variance in EST gene frequency increased towards the west. Increased variance might relate to the greater area and diversity of productive habitat suitable for reproduction in the western Pacific, particularly the wider latitudinal range (evidenced by various factors such as larval distribution, favourable water temperatures, presence of land masses, etc.) of such habitat, and thus the greater potential for reproduction in environmentally different areas. A suggestion was made that skipjack blood samples from east and west extremes of the Indian Ocean might prove useful, since environmental heterogeneity, due to presence of mid ocean land masses and seamounts, was considerably less in the Indian Ocean, and accordingly one might expect less variability in gene frequency.

8.0 CONCLUSIONS AND RECOMMENDATIONS FROM THE WORKSHOP

At the very least, the blood genetics data showed that skipjack follow some form of population structuring across the Pacific, i.e. Pacific skipjack are not a panmictic population. When tagging data are included, it is reasonable to conclude that short-term mixing, sufficient to affect the overall abundance of skipjack, is negligible between longitudinal extremes in the study area.

One common feature of all but the first of four models that were described is the isolation-by-distance concept. This states that reproducing fish in any one region mingle with reproducing fish in neighbouring regions, and that the degree of mingling between any two regions decreases with increasing distance between these regions. The workshop could not identify genetically isolated skipjack subgroups, separated by stable geographical boundaries, as had been advanced by Fujino (1972 and (1976). Furthermore, we would not, on the basis of the evidence available, propose any permanent barriers to the interaction of fisheries between neighbouring regions. However, the geographical extent of neighbouring regions cannot be defined on the basis of present blood genetics data. Further analysis of tagging data may allow some refinement of the extent of these regions, but will not on its own allow description of population structuring.

More precise inferences on relatedness between skipjack in sub-areas of the region, their inter-location movement, their breeding area origin, and so on, cannot be made at this time with any reasonable degree of certainty using the blood genetics data alone, or with, at this stage in the analyses, the combination of available blood genetics, tagging and ancillary data. This should be readily apparent from the wide range of models and interpretations presented.

Before settling on a single hypothesis that best describes skipjack population structuring, it is recommended that certain important research questions be addressed. For example: (i) what is the interplay between, and magnitude of, selection and dispersal in the study area? In fact, is there any selection, or could the cline be a result of genetic drift? (ii) Is there net directional dispersal of spawning adults and reciprocal dispersal of immature stages; do spawners home to spawning locations; and is spawning concentrated in time and space? (iii) Other questions relate to the nature of EST selection - when does it occur in the skipjack's life cycle; what is the environmental vector? Obviously of considerable value would be samples of

gene frequencies for larval or juvenile skipjack to contrast with data for adults from similar locations and times. (iv) There are important questions related to skipjack schooling: do schools represent breeding groups; are schools integral units, and for how long?

In summary, analyses to date of blood samples from skipjack caught by pole-and-line gear, when considered alone, only provide a very general view of skipjack intermingling, and add little insight into aspects of population structuring which are relevant to management questions. Thus it was concluded that continuation of the present strategy of skipjack blood sampling would not significantly improve our understanding of skipjack population structure in the Pacific Ocean.

The workshop recommended some further analysis of all available genetic data in combination with tagging data. This may give more insight into timing and geographical extent of skipjack intermingling. Nevertheless, it must be recognized that there will continue to be serious gaps in our knowledge of skipjack population structure and ecology. In order to provide the best possible advice to those formulating fisheries policy for skipjack resources in the SPC area, some of these gaps will have to be filled. This should be possible after careful, comprehensive analysis of all blood genetics, tagging and ancillary data resulting from well-designed field experiments.

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APPENDIX A

WORKSHOP PARTICIPANTS

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Professor J.S.F. Barker	University of New England, Australia
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APPENDIX B

SOUTH PACIFIC COMMISSION SKIPJACK BLOOD SAMPLES

Sample No.	Sample Code	Country	Area	Number Specimens	Date	Position	Time	Skipjack Tags			Gene Frequency				Average Fork Length	Standard Deviation in Length	Coefficient Variation in Length	Sea Surface Temp.	Immature Females/Total Females Examined
								Number Released	Recovered[1] No.	%	Actual EST	Est. EST	TSF	GDA					
1	A	CAL1	A	78	8/01/78	21°46'S 166°42'E	1545	261	1	.38	.54	.60	.68	.112	462	33	.07	26.9	4/10
2	B	CAL1	A	116	15/01/78	20°58'S 164°24'E	0840	842	4	.48	.61	.61	.70	-	492	28	.06	25.2	2/9
3	C	VAN1	A	22	20/01/78	17°36'S 167°42'E	1015	79	-	-	-	-	-	-	-	-	-	28.3	0/17
4	D	VAN1	A	73	21/01/78	16°15'S 167°51'E	1200	418	3	.72	.75	.60	.70	-	490	27	.06	28.9	1/13
5	E	FIJ1	B	100	31/01/78	18°55'S 178°24'E	0655	599	8	1.34	.55	.56	.71	-	466	29	.06	27.8	0/12
6	F	FIJ1	B	120	10/02/78	17°13'S 179°17'W	1550	500	11	2.20	.60	.55	.71	.083	470	33	.07	28.8	2/11
7	T1	TON1	B	95	21/04/78	18°19'S 174°25'W	1100	63	-	-	.60	.53	.87	-	496	27	.05	27.7	7/15
8	W1	WAL1	B	114	06/05/78	13°26'S 176°02'W	0930	601	2	.33	.50	.54	.74	-	530	43	.08	28.6	0/8
9	W2	WAL1	B	87	15/05/78	13°09'S 176°22'W	1035	410	1	.24	.51	.54	.69	-	594	31	.05	28.8	1/12
10	W3	WAL1	B	119	17/05/78	13°29'S 176°07'W	1150	1,034	6	.58	.50	.54	.75	-	513	25	.05	28.3	8/13
11	W4	WAL1	B	95	19/05/78	13°30'S 176°05'W	1030	1,027	12	1.17	.53	.54	.75	.124	520	31	.06	28.4	6/9
12	W5	WAL1	B	49	26/05/78	13°19'S 176°17'W	0815	122	-	-	-	-	-	-	346	12	.03	28.9	10/10
13	W6	WAL1	B	144	29/05/78	14°13'S 178°03'W	1045	38	-	-	.59	.55	.71	-	604	16	.03	28.5	6/8
14	H	WES1	B	105	14/06/78	13°42'S 171°45'W	1000	1,635	6	.37	.62	.52	.77	.089	485	28	.06	28.7	0/9
15	J	TUV1	B	158	25/06/78	10°23'S 178°48'E	1220	486	3	.62	.60	.56	.70	-	510	24	.05	29.2	8/12
16	K	TUV1	B	108	27/06/78	08°40'S 179°13'E	1703	426	2	.48	.56	.56	.74	-	519	31	.06	29.9	10/10
17	L	TUV1	B	103	01/07/78	08°42'S 179°10'E	1710	470	3	.64	.65	.56	.70	-	529	25	.05	29.4	6/6
18	M	KIR1	B	122	16/07/78	02°57'N 172°45'E	0803	573	24	4.19	.49	.58	.68	-	477	18	.04	29.2	2/8
19	N	KIR1	B	115	22/07/78	03°00'N 172°48'E	1150	842	18	2.13	.53	.58	.66	.116	476	20	.04	29.4	0/13
20	P	TRK1	A	100	10/08/78	07°42'N 151°44'E	1357	720	30	4.17	.62	.65	.71	-	503	18	.04	30.9	0/9
21	R	PAL1	A	128	20/10/78	07°06'N 134°54'E	1300	440	8	1.82	.68	.71	.69	.165	589	33	.06	29.1	0/11
22	T	NCK1	C	81	04/12/78	09°07'S 157°43'W	1540	32	-	-	.46	.47	.64	.081	504	23	.05	29.1	0/16
23	U	TUA1	C	107	19/12/78	15°38'S 145°34'W	0955	686	-	-	.40	.43	.65	.101	524	31	.06	28.2	0/6
24	X	MAQ1	C	102	11/01/79	08°58'S 140°20'W	1600	182	-	-	.38	.41	.71	.125	473	23	.05	28.0	1/11
25	Y	TUA1	C	88	13/01/79	12°35'S 143°26'W	1630	256	-	-	.47	.42	.70	.131	473	14	.03	28.4	0/8
26	Z	TUA1	C	99	22/01/79	16°15'S 145°58'W	1645	384	-	-	.43	.43	.71	.094	535	19	.04	27.9	0/12
27	AA	TUA1	C	140	24/01/79	16°07'S 146°06'W	1435	299	-	-	.44	.43	.68	.111	523	31	.06	27.8	1/12

28	AB	ZEA1	B	106	6/03/79	35°51'S 175°30'E	0700	690	12	1.74	.54	.57	.72	.086	467	11	.02	20.7	11/11
29	AC	ZEA1	B	90	8/03/79	37°41'S 177°26'E	0920	556	1	.18	.45	.56	.70	.108	548	23	.04	20.9	10/10
30	AE	ZEA1	B	99	20/03/79	35°47'S 175°20'E	0810	976	8	.82	.48	.57	.66	.142	463	13	.03	21.0	10/10
31	AF	NSW1	A	87	5/04/79	36°04'S 150°24'E	1030	218	1	.46	.66	.66	.72	.171	431	10	.02	21.3	12/12
32	AG	NSW1	A	144	8/04/79	35°06'S 151°04'E	0920	745	3	.40	.71	.66	.71	.132	464	29	.06	21.1	9/9
33	AH	NSW1	A	98	9/04/79	34°58'S 151°05'E	1605	764	6	.79	.56	.66	.72	.120	459	11	.02	21.6	13/13
34	AJ	QLD1	A	109	1/05/79	17°56'S 148°22'E	0800	457	3	.66	.69	.67	.67	-	639	24	.04	26.2	0/4
35	AK	QLD1	A	98	2/05/79	17°31'S 148°05'E	1615	123	-	-	.66	.67	.63	-	477	14	.03	26.2	9/10
36	AL	QLD1	A	110	3/05/79	16°22'S 150°12'E	1105	726	11	1.52	.67	.66	.70	-	480	17	.04	27.2	19/19
37	AM	PNG2	A	110	20/05/79	7°36'S 149°47'E	1700	482	6	1.25	.67	.66	.75	-	511	16	.03	28.8	5/6
38	AN	PNG2	A	109	3/06/79	4°04'S 151°01'E	1500	904	11	1.22	.70	.66	.69	-	542	27	.05	31.0	0/10
39	AO	TRK2	A	73	10/11/79	8°41'N 152°08'E	1215	334	6	1.80	.56	.65	.69	.139	512	31	.06	28.9	4/8
40	AP	PON2	A	63	11/11/79	7°38'N 155°22'E	1330	99	-	-	.56	.64	.74	.145	337	21	.06	29.0	10/10
41	AQ	PON2	A	76	11/11/79	7°39'N 155°20'E	1630	196	-	-	.63	.64	.69	.083	335	18	.05	29.0	10/10
42	AR	PON2	A	165	16/11/79	7°02'N 158°25'E	1245	396	1	.25	.63	.63	.69	.110	306	19	.06	29.5	10/10
43	AS	PHO2	B	61	02/12/79	3°35'S 174°15'W	1715	184	1	.54	.47	.53	.66	.120	395	32	.08	29.4	11/11
44	AV	MAQ2	C	83	23/12/79	10°02'S 139°30'W	1810	406	-	-	.43	.41	.72	.090	519	35	.07	29.1	0/9
45	AW	MAQ2	C	71	16/01/80	8°53'S 139°56'W	1600	902	1	.11	.42	.41	.66	.136	469	21	.04	28.9	0/9
46	AX	MAQ2	C	112	17/01/80	8°53'S 139°51'W	1400	789	-	-	.41	.41	.70	.097	424	19	.04	28.6	2/10
47	AY	GAM2	C	53	5/02/80	23°49'S 133°49'W	1700	174	-	-	.35	.39	.78	.125	459	27	.06	26.7	8/9
48	AZ	TUA2	C	75	12/02/80	15°44'S 146°55'W	1750	259	-	-	.50	.44	.67	.122	448	17	.04	28.5	8/8
49	BB	AMS2	B	117	21/02/80	14°20'S 169°23'W	1415	610	-	-	.47	.59	.72	.098	504	24	.05	30.2	0/6
50	BC	NOR1	A	65	26/03/80	29°28'S 168°20'E	1520	239	-	-	.49	.60	.67	.144	618	34	.06	22.0	12/12
51	BD	FIJ2	B	103	10/04/80	18°37'S 177°49'E	1030	417	-	-	.52	.56	.72	.137	507	34	.07	27.6	0/6
52	BE	WAL2	B	121	22/05/80	14°20'S 178°16'W	1545	477	-	-	.53	.55	.74	.115	449	32	.07	28.2	3/8
53	BG	SOL2	A	113	18/06/80	8°40'S 159°36'E	1030	1,533	-	-	.71	.63	.69	.113	445	21	.05	28.4	7/11
54	BH	PON3	A	128	18/07/80	7°07'N 158°18'E	0810	959	-	-	.68	.63	.75	.119	621	22	.04	29.1	0/11
55	BJ	PON3	A	80	20/07/80	7°03'N 157°55'E	0830	394	-	-	.56	.63	.67	.084	499	18	.04	29.3	0/10
56	BK	PAL3	A	88	9/08/80	3°00'N 131°36'E	1615	282	-	-	.77	.74	.76	.233	332	12	.04	29.2	10/10
57	BL	PAL3	A	116	18/08/80	7°41'N 134°10'E	1340	2,348	-	-	.64	.73	.75	.174	379	18	.05	28.7	10/10
58	BM	PAL3	A	121	19/08/80	7°48'N 134°18'E	1045	1,363	-	-	.67	.73	.68	.213	383	19	.05	28.6	10/10

[1] Tags at large for 90 or more days as of 31 November 1980.