1982

The ecological genetics of Daphnia species.

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THE ECOLOGICAL GENETICS OF *DAPHNIA* SPECIES

by

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A Thesis
submitted to the Faculty of Graduate Studies
through the Department of Biology
in Partial Fulfillment of the requirements
for the Degree of Master of Science at
The University of Windsor

Windsor, Ontario, Canada

1982

777472

To my mother

According to the principle of competitive exclusion, ecologically similar species cannot achieve stable coexistence in a natural habitat. Since ecological differentiation is a consequence of genetic diversification, it was assumed that the probability of coexistence and the degree of genetic similarity would be inversely related. In order to test this assumption, the competitive abilities of ten electrophoretically distinct D. pulex clones with known genetic similarites were studied under laboratory conditions. In contrast to expectations, genetic divergence did not foster clonal coexistence; coexistence occurred most frequently between genetically similar clones. Moreover, the competitive abilities of several stocks of electrophoretically identical clones isolated from different habitats were measured and found to be quite similar.

Previous allozyme studies carried out on populations of the cyclic parthenogen Daphnia magna revealed that populations from central Canada (Churchill) were nearly invariant, while those from England were segregating at about one-third of their loci. The lack of variation in Churchill populations was attributed to the isolation of this site from glacial refuges in which D. magna survived the Pleistocene. In order to confirm this hypothesis, the genetic diversity of populations of D. magna from a locality near the Yukon

glacial refuge and from two more southerly locales were estimated and compared to that of populations from Churchill and England. All populations surveyed were more variable than those at Churchill and the degree of genetic divergence observed between populations from different localities was consistent with the notion of a Bering land bridge colonization of North America by Asian stocks of D. magna.

Earlier work indicated that populations of D. magna inhabiting permanent ponds show marked heterozygote excesses and linkage disequilibria as a consequence of extensive periods of uninterrupted parthenogenetic reproduction. In order to determine the time frame required for such heterozygote excesses to develop, a laboratory aquarium was inoculated with a population of D. magna in Hardy-Weinberg equilibrium and genotypic frequencies were monitored regularly for a 4 month period. Large heterozygote excesses and marked linkage disequilibrium developed very rapidly in this simulated permanent habitat.

Many hypotheses have been generated in an attempt to account for species (or clonal) richness differences between habitats. It has been suggested that species (or clonal) diversity is positively correlated with habitat age. This hypothesis was not supported by studies of clonal diversity in obligate parthenogenetic

D. pulex populations inhabiting localities of different ages. Diversity levels in populations from a glacial refuge were high but were similar in magnitude to those from glaciated habitats. The lack of variation in clonal diversity levels was explained by assuming a rapid asymptotic approach to an equilibrium diversity.

Studies of the genetic diversity in populations of D. pulex and D. middendorffiana from the eastern and central arctic have revealed that these two forms are closely related genetically and may comprise a single agamic complex. Further genetic studies of populations of D. pulex and D. schodleri from the western arctic suggested that they might also be included in this agamic complex. Furthermore, populations of D. curvirostris were identified in the western arctic. Despite its morphological similarity to D. pulex, these two species were only distantly related. D. curvirostris appeared to reproduce by cyclic parthenogenesis.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to the chairman of my committee, Dr. P. Hebert, for his invaluable direction and for providing the unique opportunity of living and working in several areas across the Canadian arctic. The other members of my committee, Dr. I. M. Weis and Dr. K. Fung, were extremely helpful with various aspects of the data analysis and computer work. I also wish to acknowledge Dr. W. Benedict and Mr. J. Robinson for their assistance in the photomicroscopy of my specimens. In addition, the help in data collection given by Hugh MacIsaac, Bhooma Chandna, David McWalter and Dr. B. Hann was greatly appreciated.

Logistics support, room and board were willingly provided by Mr. G. Hobson, Director of the Polar Continental Shelf Project (Tuktoyaktuk Base Camp), Mr. J. Ostrick, Scientist-in-charge of the Inuvik Scientific Resource Centre and Mr. S. Hurry, Manager of the Hudson Bay Co-op in Old Crow, Y.T.

Finally, I wish to offer my heartfelt thanks to my family and friends, especially Hugh MacIsaac and Jess Zimmerman, for providing a wealth of support and good times throughout my graduate career.

I also acknowledge receipt of an NSERC Postgraduate Scholarship for the years 1980-1982.

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THE DYNAMICS OF COMPETITION BETWEEN *DAPHNIA PULEX* CLONES

CHAPTER I

INTRODUCTION

Allozyme studies have revealed abundant genotypic diversity in natural populations of Daphnia pulex reproducing by obligate parthenogenesis (Hebert and Crease 1982). Of the eleven southwestern Ontario habitats surveyed, ten contained two or more clones. Genetic distances among the clones were often substantial, indicating that clonal diversity had not arisen via mutation in each habitat. Evidently separate clones had been introduced and become established. The prevalence of within habitat clonal diversity conflicted with the widely held notion that competitive interactions should prevent the coexistence of closely related species or clones. Subsequent laboratory studies (Loaring & Hebert 1981) on four Daphnia pulex clones revealed the existence of ecological differences among them. In contrast to the field situation, the clones showed rapid competitive exclusion in the laboratory. It can be argued that the constant environmental conditions and the small size of the containers in which the experiments were conducted may have promoted competitive exclusion. Two clones coexisting in a pond through microhabitat differentiation might not be able to do so in the homogenous environment provided by a glass jar containing only a litre of water. It would be useful to demonstrate that the same outcomes

of simplified laboratory competition experiments would also be obtained in a more heterogenous environment. It was towards this end that competition experiments between two pairs of Daphnia pulex clones were set up in large aquaria maintained under natural conditions. In addition, these large tanks permitted a more detailed analysis of the nature of clonal interactions and shifts in fitness.

When competition experiments were carried out in jars, the results were only surveyed on a single occasion, as determining clonal frequencies required destroying most of the population. However, the aquarium populations could be analyzed on a frequent basis. Thus, it was possible to plot the trajectories of clone frequency change. Such trajectories should make it possible to determine if the relative fitnesses of clones are stable or variable. When coupled with population density estimates, it can be ascertained whether shifts in clone frequencies occur at times of population growth or collapse. Additional measurements of the reproductive distributions in the populations would reveal any associations existing between reproductive phenotype (parthenogenetic, ephippial, non-reproductive or male) and genotype. Finally, supplementary data on the physical and biological environment may permit the identification of the selective agents important in competitive interactions.

In summary, the purpose of the present study was to determine if experiments between clones of Daphnia pulex

performed in small containers at constant temperatures are meaningful representations of the competitive interactions in larger and more variable environments. In addition, this work aimed to elucidate the mechanism by which competitive displacement occurred, and the possible selective agents involved. To achieve these goals, genotype frequencies were monitored in competition experiments involving two pairs of Daphnia pulex clones studied by Loaring and Hebert (1981). Clone 1 was competed against clone 13 because their studies revealed clone 1 to be the best competitor, and clone 13 to be the worst; yet these two clones coexist in nature. Clone 4 was paired with clone 6 as this was the only combination which coexisted in the earlier laboratory studies. In addition to determining clone frequencies, the density of the Daphnia populations and the reproductive condition of the adults were surveyed on a weekly basis. The Daphnia studies were supplemented by surveys of algal densities and water temperatures.

MATERIALS AND METHODS

On April 14, 1981, four aquaria containing 300 l of artificial pond water (for composition see Hebert and Crease 1980) were placed on the roof of the University of Windsor Biology building. No attempt was made to shelter the tanks; they were exposed to direct sunlight, rainfall and colonization by airborne propagules. Each tank was inoculated with 4 l of an aquarium cultured algal suspension composed primarily of Scenedesmus and Kirschneriella (at approx. densities of 200,000 cells/ml and 1,000,000 cells/ml respectively). Algal growth was stimulated by placing 5 goldfish in each aquarium for an 8-day period. The fish were then removed and 150 juveniles of two clones were added to each aquarium. Clones 1 and 13 were placed in tanks 1 and 2, and clones 4 and 6 in tanks 3 and 4. Twenty-six days later (week 4 of the experiment), the Daphnia populations had attained sufficient numbers for weekly sampling to commence. On each sampling date, the Daphnia were evenly distributed throughout the aquarium by thoroughly mixing the water with a metal rod, while taking care not to create a vortex. Each tank was then sampled at each end and in the centre using a plastic cylinder (diameter = 13.5 cm). Nitex netting (mesh size = 250 μ) was slid under the cylinder and securely attached to ensure complete collection of animals in the cylinder. The volume of water sampled

was calculated using the formula $\pi r^2 d$ where r = radius of the cylinder and d = depth of water in each aquarium. The daphnids in each sample were enumerated and categorized as adult or juvenile on the basis of body size. Mean population sizes and standard errors were estimated for each aquarium. Supplementary samples were taken if the ratio of standard error/mean population size exceeded 0.25 in the initial three samples.

The maximum and minimum water temperatures for the 7-day period previous to the sampling day were recorded. Since water temperature readings did not begin until 5 weeks after sampling commenced, the maximum and minimum water temperatures for weeks 9-25 were regressed on the weekly high and low mean air temperatures respectively. Regression equations accounted for 84.9% of the variation in maximum water temperature and 85.5% of the variation in minimum water temperature (Appendix I). Maximum and minimum water temperatures for weeks 4-8 were then estimated using these regression equations. Water temperature estimates for weeks 1-3 may not be reliable since the weekly high and low mean air temperatures recorded for these weeks extended beyond the range of values used in the regression analysis. Mean weekly water temperatures were defined as the mean of the maximum and minimum water temperatures for that week. Statistical analyses were carried out on an IBM 3031 computer using Statistical Analysis System programs.

In addition, each week, five 10 ml algal samples were taken from randomly selected positions throughout each aquarium, mixed together, and added to 2 ml of Lugol's iodine. Algae sampling commenced two weeks prior to the first week of Daphnia sampling. Organisms present in the algal samples were later enumerated and classified to the division level using a Nikon inverted microscope (magnification 200X). In order to facilitate data analysis, two algal categories were defined: group one consisted of all Chlorophytes, Chrysophytes and Cryptophytes, while group two consisted of the Cyanophytes. One algal sample collected from tank 1 was improperly preserved and could not be enumerated.

To determine clonal frequencies, 96 adult D. pulex randomly selected from the weekly sample taken from each aquarium were electrophoresed. Remaining individuals were returned to their respective tanks. When 96 adults were not present in the sample, fewer animals or large juveniles were chosen for electrophoresis. Prior to electrophoresis, adults were classified according to their reproductive phenotype. Electrophoretic techniques were similar to those used by Hebert and Crease (1980); lactate dehydrogenase and phosphoglucose isomerase phenotypes were used to distinguish the clones.

2x2 tests of independence using the G-statistic were performed on 21 samples of individuals in order to determine if similar proportions of each clone were carrying

parthenogenetic broods. Contingency tables were discarded if the expected frequency of any cell was less than 1 or if greater than 25% of the expected frequencies were less than 5.

Experiments were terminated when the proportion represented by one clone was less than or equal to 0.02 for four consecutive weeks or, if this never occurred, at the end of the first week in October (week 25). No samples were taken in week 24.

Correlation and stepwise regression analyses were made on the weekly samples taken from each aquarium in order to determine the effects of environmental variables on Daphnia population size and reproductive distribution. Daphnia densities were regressed on algal densities and mean water temperature; and reproductive phenotype proportions were regressed on algal densities, mean water temperature and Daphnia densities. Values of regressor variables measured prior to week 4 (the first week of Daphnia sampling) were not included in the analyses since reliable water temperature estimates could not be obtained for these weeks.

All regression and correlation analyses incorporated a timelag inasmuch as a certain time period elapses between the environmental stress on the animals and their reaction to it (Seitz 1980; Hazelwood and Parker 1961 and 1963; Slobodkin 1954; Frank 1952; Edmondson, Comita and Anderson 1962). The choice of a timelag should depend on the

metabolic rate of the animals which is, in turn, a function of temperature. Since water temperatures were generally quite warm throughout the duration of this experiment, all analyses incorporated a lag of only one week. In order to lag the dependent variable the values of the regressor variables for week n were advanced to week n+1. By these means, the relationship between the values of the environmental variables measured one week prior to the values of the dependent variables may be estimated.

RESULTS

Genotype Frequency Shifts

Competitive exclusion of clone 13 by clone 1 was observed in both tanks 1 and 2 (Fig. 1.1 and 1.2) (Appendix II). Similarly, clone 4 completely displaced clone 6 in tank 3 and was present in significantly higher densities than clone 6 in tank 4 (Fig. 1.3 and 1.4) when the experiment was terminated in early October. Despite the lack of variation in final outcome, significant differences in clone frequency shifts were observed between replicates. Clone 13 persisted for a much longer period of time in tank 1 than in tank 2. In fact, in tank 1, clones 1 and 13 coexisted at relatively equal densities until week 14 when clone 13 sustained a substantial frequency reduction. In contrast, in tank 2, clone 13 was present in significantly lower densities than clone 1 by week 4, and was completely excluded by week 11. Differences between replicates were even more pronounced in tanks 3 and 4 (Appendix II). Clones 4 and 6 coexisted in tank 3 in roughly equivalent proportions until week 7 when the frequency of clone 4 increased drastically. No major genotype frequency shifts were observed beyond this point and exclusion of clone 6 was attained by week 20. However, in tank 4, clones 4 and 6 coexisted in similar densities until week 9, when clone 6 underwent a density increase.

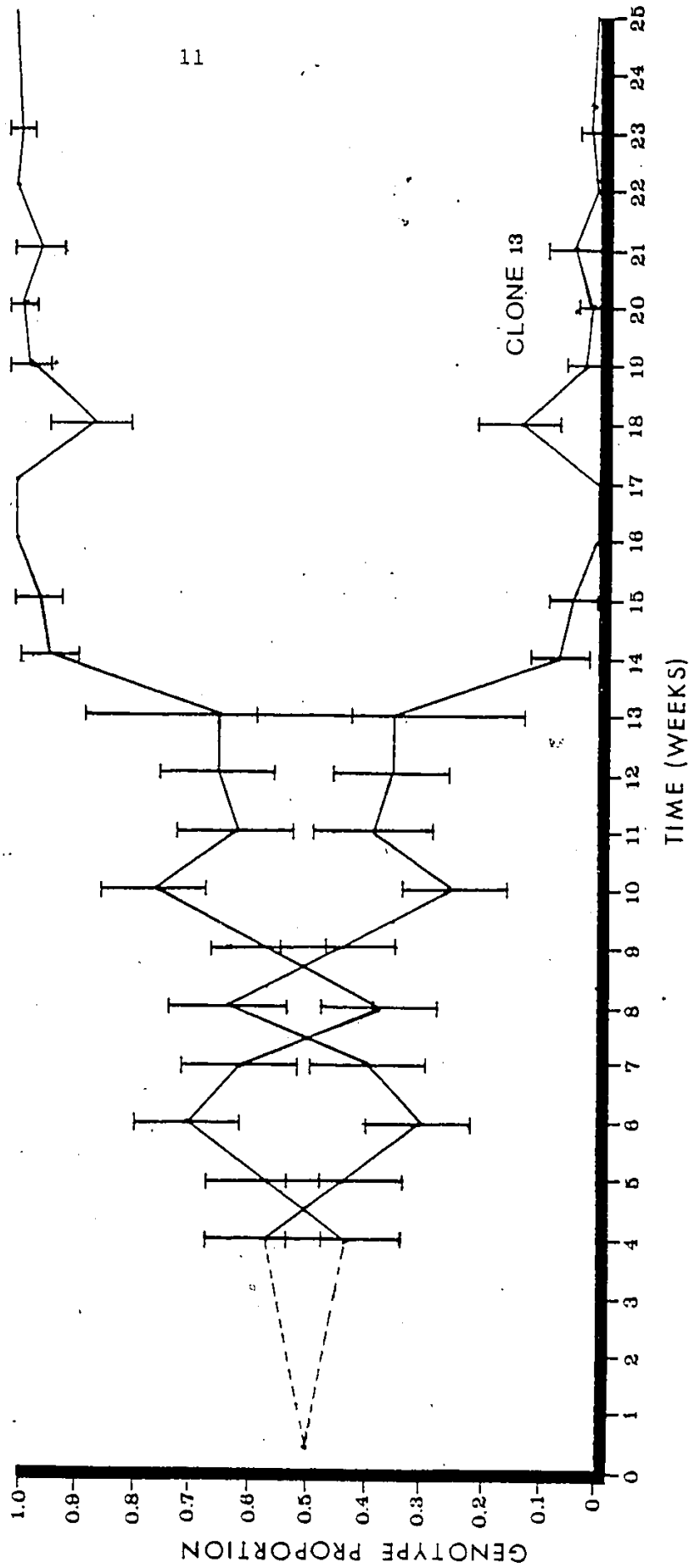


Figure 1.1 Temporal variation in genotype frequencies in aquarium 1 (with 95% confidence limits).

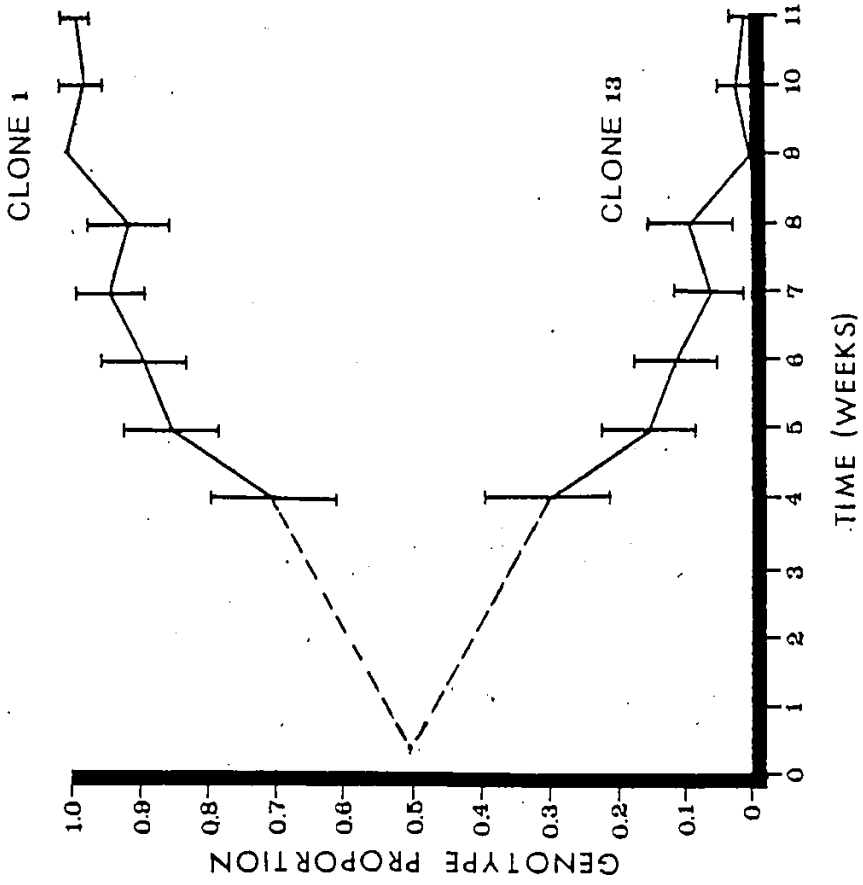


Figure 1.2 Temporal variation in genotype frequencies in aquarium 2 (with 95% confidence limits).

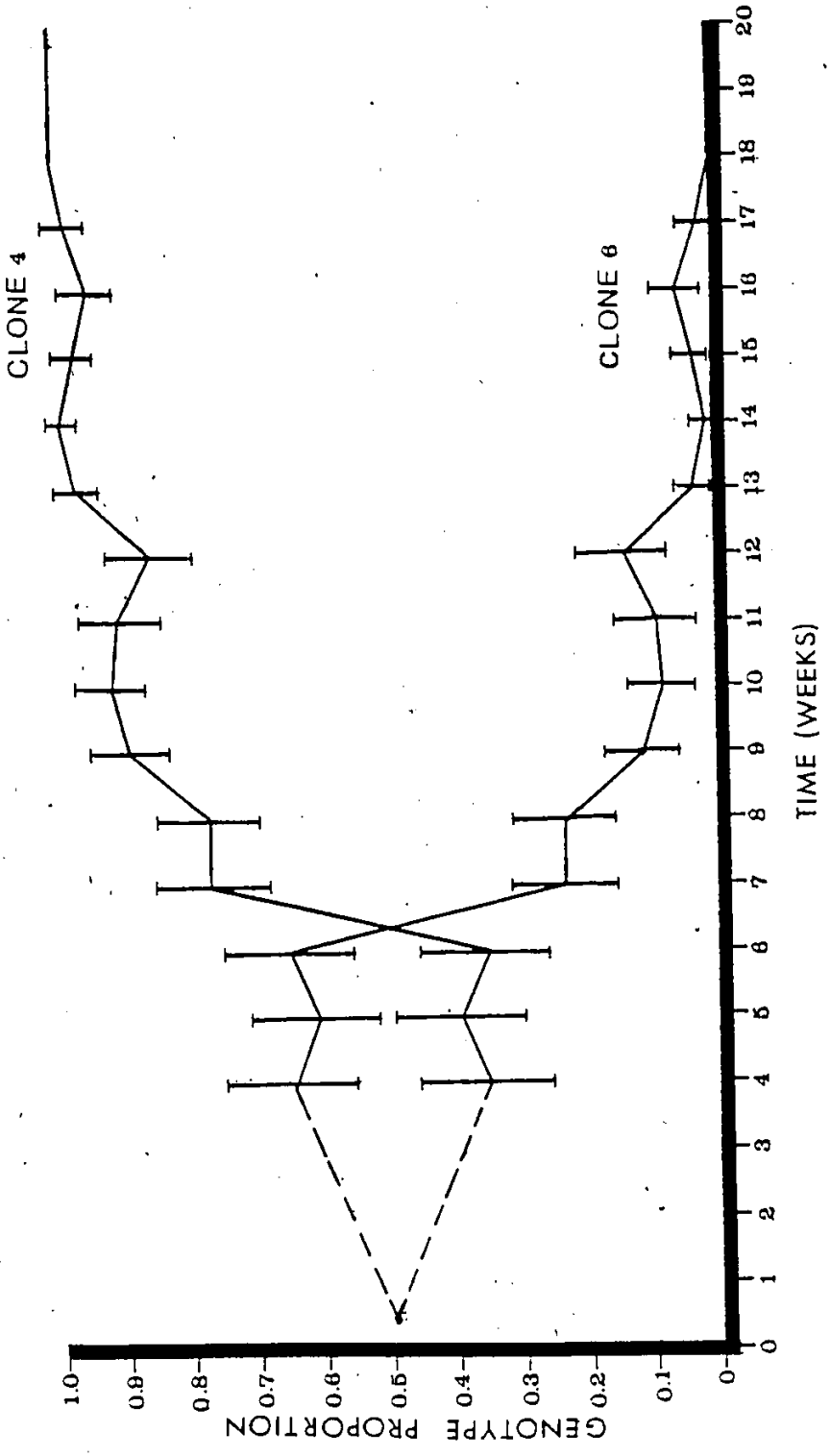
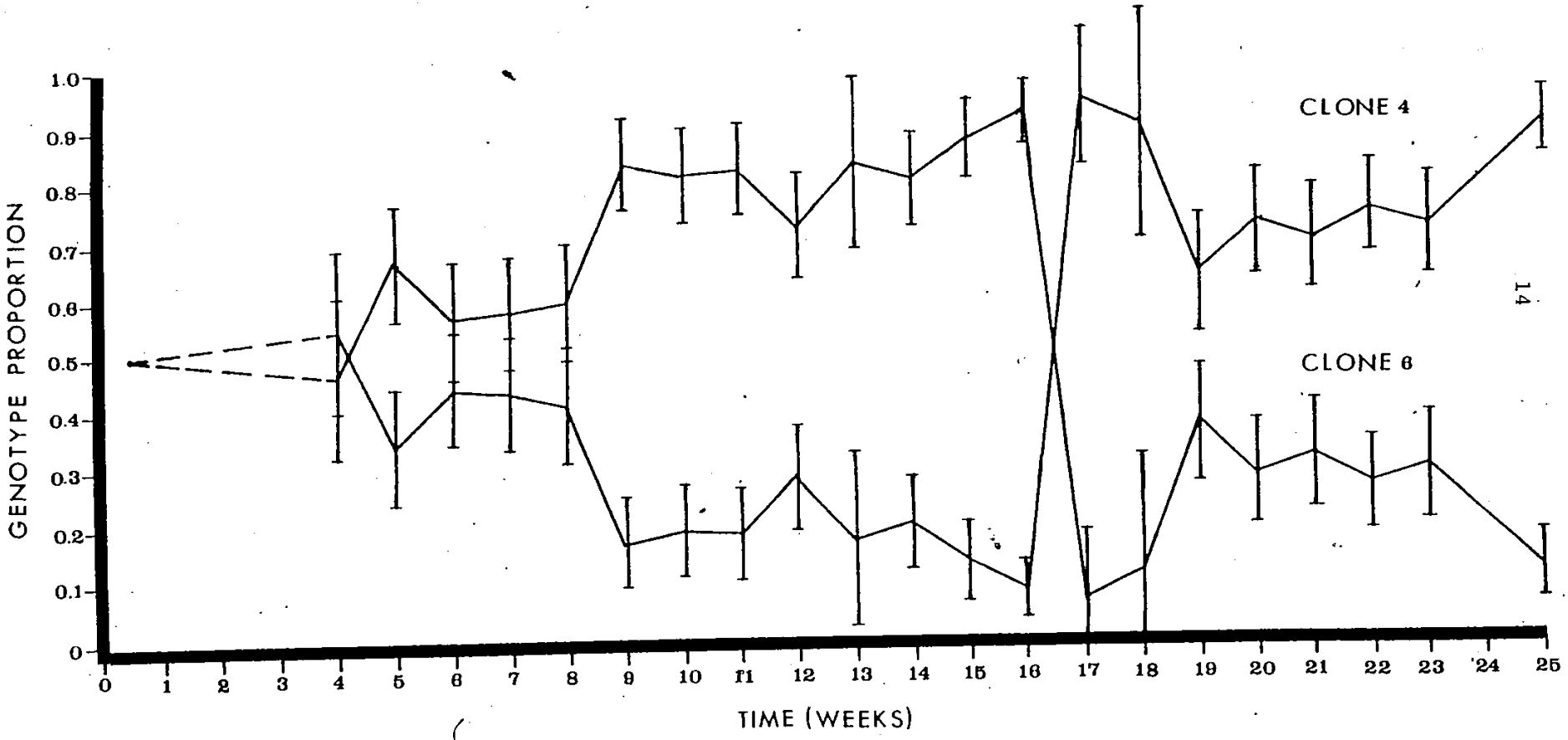


Figure 1.3 Temporal variation in genotype frequencies in aquarium 3 (with 95% confidence limits).

Figure 1.4 Temporal variation in genotype frequencies in aquarium 4 (with 95% confidence limits).



The frequency of clone 6 exceeded that of clone 4 until a major genotype frequency shift occurred between weeks 16 and 17. After week 17, clone 4 coexisted with clone 6, but in significantly higher densities.

With the exception of tank 2, all tanks exhibited periods of relative stability that were occasionally interrupted by large shifts in clonal frequencies. Presumably, these divergences corresponded to crisis periods in which the fitness of one clone greatly exceeded that of the other. Therefore, the relative fitnesses of competing clones were not constant but fluctuated throughout the course of the study.

Daphnia Population Variables

All aquaria exhibited marked temporal fluctuations in Daphnia densities (Fig. 1.5-1.8) (Appendix II). Of the 1-3 total density peaks observed in each tank, the first peak in late spring-early summer was invariably the largest. Generally, juvenile maxima were closely followed by adult maxima, but adult Daphnia densities seldom reached the high levels achieved by the juveniles, indicating juvenile mortality.

Little variation in the patterns of daphnid abundance was observed between replicate tanks 1 and 2. Both the timing of the population maxima and the densities attained were roughly similar. However, this was not the case in replicate tanks 3 and 4. The first density peak of the

Figure 1.5 Temporal variation in adult and total Daphnia densities (with standard errors) in aquarium 1. Juvenile densities are represented by the difference between total and adult densities.

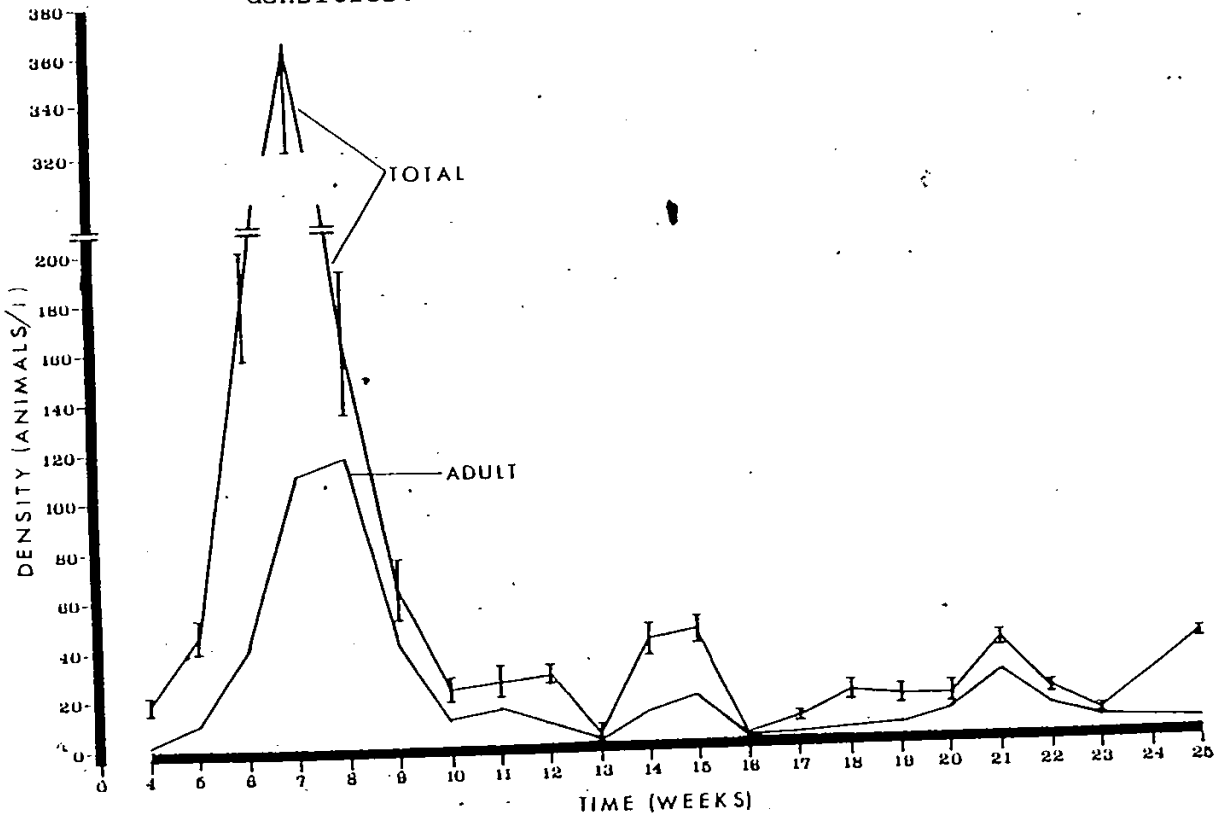


Figure 1.6 Temporal variation in adult and total Daphnia densities (with standard errors) in aquarium 2. Juvenile densities are represented by the difference between total and adult densities.

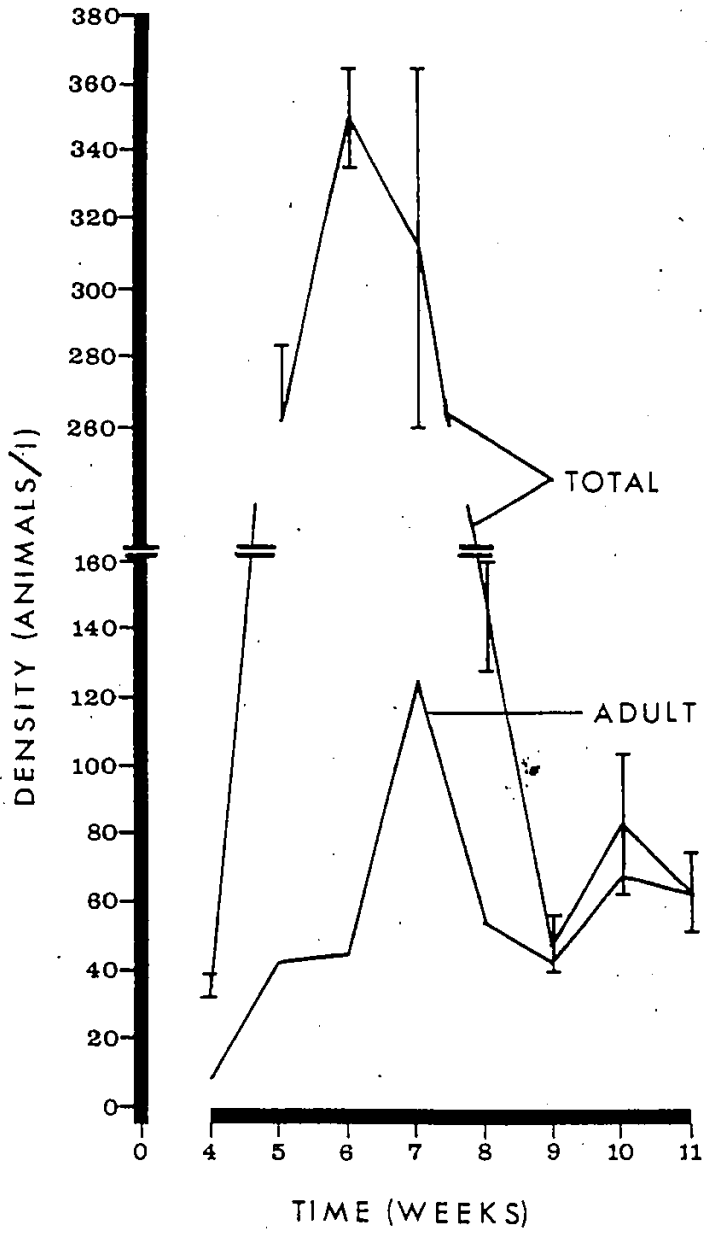
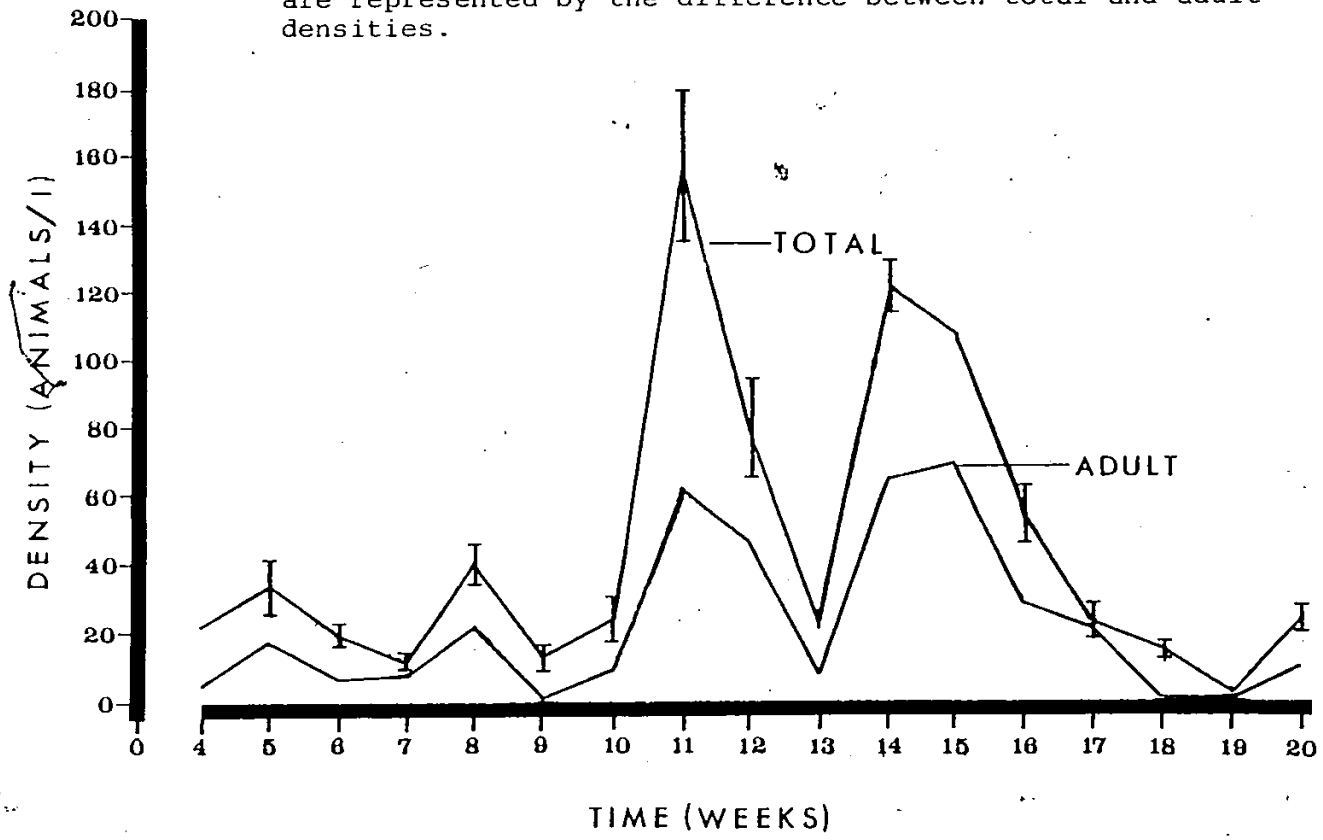


Figure 1.7 Temporal variation in adult and total Daphnia densities (with standard errors) in aquarium 3. Juvenile densities are represented by the difference between total and adult densities.



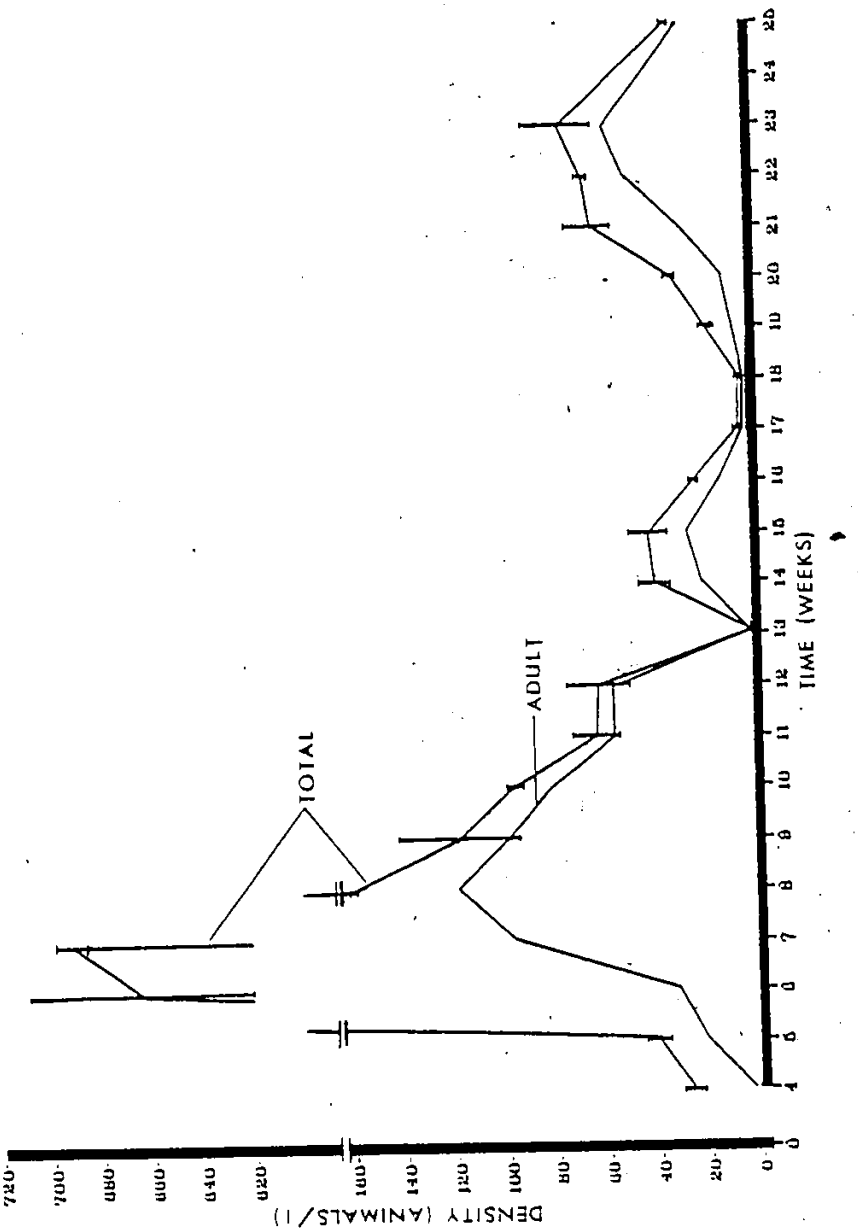


Figure 1.8 Temporal variation in adult and total Daphnia densities (with standard errors) in aquarium 4. Juvenile densities are represented by the difference between total and adult densities.

summer, consisting primarily of juveniles in both tanks, was 4.5 times larger and occurred four weeks earlier in tank 4 than in tank 3. Subsequently, the development of the first adult density peak in tank 3 lagged several weeks behind that in tank 4, although the densities attained in these peaks were not vastly different. Therefore, populations composed of the same clones, set up at the same time and in the same location, showed substantially different demographic changes.

The distribution of reproductive phenotypes is illustrated in Fig. 1.9-1.12 for each aquarium (Appendix II). Parthenogenesis continued throughout the summer and was a major form of reproduction. Ephippial production commenced after the fourth or fifth week while females with empty brood pouches were observed only after week 5. Males were never found in tanks 1 or 2 and they comprised a very small fraction of the adult population in tank 4. During week 9, males comprised a substantial proportion of the adult population in tank 3. This is somewhat misleading since the population was composed almost entirely of juveniles at this time.

The timing of changes in population reproductive distribution and the magnitude of these changes were generally similar between replicate tanks. The only notable exception was the earlier commencement of male production in tank 3. Electrophoretic analysis of the males indicated that they

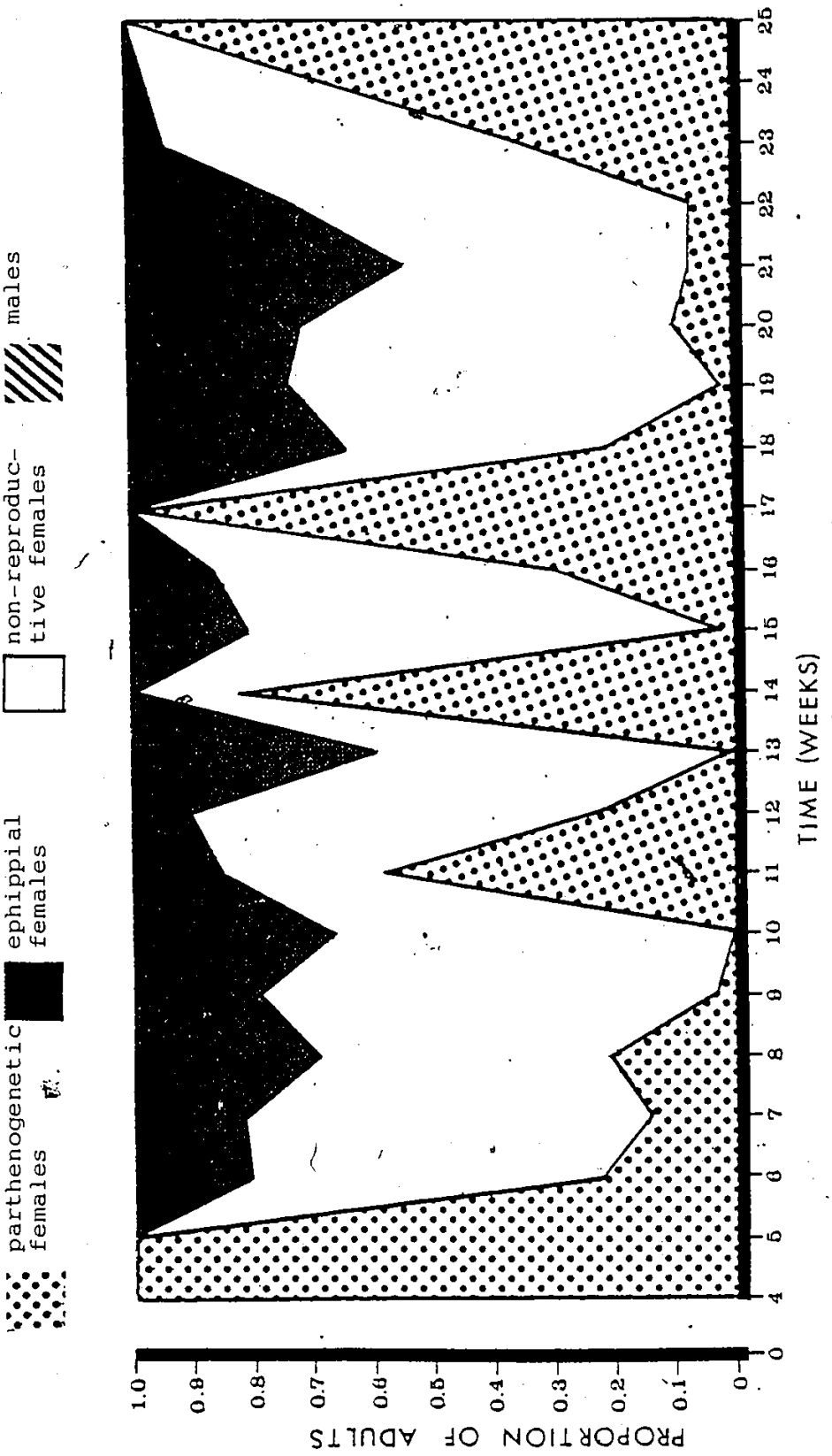
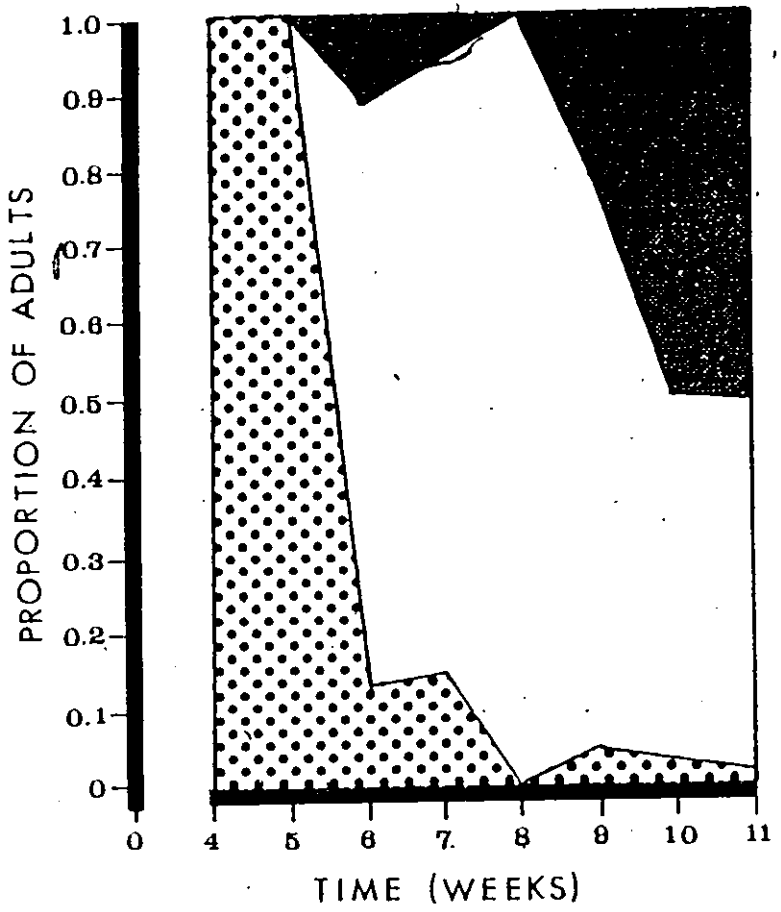


Figure 1.9 Distribution of reproductive phenotypes in aquarium 1.

Figure 1.10 Distribution of reproductive phenotypes in aquarium 2.



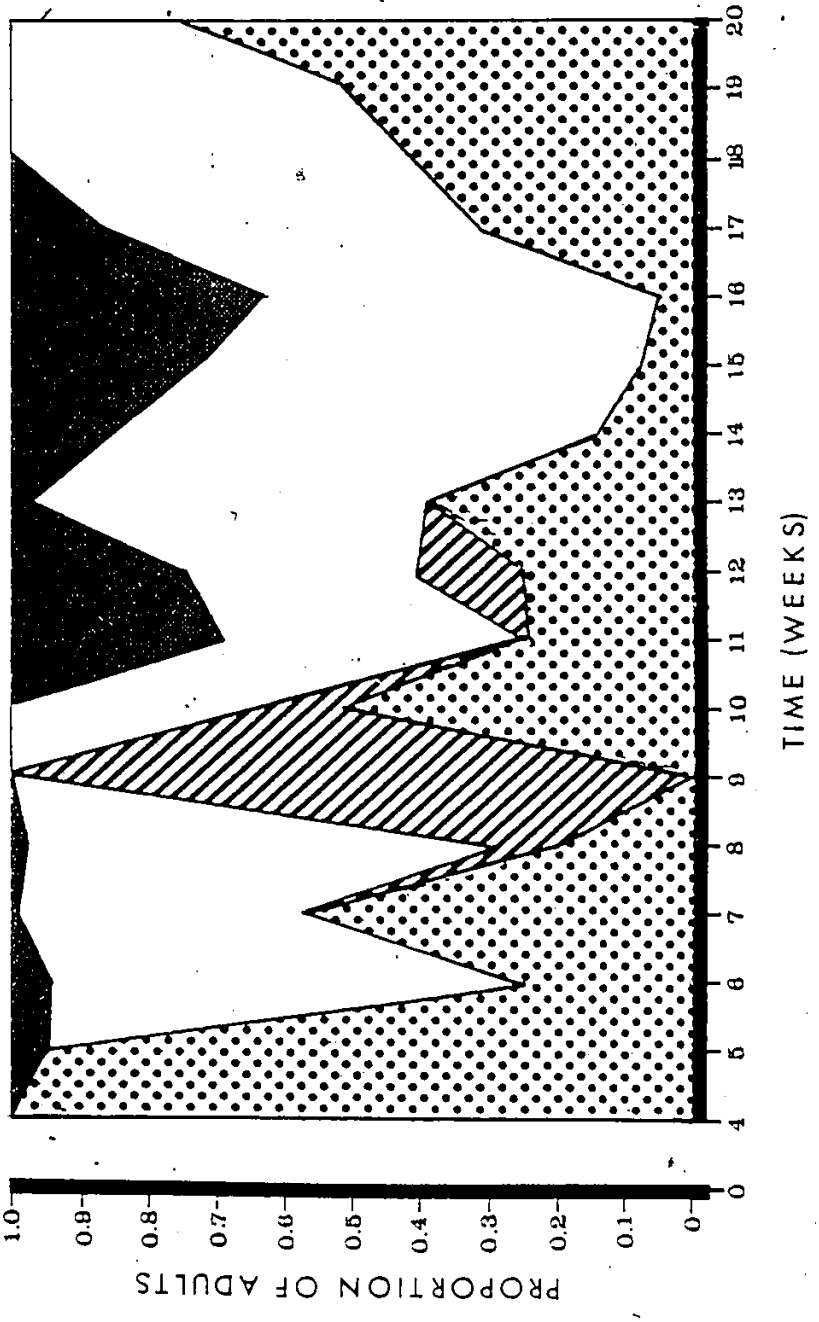


Figure 1.11 Distribution of reproductive phenotypes in aquarium 3.

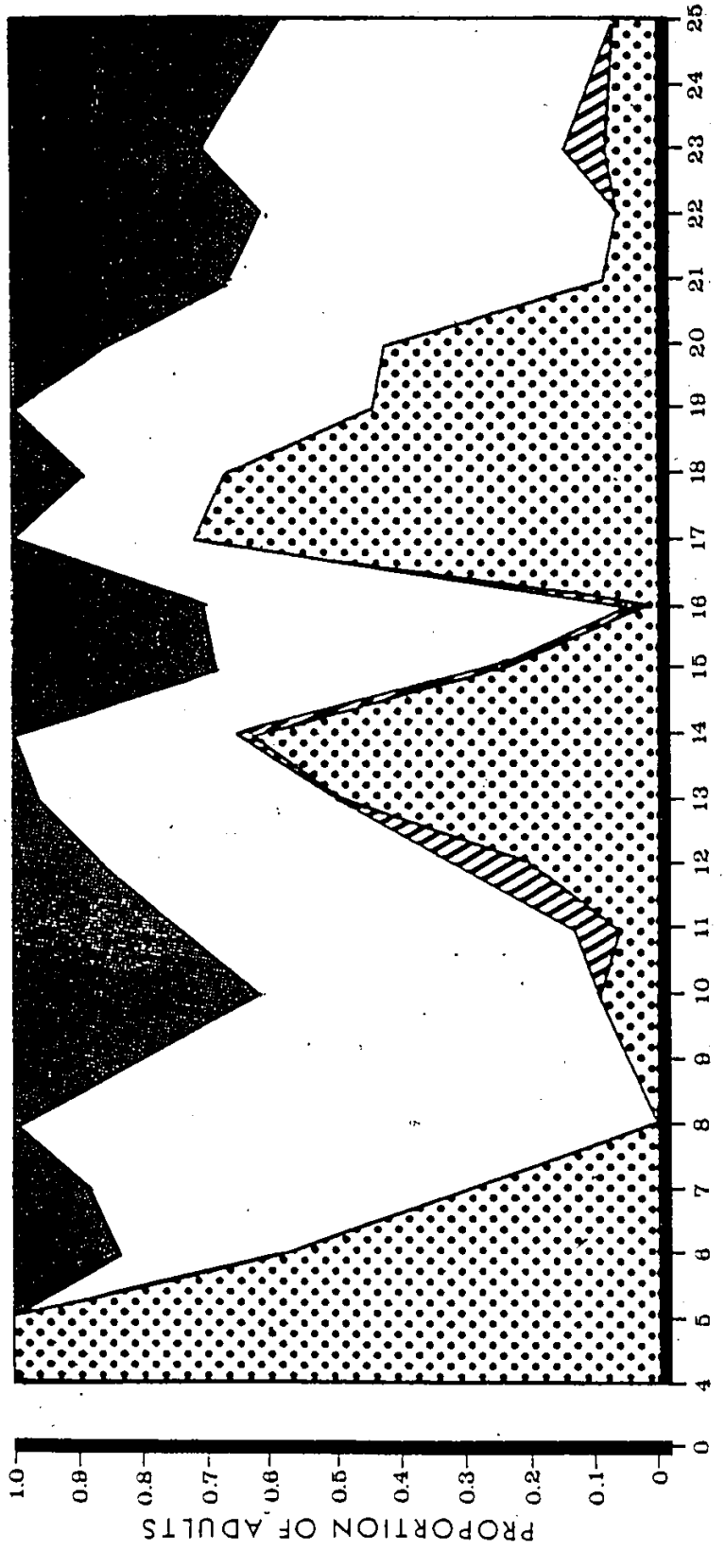


Figure 1.12 Distribution of reproductive phenotypes in aquarium 4.

all belonged to clone 4.

Eight of 21 samples revealed a significant (at the 5% level) association between reproductive phenotype and clone type (Table 1.1). In tank 1, clone 13 had a significantly higher proportion of parthenogenetic females than clone 1 in both weeks 6 and 7. The single sample tested from tank 2 showed no clonal differences. The proportion of clone 4 females with parthenogenetic eggs was significantly higher than the proportion of clone 6 in 3 samples (weeks 5, 6 and 8) from tank 3. Similarly, 3 samples (weeks 6, 7 and 20) from tank 4 revealed significantly higher proportions of parthenogenetic clone 4 females than clone 6.

Environmental Variables

Extreme fluctuations in algal densities (groups 1 and 2) were observed in all 4 aquaria (Fig. 1.13-1.16) (Appendix II). The timing of algal population peaks and the densities attained in these peaks, varied between replicate tanks. Preliminary algal samples taken during weeks 2 and 3 revealed that the algal population had attained very high densities and was already declining by the time Daphnia sampling commenced in tank 2. In contrast, the first algal peak observed in tank 1 did not occur until week 6 and was only half the magnitude of the first peak in tank 2. Similarly, algal densities had achieved very high levels and were declining when sampling began in tank 4. Algal densities never achieved very high levels in tank 3, and the

Table 1.1 2x2 Independence tests testing association between genotype and reproductive phenotype. Observed numbers of individuals are shown beside bracketed expected values in contingency tables.

*indicates significance at the 5% level.

Week	Aquarium	Contingency Table	G-statistic
5	3	Phenotype Clone | Parthenogenetic | Non-Parthenogenetic 6 | 46(51.01) | 13(7.99) | 59 4 | 37(31.99) | 0(5.01) | 37 83 | 13 | 96	13.92*
6	1	Phenotype Clone | Parthenogenetic | Non-Parthenogenetic 1 | 11(15.35) | 56(51.65) | 67 13 | 11(6.65) | 18(22.35) | 29 22 | 74 | 96	5.02*
6	2	Phenotype Clone | Parthenogenetic | Non-Parthenogenetic 1 | 12(10.63) | 73(74.38) | 85 13 | 0(1.38) | 11(9.63) | 11 12 | 84 | 96	2.94
6	3	Phenotype Clone | Parthenogenetic | Non-Parthenogenetic 6 | 6(15.75) | 57(47.25) | 63 4 | 18(8.25) | 15(24.75) | 33 24 | 72 | 96	22.86*

Table 1.1 Continued

Week	Aquarium	Contingency Table			G-statistic
		Phenotype			
		Clone	Parthenogenétic	Non-Parthenogenetic	
		6	39(45.00)	15(9.00) 54	
6	4	4	41(35.00)	1(7.00) 42	13.26*
			80	16 96	
		Phenotype			
		Clone	Parthenogenetic	Non-Parthenogenetic	
		1	14(19.05)	45(39.95) 59	
7	1	13	17(11.95)	20(25.05) 37	5.08*
			31	65 96	
		Phenotype			
		Clone	Parthenogenetic	Non-Parthenogenetic	
		6	15(16.5)	7(5.5) 22	
7	3	4	57(55.5)	17(18.5) 74	0.68
			72	24 96	
		Phenotype			
		Clone	Parthenogenetic	Non-Parthenogenetic	
		6	5(11.15)	52(45.85) 57	
7	4	4	13(6.85)	22(28.15) 35	10.88*
			18	74 92	
		Phenotype			
		Clone	Parthenogenetic	Non-Parthenogenetic	
		1	4(6.38)	32(29.63) 36	
8	1	13	13(10.63)	47(49.68) 60	1.82
			17	79 96	

Table 1.1 Continued

Week	Aquarium	Contingency Table			G-statistic
			Phenotype		
		Clone	Parthenogenetic	Non-Parthenogenetic	
8	3	6	12(18.76)	10(3.24)	22
		4	69(62.24)	4(10.76)	73
			81	14	95
			Phenotype		
		Clone	Parthenogenetic	Non-Parthenogenetic	
9	4	6	6(8.33)	74(71.67)	80
		4	4(1.67)	12(14.33)	16
			10	86	96
			Phenotype		
		Clone	Parthenogenetic	Non-Parthenogenetic	
10	4	6	10(8.94)	68(69.06)	78
		4	1(2.06)	17(15.94)	18
			11	85	96
			Phenotype		
		Clone	Parthenogenetic	Non-Parthenogenetic	
11	1	1	37(35.63)	23(24.68)	60
		13	20(21.38)	16(14.63)	36
			23	39	96
			Phenotype		
		Clone	Parthenogenetic	Non-Parthenogenetic	
12	1	1	3(5.22)	57(54.78)	60
		13	5(2.78)	27(29.22)	32
			8	84	92

18.12*

3.56

0.90

0.36

1.96

Table 1.1 Continued

Week	Aquarium	Contingency Table			G-statistic
			Phenotype		
		Clone	Parthenogenetic	Non-Parthenogenetic	
12	3	6	5(3.00)	7(9.00)	12
		4	13(15.00)	47(45.00)	60
			18	54	72
					1.96
			Phenotype		
		Clone	Parthenogenetic	Non-Parthenogenetic	
14	4	6	47(47.82)	30(29.18)	77
		4	12(11.18)	6(6.82)	18
			59	36	95
					0.20
			Phenotype		
		Clone	Parthenogenetic	Non-Parthenogenetic	
15	4	6	20(21.00)	64(63.00)	84
		4	4(3.00)	8(9.00)	12
			24	72	96
					0.48
			Phenotype		
		Clone	Parthenogenetic	Non-Parthenogenetic	
18	1	1	14(13.13)	49(49.88)	63
		13	1(1.88)	8(7.13)	9
			15	57	72
					0.66
			Phenotype		
		Clone	Parthenogenetic	Non-Parthenogenetic	
20	4	6	5(12.66)	22(14.34)	27
		4	40(32.34)	29(36.66)	69
			45	51	96
					12.96*

Table 1.1 Continued

Week	Aquarium	Contingency Table			G-statistic
		Phenotype			
		Clone	Parthenogenetic	Non-Parthenogenetic	
21	4	6	2(3.44)	28(26.56)	30
		4	9(7.56)	57(58.44)	66
			11	85	96
					1.08
		Phenotype			
		Clone	Parthenogenetic	Non-Parthenogenetic	
22	4	6	3(1.82)	22(23.18)	25
		4	4(5.18)	67(65.82)	71
			7	89	96
					1.02

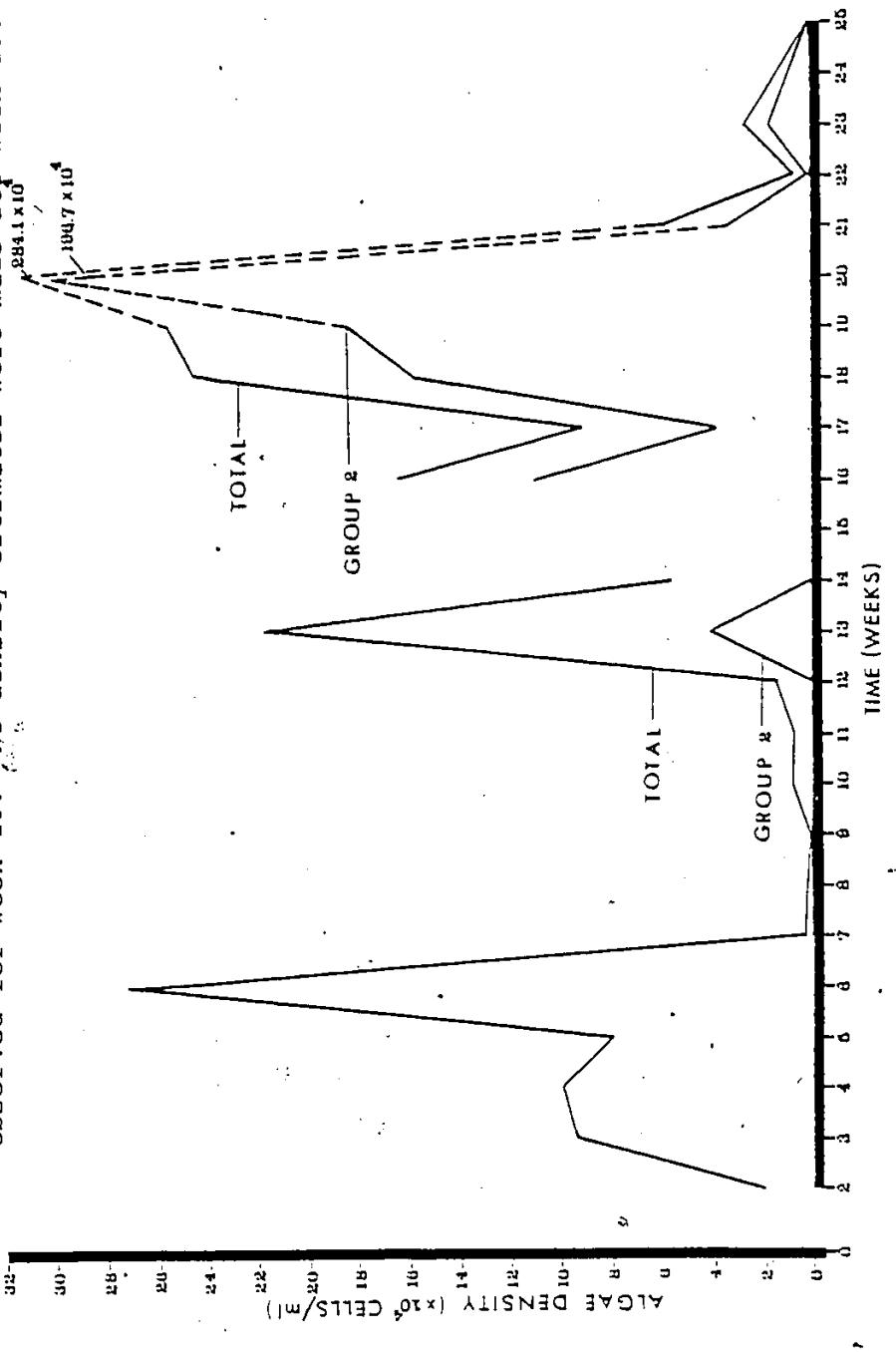


Figure 1.13 Temporal variation in group 2 and total algae densities in aquarium 1. Group 1 algae densities are represented by the difference between total and group 2 densities. Dotted lines indicate extremely high densities observed for week 20. No density estimates were made for week 15.

Figure 1.14 Temporal variation in total algae densities in aquarium 2. All algae belonged to group 1.

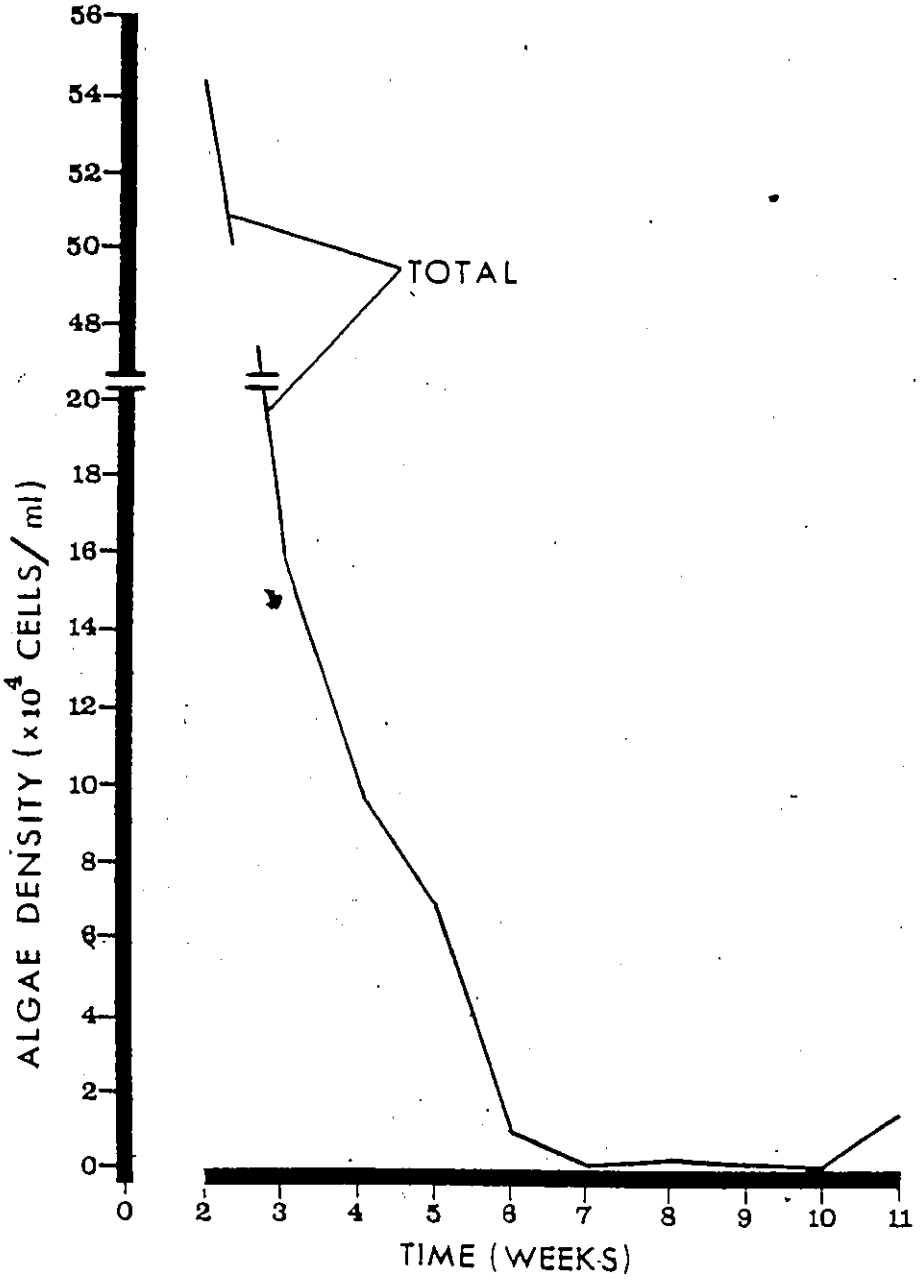
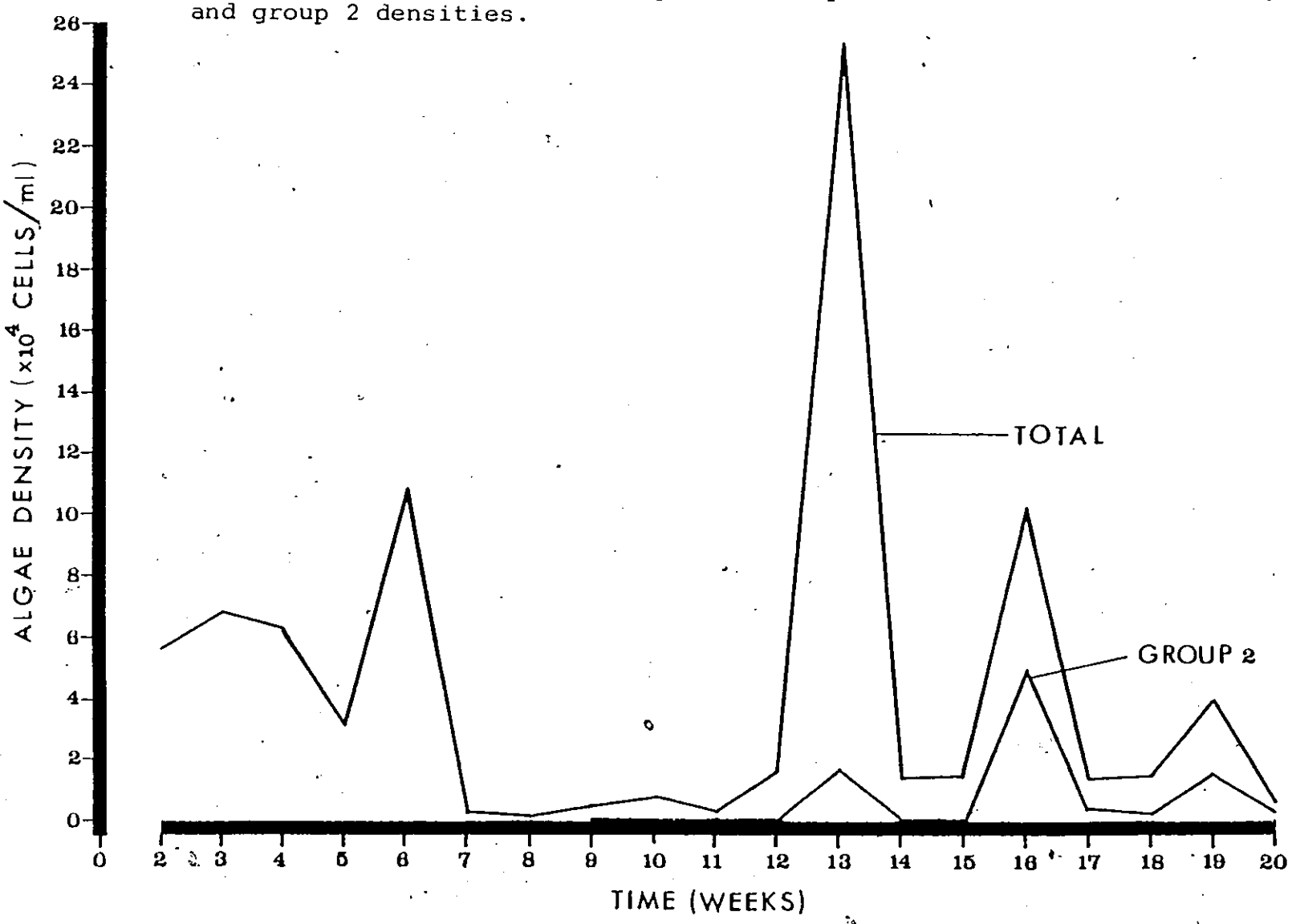


Figure 1.15 Temporal variation in group 2 and total algae densities in aquarium 3. Group 1 algae densities are represented by the difference between total and group 2 densities.



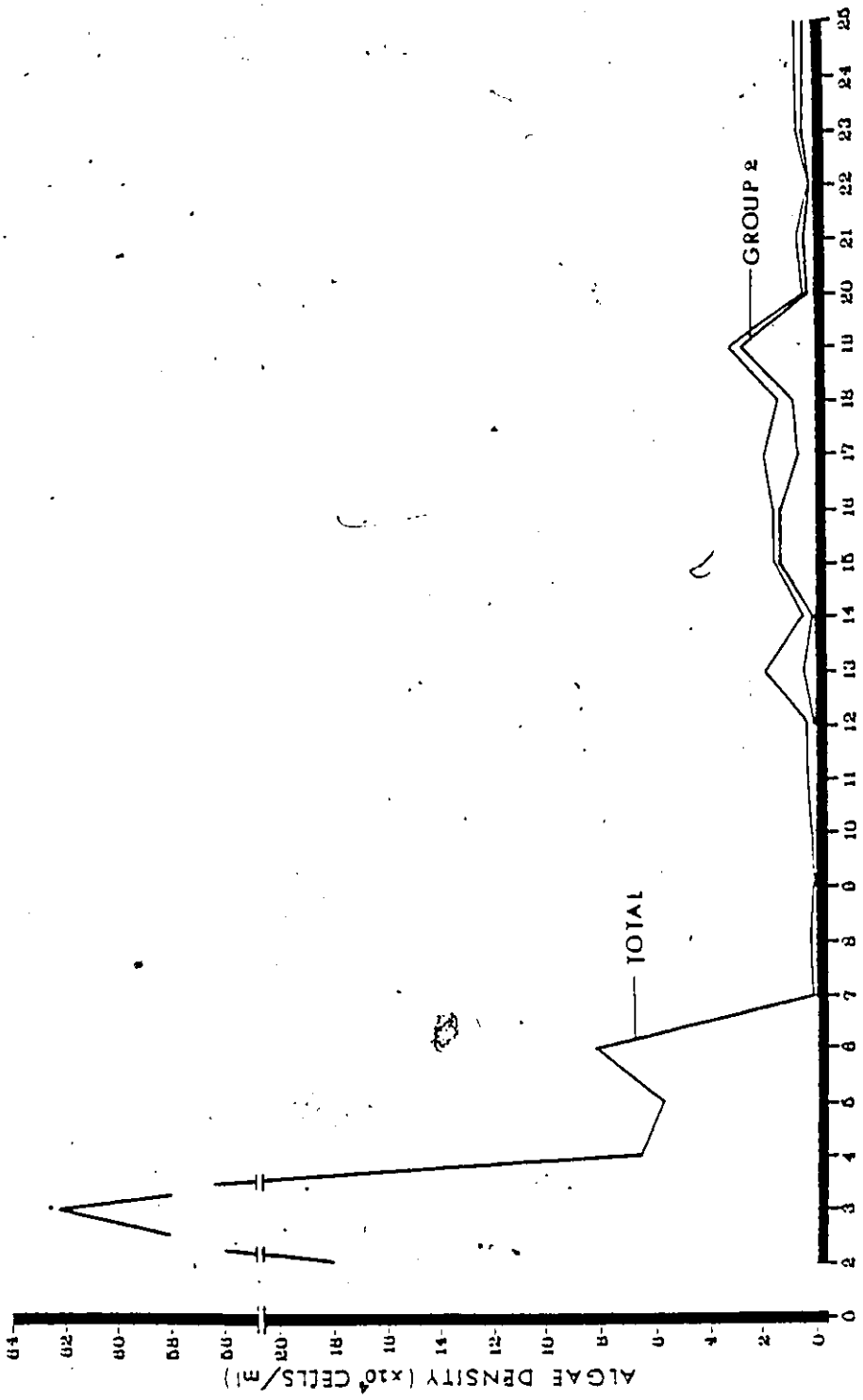


Figure 1.16 Temporal variation in group 2 and total algae densities in aquarium 4. Group 1 algae densities are represented by the difference between total and group 2 densities.

highest peak was not observed until week 13. Species of blue-green algae did not appear in significant numbers until week 13, but they comprised a large fraction of the algal populations in all tanks after this date.

Maximum, minimum and mean water temperatures are plotted against time in Fig. 1.17. Mean water temperatures climbed steadily through the early weeks of the experiment, but remained between 20°C and 26°C from week 8 to week 20. After this date, mean water temperatures slowly declined. While mean temperatures were within the tolerance limits of the species, diurnal temperature exceeded 30°C in weeks 9, 12 and 13.

Stepwise Regression Analysis

Correlation matrices (not presented) revealed strong intercorrelations between many of the environmental and population variables. The results of stepwise regression analyses performed for each aquarium using Daphnia densities as the dependent variable and environmental variables as the regressors, are summarized in Table 1.2. Regressor variables, listed according to their order of entry into the model, were retained only if their inclusion resulted in a significantly larger R^2 at the 5% level. The F values given are those calculated for each regressor variable included in the final model.

These analyses show that mean water temperature accounted for significant amounts of variation in Daphnia densities in 3 of 4 tanks (1, 2 and 4). As water temperatures climbed

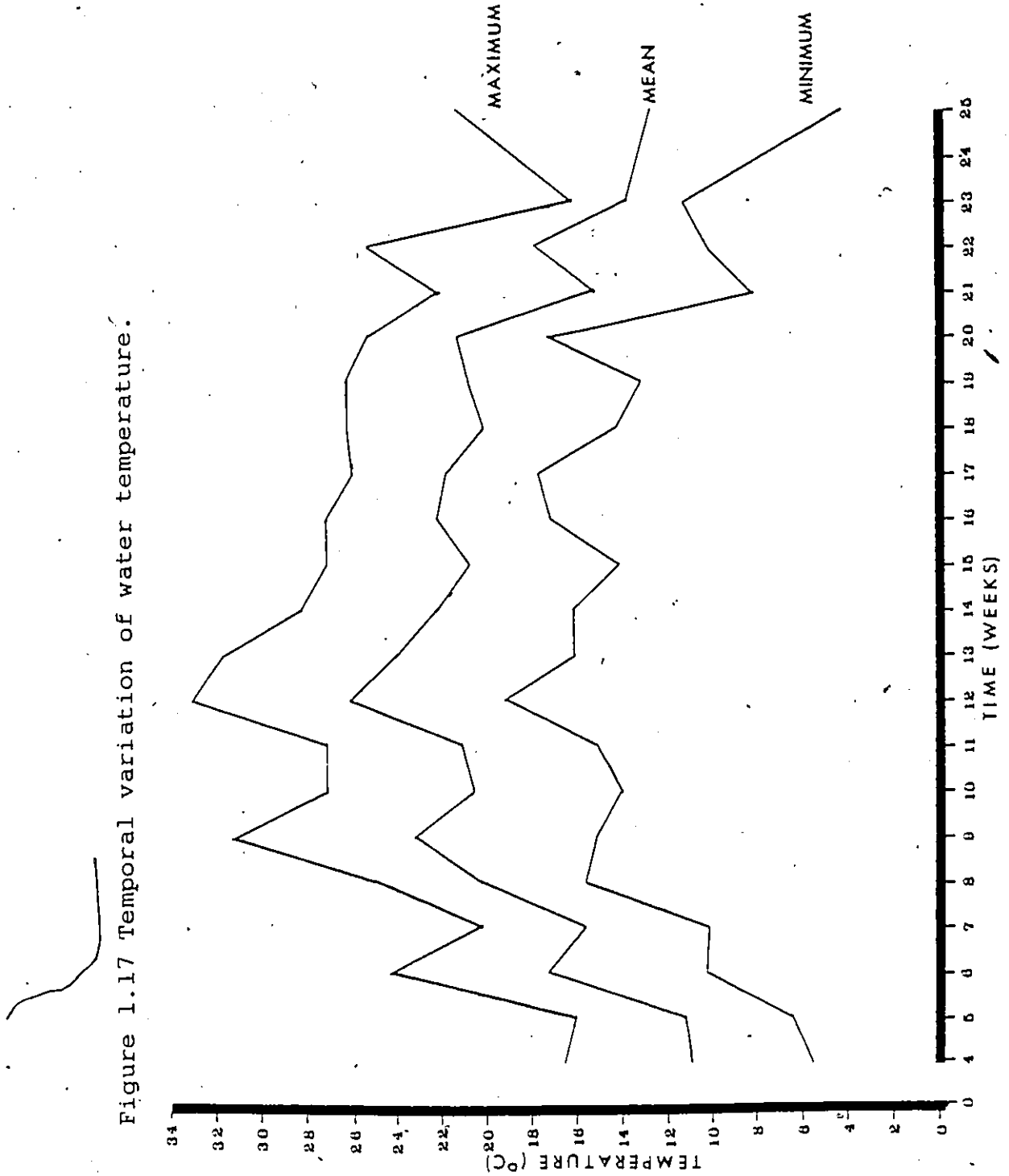


Figure 1.17 Temporal variation of water temperature.

Table 1.2 Stepwise regression of ln total *Daphnia* densities on mean water temperature, ln group 1 algal densities, and ln group 2 algal densities.
* indicates significance at the 5% level.

Aquarium	n	Regressor Variable	r	Intercept	Regression Coefficient	F	P value	R^2
1	19	mean water temperature	-0.48	6.01	-0.14	6.14*	0.0240	0.2653
2	7	mean water temperature	-0.81	7.26	-0.13	9.71*	0.0264	0.6600
3	16	mean water temperature	0.16	2.64	0.04	0.38	0.5458	0.0266
4	20	mean water temperature	-0.55	8.01	-0.23	7.93*	0.0114	0.3059

Daphnia population size was reduced. The influence of groups 1 and 2 algal densities on total Daphnia densities was insignificant when mean water temperature was taken into account.

The results of stepwise regression analyses using reproductive phenotype proportions as the dependent variables and mean water temperature, groups 1 and 2 algal densities and total Daphnia densities as regressors are presented in Tables 1.3-1.6.

There was a significant negative correlation between total Daphnia density and the proportion of parthenogenetic adults in 3 of the 4 tanks (1,3 and 4). Group 1 algal density exerted a significant effect on the proportions representing this phenotype in 2 tanks (2 and 4), while group 2 algal density and mean water temperature were retained in the model only in tank 1.

Total Daphnia density significantly influenced the proportion of adults carrying ephippia in tanks 1 and 3. Group 2 algal density was also retained in the model in tank 1, while mean water temperature significantly influenced the proportion of ephippial females in tank 2. No significant model was obtained in tank 4.

Group 1 algal density and total Daphnia density significantly influenced the proportion of non-reproductive females in tanks 2 and 4. Total Daphnia density, group 2 algal density and mean water temperature exerted a significant effect on this dependent variable in tank 1. No significant model was obtained in tank 3.

Group 1 algal density was the most important variable

Table 1.3 Stepwise regression of proportion of adult population represented by parthenogenetic females (arcsine-square root transformed) on mean water temperature, ln group 1 algal densities, ln group 2 algal densities, and ln total *Daphnia* densities.
* indicates significance at the 5% level.

Aquarium	Regressor Variable	r	Intercept	Regression Coefficient	F	P value	R^2
1	ln total *Daphnia* density	-0.60	3.15	-0.44	42.63*	0.0001	0.7541
	ln group 2 algal density	-0.01		-0.06	14.97*	0.0015	
	mean water temperature	-0.23		-0.04	5.71*	0.0304	
2	ln group 1 algal density	0.76	-1.12	0.18	7.05*	0.0452	0.5849
3	ln total *Daphnia* density	-0.62	1.40	-0.23	8.68*	0.0106	0.3826
4	ln group 1 algal density	0.73	-0.51	0.17	17.34*	0.0007	0.6477
	ln total *Daphnia* density	-0.54		-0.09	5.84*	0.0272	

Table 1.4 Stepwise regression of proportion of adult population represented by ephippial females (arcsine-square root transformed) on mean water temperature, ln group 1 algal densities, ln group 2 algal densities and ln total Daphnia densities.
*indicates significance of variable at 5% level.

Aquarium	Regressor Variable	r	Intercept	Regression Coefficient	F	P value	R^2
1	ln total Daphnia density	0.50	-0.52	0.21	25.99*	0.0001	0.6250
	ln group 2 algal density	0.13		0.04	15.89*	0.0011	
2	mean water temperature	0.78	-0.56	0.06	7.73*	0.0389	0.6071
3	ln total Daphnia density	0.66	-0.28	0.16	10.59*	0.0058	0.4307
4	ln group 1 algal density	-0.40	0.95	-0.07	3.35	0.0839	0.1569

Table 1.5 Stepwise regression of proportion of adult population represented by non-reproductive females (arcsine-square root transformed) on mean water temperature, ln group 1 algal densities, ln group 2 algal densities and ln total *Daphnia* densities.
*indicates significance at the 5% level.

Aquarium	Regressor Variable	r	Intercept	Regression Coefficient	F	P value	R^2
1	ln total *Daphnia* density	0.62	-0.97	0.28	26.69*	0.0001	0.6711
	ln group 2 algal density	-0.10		0.03	5.92*	0.0280	
	mean water temperature	0.25		0.03	5.31*	0.0359	
2	ln total *Daphnia* density	0.89	-0.58	0.45	51.82*	0.0020	0.9466
	ln group 1 algal density	-0.51		-0.09	11.96*	0.0259	
3	ln total *Daphnia* density	0.25	0.42	0.08	0.97	0.3408	0.0650
4	ln group 1 algal density	-0.62	1.40	-0.10	8.54*	0.0095	0.5128
	ln total *Daphnia* density	0.52		0.07	4.50*	0.0490	

Table 1.6 Stepwise regression of proportion of adult population represented by males (arcsine-square root transformed) on mean water temperature, ln group 1 algal densities, ln group 2 algal densities and ln total Daphnia densities.
* indicates significance at the 5% level.

Aquarium	Regressor Variable	r	Intercept	Regression Coefficient	F	P value	R^2
3	ln group 1 algal density	-0.61	1.97	-0.19	8.46*	0.0114	0.3768
4	ln group 1 algal density	-0.35	0.27	-0.03	2.56	0.1267	0.1247

influencing male densities in both tanks 3 and 4. However, this regressor explained significant amounts of variation in the dependent variable only in tank 3.

Associations of Genotype Frequencies With Environmental Variables

Clone 13 was excluded by clone 1 from tank 2 in a much shorter period of time than in tank 1. Nevertheless, it is hypothesized that the exclusion of clone 13 from both tanks was the result of the greater fitness of clone 1 at low Daphnia densities and corresponding high algae levels. The following is a summary of the events that may have transpired in tank 1:

During weeks 4 and 5, the fitness of clone 1 exceeded that of clone 13 due to low Daphnia densities and increasing algae levels. This fitness differential may have been manifested as differences in clonal brood size since the proportion of parthenogenetic females represented by each clone were roughly equal and mortality was low at this time. By week 6, the population size of clone 1 significantly exceeded that of clone 13. Between weeks 6 and 7, Daphnia density peaked and algae levels decreased dramatically. Consequently, clone 1's fitness advantage was lost. This was reflected in the excess of parthenogenetic clone 13 females observed in weeks 6 and 7 (Table 1.1). By week 8, clone 13 was found in greater densities than clone 1. Between weeks 8 and 10, clone 1 regained its fitness advantage

as Daphnia population size had decreased and algae levels slowly began to rise. Accordingly, the majority of the dying daphnids observed at this time were probably clone 13. By week 10, the frequency of clone 1 exceeded that of clone 13. High temperatures in week 12 resulted in high algae levels and very low Daphnia densities by week 13. The fitness advantage of clone 1 was greatly pronounced under these conditions and was probably reflected in a clonal brood size differential. Therefore, the increase in Daphnia density between weeks 13 and 15 was probably due primarily to the increase in the proportion of clone 1.

Clone 1 was able to secure an early fitness advantage in tank 2 due to the early development of high algal densities while Daphnia density was still very low. Before sampling commenced, clone 1 females probably carried larger broods than clone 13 females such that by week 4, clone 1 was already present in significantly higher densities. Cooler temperatures prevented an increase in the Daphnia population until weeks 5 and 6. By this time, clone 13 comprised less than 15% of the total Daphnia population. Clone 13 may have maintained a fitness advantage from weeks 6-8 when high Daphnia levels and low algae densities prevailed, but its extremely low densities prevented it from increasing significantly within such a short time period. Although the frequency of clone 13 had increased slightly by week 8, the Daphnia population had been substantially reduced due to great amounts of juvenile mortality (60%-70%).

Consequently, clone 13 lost its fitness advantage and was rapidly excluded.

The results of this study suggest that clone 4 has a slight fitness advantage over clone 6 under conditions of low bluegreen algal densities, but that this fitness advantage is greatly magnified when bluegreen algae are abundant.

In tank 3, clonal frequencies remained stable for the first 3 weeks of sampling. By weeks 5 and 6, clone 4 had significantly more parthenogenetic females than expected (Table 1.1) due to its slight fitness advantage at low bluegreen algal densities. Since the time from birth to reproductive maturity averages about 8-9 days for these clones (pers. obs.) at the cooler spring temperatures (mean temperature 11°C-17°C), the increased production of clone 4 juveniles in weeks 5 and 6 was not apparent in the adult population until week 7. In week 8, most of the parthenogenetic females in tank 3 were clone 4 and as a result, a second increase in the frequency of clone 4 was observed by weeks 9 and 10. Clone 4 was already approaching fixation in week 13 when the bluegreen algae first appeared, but the effects of the bluegreen toxins may have accelerated the exclusion of clone 6.

As in tank 3, initial samples in tank 4 revealed similar clonal frequencies. The appearance of greater than expected numbers of parthenogenetic clone 4 females (Table 1.1) in

weeks 6 and 7 suggested that by weeks 8 and 9 an increase in the frequency of clone 4 adults would be observed. In contrast to expectations, clone 4 decreased in frequency in week 9. A drastic decrease in juvenile density had occurred between weeks 7 and 8, suggesting that the decline of clone 4 in week 9 may have been a consequence of selective juvenile mortality. Clone 6 was able to maintain high densities throughout this period since there were few clone 4 individuals to compete with. Bluegreen algae became dominant for the first time in week 15. The simultaneous appearance of bluegreen algae and the probable disappearance of the force selecting against clone 4 (perhaps oxygen tension or pH of the water in tank 4) placed clone 6 at a great disadvantage and by week 17, most of them had died out. Clone 4 maintained significantly higher frequencies throughout the remainder of the experiment, but clone 6 was never completely excluded.

DISCUSSION

Conditions in the aquaria were probably not atypical of those in a newly formed pond habitat in southwestern Ontario. The temporal fluctuations in algal densities and the patterns of algal succession observed in this study were characteristic of most temperate water bodies (George and Edwards 1974; Jacobs 1977; Nadin-Hurley and Duncan 1976). The increasing light and temperature levels promoted a springtime algae bloom consisting primarily of small Chlorophytes. When the spring bloom subsided, there were several irregular periods of increase and decrease in total algal densities which occurred in close association with Daphnia population oscillations. This relationship is explained by the stimulatory effects of elevated algal levels on zooplankton population growth (Slobodkin 1954; Clark and Carter 1974) and the control of phytoplankton population size by zooplankton grazing activity (George and Edwards 1974; Anderson, Comita and V-Engstrom-Heg 1955; Porter 1973). Finally, through the latter part of the summer and early autumn, the phytoplankton population was characteristically dominated by filamentous bluegreens and gelatinous green algae.

The Daphnia population dynamics were also typical of those observed in natural habitats. Populations were initiated in early spring, at a time when natural populations emerge from ephippial eggs. The densities in the tanks

rapidly increased and this increase was accompanied by the appearance of ephippial and non-reproductive females and decreases in the parthenogenetic fraction of the population. Other workers have shown that increased density is followed closely by increased numbers of ephippial females (Elborn 1966; Slobodkin 1954) and a decreased birth rate (Frank, Boll and Kelly 1957; Frank 1952). Mean water temperature and algal densities also exerted a signficiant effect on the distribution of reproductive phenotypes. Decreasing group 1 algal densities (green algae and desmids) and increasing group 2 algal densities (bluegreens) and increasing water temperatures resulted in fewer females carrying parthenogenetic broods and greater numbers of non-reproductive and ephippial females. Related studies have demonstrated that reduced food ration decreases brood size (George and Edwards 1974; Lampert 1978; Green 1956), number of broods per lifetime (Weglenska 1971) and birth rate (Wright 1965).

Indications of substantial juvenile mortality during periods of low food levels were noted in this study. Other workers (Lynch 1980; Goulden et al. 1980) have suggested that under conditions of food limitation, juveniles are more susceptible to starvation than adults and the physiological response to low food supply prolongs exposure of small juveniles to the adverse conditions associated with food limitation. That is, as food becomes scarce, the growth

rate decreases while the age at reproductive maturity increases. In addition, Neill (1975) attributed heavy mortality among juveniles of filter feeding species to the effects of competing for food with Ceriodaphnia. Therefore, taking into account the food-limiting conditions of the aquaria, the high rates of juvenile mortality observed in this study are not surprising. Presumably, the competitively inferior clones suffered greater juvenile mortality than the competitively superior clones. Consequently, juvenile mortality may be a key factor in the means by which one clone excludes another.

Daphnia density was significantly negatively correlated with temperature in 3 of the 4 tanks. Laboratory studies have shown that water temperatures higher than 20°C-25°C can severely restrict development, egg production and filtering capacity in species of Daphnia (Kibby 1971; Burns and Rigler 1967; Burns 1969; Tauson 1931; Green 1956; Ivleva 1969; Craddock 1976). Temperatures in the tanks were frequently greater than 25°C, and on occasion, as high as 33°C. Samples collected the week following the occurrence of such high temperatures generally exhibited low Daphnia densities. Similarly, natural Daphnia populations from southwestern Ontario living in unshaded ponds usually die out during the first period of high temperature (pers. obs.). Therefore, it seems likely that high temperatures exert a significant selective impact on natural populations. It is

suggested that within an optimal temperature range, natural Daphnia densities would be most strongly influenced by food levels (Clark and Carter 1974) and positively correlated with temperature (Moore 1980; Hazelwood and Parker 1963; George and Edwards 1974). However, the high water temperatures observed in this study restricted Daphnia population growth such that food levels were able to exert little influence.

Eight of twenty-one samples of D. pulex revealed significant genotypic differences in the proportion of parthenogenetic females. Large genotype frequency changes were often, but not always, observed within the following 2 week period. Similarly, Hebert (1974) found strong correlations between the parthenogenetic fecundities of different D. magna genotypes and their frequencies one month later. In the present work, there were several occasions during periods of population increase, that large changes in genotype frequencies were observed in the absence of clonal differences in the proportion of parthenogenetic females. The frequency shifts may have been caused by clonal variation in brood size, but unfortunately, egg production was not measured in this study. Some of the major shifts in genotype frequencies occurred during periods of population decline, indicating that differential mortality as well as natality must be considered.

It is noteworthy that clone 4 was apparently the only

clone to retain male production. As D. pulex reproduces by obligate parthenogenesis (Hebert and Crease 1980), males serve no function and their production is possible evidence of a lack of adaptation. Other workers (Frank 1957) have attributed the competitive exclusion of a species to its excessive production of male offspring. It is significant then, that clone 4 was a superior competitor to clone 6. Evidently, the small amounts of male production by clone 4 was offset by other fitness attributes.

This study has provided firm evidence of ecological differences between Daphnia pulex clones maintained under conditions closely approximating those found in nature. Clones competitively superior under constant laboratory conditions at temperatures common in a temperate habitat, were also competitively superior in a more natural setting subject to fluctuating environmental factors. However, competitive exclusion was not achieved in these experiments through a constant selective force acting against one genotype causing a slow steady decline in its frequency. Instead, large genotype frequency changes occurred during periodic "crisis" situations. Critical events such as algae or Daphnia population crashes or bluegreen blooms appeared to trigger large shifts in genotype frequencies. It seems likely that the selective forces acting in nature are also variable and their effects are manifested most clearly under conditions of environmental change.

Differing reactions of species and clones to environmental change have been previously documented. In a study of the midsummer dynamics of Daphnia pulicaria and D. galeata mendotae in Wintergreen Lake, Michigan, Threlkeld (1979) suggested that an observed decrease in reproduction by D. pulicaria in mid-July was the result of an interaction of high temperatures, declining standing crops of small algae and increasing amounts of Anabaena, Ceratium and Volvox. D. galeata mendotae did not show any adverse response to these mid-July algae-temperature conditions. Jacobs (1978) speculated that a seasonal succession of available food particles altered the competitive abilities of two Daphnia species. Similarly, it is hypothesized that the fitness of clone 1 exceeded that of clone 13 under conditions of low Daphnia densities and corresponding high algae levels.

The coexistence of clones 4 and 6 in a jar at 23°C (Loaring and Hebert 1981) suggested that fitness differences between these clones were very slight. The results of this study suggested that when bluegreen algae density was low, the fitness of clone 4 slightly exceeded that of clone 6. Under such conditions, clone 6 would eventually be excluded, but only after a rather lengthy period of coexistence. However, when Cyanophytes became prevalent, clone 6 underwent a large fitness reduction. The rapid reaction of clone 6 suggested that the bluegreens may have liberated some toxin

which selectively killed clone 6 individuals. The toxic effect of bluegreen algae on cladocerans has been well documented (Porter 1977; Stangenberg 1968; Arnold 1971; Porter and Orcutt 1980; Carmichael and Gorham 1977). Porter and Orcutt (1980) reported that bluegreen algal toxins may be detoxified or tolerated by animals maintaining a high food intake. It is possible that clone 6 has reduced detoxification capabilities in comparison with clone 4. In support of this hypothesis, Snell (1980) found that rotifer genotypes differ in their sensitivies to bluegreen toxins such that the reproductive rates of certain genotypes are reduced to a greater extent than others during a bluegreen bloom.

Replicate tanks showed variation in Daphnia densities, algal densities and the time required for one clone to competitively exclude another. This between replicate variation may have been due to a large number of factors. Predaceous dytiscid beetle larvae and ostracods managed to colonize each tank at different times throughout the experiment. Although the beetle larvae were removed, they may have selectively preyed upon one clone during their short stay. The ostracods were virtually impossible to remove once they were established. Some tanks had larger ostracod populations than others. It is possible that the ostracods may have exerted enough of a competitive effect on the Daphnia population so as to account for some of the variation observed between tanks. Moreover, unmeasured factors such as pH or

oxygen tension may have differed between aquaria. Finally, other workers have found that Daphnia feed not only on algae, but on detritus and bacteria as well (Porter 1977; Nadin-Hurley and Duncan 1976; Taub and Dollar 1968; Gophen 1977; Edmondson 1957; Lampert 1974; Peterson and Hobbie 1978). No estimate was made on the amount of food provided by detritus or bacteria, nor of the extent to which Daphnia fed on them. If these two fractions differed either qualitatively or quantitatively between tanks, then this would account for some variation observed between replicates.

In conclusion, the present study has confirmed the existence of ecological differences between clones of Daphnia pulex maintained under semi-natural conditions. Moreover, the outcomes of the present competition experiments were similar to those obtained in jars. Competitive exclusion of one clone by another occurred through a series of crisis situations in which sudden environmental change brought great selective pressures to bear on one clone. Selection coefficients were variable both in magnitude and in direction and depended upon the type of environmental conditions encountered. It is clear that elucidation of the nature of fitness differences between clones in a natural habitat requires co-ordinate sudies on several environmental variables (food densities, water temperature, pH, oxygen tension), population variables (densities, egg production) as well as surveys of genotypic frequencies.

SUMMARY

Competitive interactions between four Daphnia pulex clones were studied under conditions closely approximating those found in nature. Competitive exclusion of one clone by another was brought about by large clone frequency shifts which occurred during periodic crisis situations. Thus, the relative fitnesses of competing clones were not constant, but shifted in response to environmental change. Clones found to be competitively superior in laboratory studies were also competitively superior in these more natural conditions.

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CHAPTER II

THE RELATIONSHIP BETWEEN GENETIC SIMILARITY AND THE INTENSITY OF COMPETITION IN CLONES OF *DAPHNIA PULEX*

CHAPTER II
INTRODUCTION

The competitive exclusion principal formulated by Gause in 1935 forbids the stable coexistence of two or more species having similar ecological requirements. Work in the late 1940's and 50's supported the notion that coexisting species in nature show clear niche separation. It came to be widely accepted that ecological differentiation was the principal way of escaping competitive displacement. Insofar as ecological differentiation is a consequence of genetic diversification, it has been implicitly assumed that there should be an inverse relation between the intensity of competition and the degree of genetic similarity. While this view has been accepted, no substantiating analyses have been provided; such study requires a group of reproductively isolated entities of variable genetic similarity, with similar ecological requirements. While a large sibling species complex might satisfy these criteria, none have been analyzed. Agamic complexes are more likely candidates for this type of study, as these complexes often include a number of clones with rather similar ecological characteristics. Obligate parthenogenetic populations of Daphnia pulex from southern Ontario represent such a complex. Hebert and Crease (1982) identified 39 clones of this species in their survey of 20 Ontario habitats. Cluster analysis indicated that the 39 clones fell into three distinct groups on the basis of genetic distance data. Three clones belonged

to group 1, nine to group 2 and twenty-seven to group 3. A laboratory study revealed that ecological differences existed among clones in traits such as intrinsic rates of increase and competitive abilities (Loaring and Hebert 1981). This work dealt with one clone belonging to group 1, two clones belonging to group 2 and a fourth belonging to group 3. Rather surprisingly, it was found that the two most closely related clones tended to coexist, while the more distantly related clones rapidly excluded one another. An objective of the present study was to test the generality of this observation. As a result, the competitive abilities of ten electrophoretically distinct clones were studied.

Past studies (Angus and Schultz 1979; Vrijenhoek 1979) have shown that electrophoretic analysis may overlook clonal diversity. By tissue graft studies, Angus and Schultz (1979) showed that electrophoretically identified clones of Poeciliopsis were not isogenic. To determine the extent of variation in competitive ability between electrophoretically recognized clones of D. pulex, stocks of a specific clone were isolated from 2 or more habitats, and then competed with a standard stock. Thus, the present study had two aims: to ascertain the relationship between genetic similarity and the intensity of competition in laboratory situations and also to assess the degree of ecological similarity among clones recognized as being electrophoretically identical.

MATERIALS AND METHODS

Pairwise competition experiments were set up between Windsor 1 clone 1 (group 1) and 13 stocks of 9 different group 2 and group 3 clones (Table 2.1). Windsor 1 clone 13 (group 3) was competed against 18 stocks of 9 different clones belonging to all 3 groups. Finally, Cedar Springs clone 4 (group 2) was competed against 6 stocks of clone 1, each isolated from a different pond. All pairwise combinations of clones were thus categorized into one of three combination types: group 1 vs. 2, 1 vs. 3, 2 vs. 3, 3 vs. 3.

Competition experiments were conducted in 1.8 l glass jars containing approx. 1 l of synthetic pond water (for composition see Hebert and Crease 1980). Three replicates of all pairwise clonal combinations were set up in Percival controlled environment chambers at 20 ± 1° C and twenty-four hr. light. Each replicate was initiated with 10 neonates of the two competing clones, born within the same 24 hr. period. No neonates from the brood of a primiparous female were used in these experiments since they are often smaller (Goulden et al. 1980) and may be less likely to survive. Sixty ml of an aquarium cultured algal suspension (primarily Scenedesmus and Kirschneriella at approx. concentrations of 2×10^5 cells/ml and 1×10^6 cells/ml respectively) mixed with desiccated liver (120 mg/l of algal suspension) were added to the jars three times per week, and 1 ml of vitamin concentrate was added once a

Table 2.1. Pairwise competition experiments of *Daphnia pulex* clones from southwestern Ontario.

	STANDARD COMPETITOR		
	GROUP 1 WINDSOR 1 CLONE 1	GROUP 2 CEDAR SPRINGS CLONE 4	GROUP 3 WINDSOR 1 CLONE 13
GROUP 1 COMPETITORS		Windsor 2 Clone 1 Windsor 3 Clone 1 Cedar Springs Clone 1 Charing Cross 1 Clone 1 Charing Cross 2 Clone 1 Cottam Clone 1	Windsor 2 Clone 1 Windsor 3 Clone 1 Cedar Springs Clone 1 Charing Cross 1 Clone 1 Charing Cross 2 Clone 1 Cottam Clone 1
GROUP 2 COMPETITORS	Cedar Springs Clone 4 Cedar Springs Clone 6 Charing Cross 1 Clone 6 Bloomfield Clone 6 Kingsville Clone 11 Kingsville Clone 12 Windsor 2 Clone 12		Cedar Springs Clone 4 Cedar Springs Clone 6 Charing Cross 1 Clone 6 Bloomfield Clone 6 Kingsville Clone 11 Kingsville Clone 12 Windsor 2 Clone 12
GROUP 3 COMPETITORS	Rondeau 1 Clone 8 Rondeau 2 Clone 8 Cottam Clone 10 Windsor 1 Clone 13 Rondeau 1 Clone 15 Rondeau 1 Clone 16		Rondeau 1 Clone 8 Rondeau 2 Clone 8 Cottam Clone 10 Rondeau 1 Clone 15 Rondeau 1 Clone 16

week. In order to determine if the slight temporal variation in cell densities of the algal suspension affected the outcomes of the competition experiments, 15 additional replicates were set up. These additional replicates were fed only algal solutions having optical densities of 0.30 - 0.35 at 620 nm, a criterion that was not necessarily met in feeding the other cultures. Fluctuations in algal cell densities apparently exerted little effect on clonal competitive abilities, as these additional replicates invariably had the same outcomes as the other jars. The cultures were monitored carefully during the establishment phase in order to ensure that population sizes never dropped below 15 animals. Of the 126 replicate cultures set up, 8 were discarded because of a bottle neck following establishment or a subsequent population collapse. Experiments were terminated after 80 days, or approx. 3 generations, when generation time is defined as half the median lifespan (Hebert 1978). At this time, a minimum of 24 individuals from each replicate jar were electrophoresed in order to determine clonal frequencies. In cases of clonal coexistence, 48 individuals were genotyped. Clones were distinguished by their lactate dehydrogenase (LDH), phosphoglucose isomerase (PGI) or phosphoglucomutase (PGM) electrophoretic patterns. Clones were then ranked according to their competitive abilities at 20° C under laboratory conditions.

RESULTS

The ranking of Daphnia pulex clones in terms of their competitive abilities is shown in Table 2.2. Four stocks of clone 1 (Windsor 1 clone 1, Cedar Springs clone 1, Charing Cross 1 clone 1 and Windsor 3 clone 1) coexisted with Cedar Springs clone 4 in roughly equal densities (Table 2.3). Similarly, Windsor 1 clone 1 failed to exclude any clone 6 stock (Bloomfield, Charing Cross 1 and Cedar Springs). Accordingly, all clone 6 stocks, Cedar Springs clone 4 and the 4 clone 1 stocks were all ranked equivalently as the "best competitors". The remaining clone 1 stocks (Cottam, Charing Cross 2 and Windsor 2) and the clone 12 stocks (Kingsville and Windsor 2) were ranked second highest in terms of their competitive abilities. These clone 1 stocks coexisted with Cedar Springs clone 4 at very low densities or were excluded by it. Similarly, both clone 12 stocks coexisted with Windsor 1 clone 1 at very low densities in some replicates, or were excluded by clone 1 in other replicates. Kingsville clone 11 was ranked third since it excluded Windsor 1 clone 13 in all replicates, but was outcompeted by Windsor 1 clone 1. Windsor 1 clone 13, Cottam clone 10 and Rondeau 1 clone 15 were ranked fourth in terms of competitive ability, since the latter two clones coexisted with Windsor 1 clone 13 and all three clones were completely or nearly excluded by Windsor 1 clone 1. The poorest competitors were clone 8 (from Rondeau 1 and Rondeau 2) and clone

Table 2.2 Competitive rank of D. pulex clones at 20°C.

Rank	Clones
1	Windsor 1 clone 1
	Cedar Springs clone 1
	Charing Cross 1 clone 1
	Windsor 3 clone 1
	Cedar Springs clone 4
	Bloomfield clone 6
	Charing Cross 1 clone 6
	Cedar Springs clone 6
2	Cottam clone 1
	Charing Cross 2 clone 1
	Windsor 2 clone 1
	Kingsville clone 12
	Windsor 2 clone 12
3	Kingsville clone 11
4	Cottam clone 10
	Windsor 1 clone 13
	Rondeau 1 clone 15
5	Rondeau 1 clone 8
	Rondeau 2 clone 8
	Rondeau 1 clone 16

Table 2.3 Outcome of *D. pulex* competition experiments: percentage represented by competitor 1. Percentages represent average of all replicates of each pairwise combination.

	Competitor 1		
Competitor 2	Windsor 1 clone 1	Windsor 1 clone 13	Cedar Springs clone 4
Windsor 1 clone 1		0%	
Windsor 3 clone 1		0%	44%
Cedar Springs clone 1		0%	74%
Charing Cross 1 clone 1		0%	63%
Cedar Springs clone 4	60%	0%	
Bloomfield clone 6	25%	0%	
Charing Cross 1 clone 6	54%	0%	
Cedar Springs clone 6	65%	0%	
Cottam clone 1		0%	96%
Charing Cross 2 clone 1		0%	90%
Windsor 2 clone 1		0%	100%
Kingsville clone 12	86%	4%	
Windsor 2 clone 12	93%	0%	
Kingsville clone 11	100%	0%	
Cottam clone 10	97%	16%	
Windsor 1 clone 13	100%		
Rondeau 1 clone 15	100%	67%	
Rondeau 1 clone 8	100%	96%	
Rondeau 2 clone 8	100%	86%	
Rondeau 1 clone 16	100%	96%	

16 (from Rondeau 1). These three stocks were completely or nearly excluded by Windsor 1 clone 13 in all replicates. Stocks of the five group 1 and 2 clones used in this study (clones 1, 4, 6, 11, 12) were all better competitors than any of the group 3 clones (clones 8, 10, 13, 15, 16). Also, with the exception of clone 1, all stocks of a clone isolated from different ponds were ranked at equivalent levels and even clone 1 stocks were not greatly different in their competitive abilities.

Table 2.4 shows the results of a chi-square test performed in order to determine if the four types of pairwise combinations differed in the proportion of replicates resulting in exclusion. The calculated X^2 value was significant (P value = 0.0001). Coexistence among group 3 clones and between group 1 and 2 clones was observed more frequently than expected, while clones from both groups 1 and 2 tended to exclude group 3 clones. Note that the mean genetic distances among group 3 clones (.110) and between group 1 and group 2 clones (.175) were smaller than those between clones of groups 1 and 3 (.205) and groups 2 and 3 (.215) (Crease 1980).

Table 2.4 Contingency table for 2 variables: 1) type of pairwise combination and 2) outcome of each replicate. Expected values are bracketed beside observed values.

		Mean Genetic Distance	Replicate Outcome: Coexistence	Exclusion	
Pairwise Combination Type	Group 1 vs 2	0.175	29(14.92)	15(29.08)	44
	Group 1 vs 3	0.205	1(12.20)	35(23.80)	36
	Group 2 vs 3	0.215	1(7.80)	22(15.20)	23
	Group 3 vs 3	0.110	9(5.08)	6(9.92)	15
			40	78	118

$X^2 = 49.20^*$ df = 3 P value = 0.0001

DISCUSSION

Earlier studies have revealed differences in competitive ability between clones of Daphnia pulex (Loaring and Hebert 1981; Good 1981). The present work has provided additional proof of the existence of such clonal variation. Other workers have also demonstrated the existence of ecological differences between clones (Mitter et al. 1979; King 1972; Snell 1979; Mort and Jacobs 1981; Hebert 1974; Young 1979; Vrijenhoek 1979; McWalter 1981; Woodrich 1980). Little between replicate variation in outcome was observed in this study and many of the replicates showed clonal coexistence upon termination of the experiment. The coexistence of clones under simplified laboratory conditions suggested that they had roughly similar fitnesses at 20°C. Notably, stocks of the same clone originating from different habitats were ranked at similar levels of competitive ability indicating that genetically similar clones were ecologically similar as well. Unfortunately, the ranking scheme described in this study was not entirely complete as all pairwise combinations of clones were not competed against each other. Moreover, the current ranking is not in complete agreement with the results obtained in an earlier study (Loaring and Hebert 1981) in which Windsor 1 clone 1 excluded two clones (Cedar Springs clone 4, Charing Cross clone 6) which it tended to coexist

with in the present experiment. This discrepancy might be attributed to the failure to adequately control certain relevant environmental variables.

Perhaps the most striking result of this study was the divergence in competitive abilities between members of groups 1 and 2 versus group 3 clones. In all but two replicates, all stocks of group 1 and 2 clones were able to competitively exclude all stocks of group 3 clones. In contrast, most combinations pairing group 1 clones with group 2 clones, or group 3 clones with group 3 clones resulted in clonal coexistence. Therefore, in general, group 1 clones were similar in terms of competitive ability to group 2 clones, and group 3 clones were similar to each other; but clones of groups 1 and 2 were competitively superior to group 3 clones. It is interesting that the mean genetic distances among group 3 clones and between group 1 and group 2 clones were smaller than those between clones of groups 1 and 3 and groups 2 and 3. Thus, in contrast to expectations, the results of this work indicated that genetic divergence does not foster clonal coexistence under laboratory conditions. Other laboratory studies have also posed apparent contradictions to the Gause principle. Miller (1964) found that larvae of 2 sibling species of Drosophila with nearly identical ecological requirements were able to coexist for long periods. Similarly, Sokoloff (1955) concluded that although competition is most intense between sibling species of Drosophila, coexistence was usually more successful between closely related species

than between more distant relatives. Additional studies on Drosophila species (Merrell 1951) and on Tribolium species (Park 1948) have documented that the coexistence of ecologically similar species may endure for a very long time under laboratory conditions.

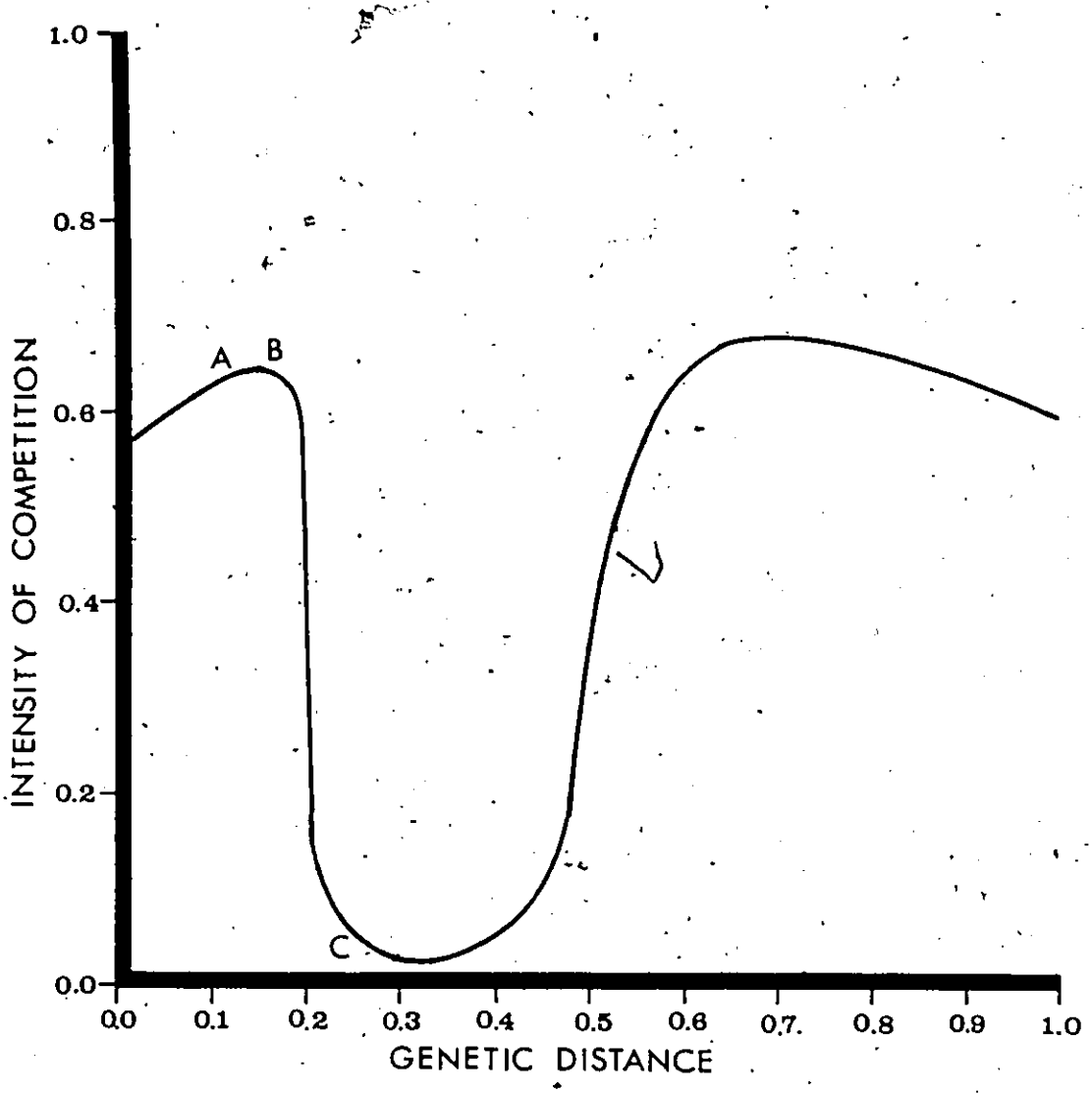
The competitive exclusion of one clone by another is more likely to occur under laboratory conditions than in a natural habitat. Theoretically, clones in nature are capable of coexisting indefinitely since ephippia produced by a competitively inferior clone may hatch every season, thereby preventing its exclusion. Nevertheless, the patterns of clonal coexistence observed in nature were in agreement with the laboratory results of the present study. Members of group 1 and 2 frequently coexisted with each other, but rarely with clones belonging to group 3. However, several group 3 clones were often present in the same habitat. In addition to D. pulex, clones of several other parthenogenetic species have been found to coexist in nature (Parker and Selander 1976; Lokki et al. 1975; Jaenike et al. 1980; Suomalainen and Saura 1973; McWalter 1981). If one ecologically distinct habitat (habitat 25) is excluded from the 64 habitats sampled by Jaenike et al. (1980), then the average genetic distance between coexisting clones of earthworms is found to be a very low value (0.09). Similarly, Suomalainen and Saura (1973) indicated that genotypes within populations of weevils resemble each other electrophoretically more

than genotypes belonging to different populations.

Early studies of zooplankton communities revealed patterns similar to those observed in vertebrate communities (Hutchinson 1951). Most habitats contained only a single species of each genus and if congeners did coexist, they tended to differ in size or in habitat preferences. In pond habitats where opportunities for spatial, vertical or temporal segregation were limited, species richness tended to be low. Yet recent work has revealed weaknesses in these earlier studies. In some cases, taxonomy was inadequate, and what had been regarded as a single species turned out to be a group of morphologically similar species coexisting in the same habitat (Hebert 1977; Manning et al. 1978). Moreover, while vertebrate communities showed separation of resource use patterns, the data on zooplankton communities suggest that the overlap in resource use is often extensive (Hebert 1978). The results of the present work also conflicts with earlier studies in that coexistence between genetically and ecologically similar clones was widespread in both laboratory and field situations.

When coupled with the results of classical competition experiments, the present data suggest the existence of a bimodal distribution between the intensity of competition and genetic similarity (Fig. 2.1). Intensity values for each pairwise combination are given by the ratio: number of replicates resulting in coexistence/total number of replicates. The first and second peaks of this distribution represent genetically similar conspecifics and coexisting genetically distant species respectively;

Figure 2.1 Relationship of the intensity of competition to genetic distance in D. pulex clones. The positions of the types of pairwise combinations are designated by A (group 3 vs. group 3), B (group 1 vs. group 2) and C (group 1 vs. group 3 and group 2 vs. group 3).



while the trough represents more distantly related conspecifics and species which do not coexist. For the most part, previous studies have involved organisms which are described by the latter half of the distribution and as such, adhere to Gause's principle. Intensity values and genetic distance values are unknown for this portion of the distribution, hence the height and placement of the second peak along the abcissa are purely arbitrary.

In conclusion, this study has shown that ecological similarity is positively correlated with genetic similarity when a clonally reproducing species is considered. In contrast to conventional ideas, genetic divergence did not foster clonal coexistence in either laboratory or field situations. Instead, a bimodal distribution was thought to describe the relationship between the probability of coexistence and genetic distance.

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CHAPTER III

GENETIC DIVERGENCE BETWEEN NORTH AMERICAN AND ENGLISH METAPOPULATIONS OF *DAPHNIA MAGNA*

CHAPTER III

INTRODUCTION

Many zooplankton species have broad ranges over which they show little morphological variability. For many years it was felt that this lack of morphological diversification was a consequence of extensive gene flow. Yet studies of genetic variation in a local group of Daphnia magna populations revealed frequent allelic substitutions and major gene frequency differences (Hebert 1975). A later study showed that genetic differences were even more pronounced between distant populations (Crease and Hebert 1982). Thus, populations of D. magna from central Canada (Churchill, Manitoba) shared only 60% of their alleles with English populations. Despite this genetic divergence, individuals from the two regions readily hybridized and their offspring showed evidence of heterosis. Therefore, these studies indicated that, despite the genetic diversity which lies hidden beneath a facade of morphological uniformity, D. magna should be regarded as a single biological species.

These earlier studies on D. magna left a number of unresolved problems. The gene pool of D. magna in central Canada was found to be nearly invariant, while English populations were segregating at about one-third of their loci. Crease and Hebert (1982) suggested that the low amounts of genetic variation at Churchill might reflect the isolation of this site from glacial refuges in which

D. magna survived the Pleistocene. Alternatively, perhaps all North American populations of D. magna are nearly invariant as a consequence of founder effects during the colonization of North America. The species range is restricted to the western half of the continent and Brooks (1957) has suggested that colonists first arrived in North America from Asia during the Pleistocene. A survey of the extent of genetic variation in D. magna populations found near the glacial refuges of northern Canada and those at more southerly locales offer the best opportunity to distinguish these alternate hypotheses. The present study presents information on the genetic diversity of populations of D. magna in immediate proximity to the glacial refuges of the Yukon, as well as two more southerly locales (Williams Lake, British Columbia and San Diego, California).

Earlier work suggested that the genetic characteristics of D. magna populations in England were greatly influenced by environmental conditions (Hebert 1974b and 1974c). In intermittent ponds, in which D. magna populations were regularly re-established from sexually produced resting eggs, genotypic frequencies were stable and approximated Hardy-Weinberg proportions. In contrast, in more permanent ponds, where populations were able to reproduce parthenogenetically for extended periods of time, genotypic frequencies were unstable and often deviated markedly from Hardy-Weinberg expectations. In temperate areas intermittent and permanent ponds co-occur and the classification of habitats is not always clear cut.

However, in arctic Canada, all ponds are intermittent since they freeze solid during winter. Daphnia populations in such ponds are undoubtedly re-established from sexually produced eggs, provided that the species is a cyclic parthenogen. Thus, arctic populations provide an ideal test for the assertion that the genotypic characteristics of intermittent populations approximate those of sexually reproducing species. The regular enforcement of sexual reproduction in arctic habitats should also ensure that disequilibrium among loci is low. As such, arctic D. magna populations are of value in learning more about the origin of the heterozygote excesses so evident in permanent populations (Hebert 1974b; Young 1979a,b; Mort and Jacobs 1981). It has been argued that these excesses are a consequence of associative overdominance resulting from linkage disequilibrium between selected loci and the allozyme loci being surveyed (Berger 1976; Angus 1978; Hebert et al. 1982; Young 1979b). There is no evidence concerning either the frequency of such linkage association or the time frame required for such heterozygote excesses to develop. In the present study, sexually produced hatchlings from an arctic pond were established in a laboratory aquarium which permitted continued parthenogenetic reproduction. Genotypic frequencies were monitored on a regular basis.

MATERIALS AND METHODS

D. magna was collected from ten ponds in the area of Tuktoyaktuk, N.W.T. (Tuk; 69.27N 133.02W), two ponds located approximately 30 miles west of Williams Lake, B.C. (52.08N 122.09W), and one pond near San Diego, California (32.43N 117.09W) (Appendix III). The Tuk ponds were shallow tundra pools, located in close proximity to the Beaufort Sea and ranging from 3 meters to greater than 100 meters in diameter. The Williams Lake and San Diego ponds were large in surface area and surrounded by grass and other low lying vegetation. The conductivities and salinities of the Tuk and Williams Lake ponds are listed in Appendix IV.

Live samples of each D. magna population were shipped to Windsor where they were frozen in distilled water for use in electrophoretic studies. Unfortunately, the San Diego collection suffered heavy mortality, but 4 individuals survived and were used to initiate laboratory cultures (for description of laboratory culture techniques, see Crease 1980).

The samples from Williams L. and the 4 San Diego clones were analyzed electrophoretically at 10 enzyme loci: phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), tetrazolium oxidase (TO), glucose-6-phosphate dehydrogenase (G6PDH), glutamate oxaloacetate transaminase (GOT), xanthine dehydrogenase (XDH), amylase-1 (AMY), and leucine aminopeptidase (LAP). LAP patterns for the Williams L. populations

were too faint to be accurately deciphered. Preliminary electrophoretic analyses of the Tuk populations carried out in 1980 revealed that all loci were monomorphic except LAP and GOT. Samples collected in 1981 provided additional gene frequency data for these two enzymes. Only 1981 data were included in this study. Details on electrophoretic procedures are outlined in Crease (1980). Electrophoretic runs in which 15% or more of the gels could not be scored with confidence were discarded. Alleles were numbered (1, 2, 3, etc.) in order of increasing migration from the origin. Genotype and gene frequencies at each locus were determined by direct count. Breeding studies were carried out on the Tuk and San Diego individuals in order to verify that genotypic differences were heritable. This verification was not made for the Williams L. populations.

Data Analysis

Genotypic frequencies at polymorphic loci in the Tuk and Williams L. populations were checked for concordance with Hardy-Weinberg expectations using a chi-square goodness-of-fit test. Populations were not included in the analysis if greater than one-third of the expected frequencies were less than 4. For each case showing a significant (at the 5% level) heterozygote deficiency, an inbreeding coefficient was calculated using the formula $F = \frac{H_o - H_f}{H_o}$, where H_o is the proportion of heterozygotes expected with random mating ($H_o = 2\sum pq$) and H_f is the observed proportion of heterozygotes in the sample (Li and Horvitz 1953). A second set of expected genotype frequency values were calculated using a population model that assumes inbreeding ($p^2+Fpq+Fpr$, $2pq-2Fpq$,

$q^2+Fpq+Fqr$, $2qr-2Fqr$, $r^2+Fpr+Fqr$, $2pr-2Fpr$) and a second X^2 test was performed. The genotypic frequencies at the LAP and GOT loci among the Tuk populations were subjected to homogeneity chi-square analysis. Since LAP genotypes 33, 44 and 24 were rare, all individuals possessing these genotypes were combined to form a single genotypic class. In addition, the coefficient of inbreeding among the Tuk populations (F_{ST}) was calculated for every allele at each polymorphic locus using the formula $F_{ST} = \frac{\sigma_i^2}{\bar{p}_i \ (\overline{1-p_i})}$ where σ_i^2 = the variance in the frequency of allele i when several populations are pooled, $\bar{p}_i$ = the mean frequency of allele i and $\overline{1 - p_i}$ = the mean frequency of the remaining alleles pooled for each population. A mean inbreeding coefficient per locus was calculated by weighting the F_{ST} value for each allele by the mean allele frequency. Average heterozygosities per individual and the proportion of polymorphic loci were calculated for the Tuk and Williams L. populations. Average heterozygosity is given by $H = \sum \underline{h}_L / \underline{r}$ where $\underline{h}_L = 1 - \sum \underline{x}^2_{Li}$ where 'L' is the L^{th} locus, $\underline{r}$ is the number of loci, and $\underline{x}_{Li}$ is the frequency of the i^{th} allele at the L^{th} locus (Nei 1975).

Nei's measures of genetic divergence I (genetic similarity) and D (genetic distance) were calculated for each pair of populations and/or clones using gene frequency data at 10 loci from the 10 Tuk populations, one Churchill Man. clone (204), two English clones (SF and MF) and the two most genetically dissimilar San Diego clones. The allozyme data for the Churchill and English clones were from

Crease (1980). A cluster analysis was performed on the matrix of genetic distances in order to construct a dendrogram illustrating the genetic relationships of the populations and clones. These procedures were then repeated using gene frequency data at 9 loci from the populations and clones mentioned previously and, in addition, the two Williams L. populations. Cluster analyses were based on average genetic distance and used the BMD P1M program.

Permanent Pond Simulation

A 100 gal. aquarium containing 300 l of artificial pond water (for composition see Crease 1980) was inoculated with algae (primarily Scenedesmus and Kirschneriella at approx. densities of 2×10^5 cells/ml and 1×10^6 cells/ml respectively) which were then allowed to grow for 10 days. At this time, 250 gravid D. magna collected from Tuk 13 were placed in the aquarium. These animals were ephippial hatchlings, as they were collected in early July, at a time when parthenogenetically produced individuals were still immature. A second sample from Tuk 13 used for electrophoretic analysis revealed this population to be in Hardy-Weinberg equilibrium. Genotypic frequencies in the simulated permanent pond were determined every 60 days. The experiment was terminated after 120 days (or 5 generations when a generation is defined as half the median lifespan, Hebert 1978).

RESULTS

Allozyme Phenotypes and Gene Frequencies

a) Tuk Populations

Populations of D. magna from the Tuk area were polymorphic at 2 of 10 enzyme loci surveyed: GOT and LAP. GOT phenotypes were characterized by single banded homozygotes and triple banded heterozygotes, as noted by Young (1979a). Three alleles were detected (1, 2 and 3) and all 6 possible phenotypes were observed (Fig. 3.1.1). Allele 2 was generally present in slightly higher frequencies than alleles 1 and 3 (Table 3.1). Tuk 7 was the only exception; in this population, allele 3 was most common. The LAP homozygotes were single banded and heterozygotes double banded as found by Crease and Hebert (1982). Three alleles were detected at Tuk (2, 3 and 4) and 5 of the 6 possible phenotypes (22, 23, 24, 33 and 44) were observed (Fig. 3.1.2). Allele 2 was invariably present in substantially higher frequencies than either allele 3 or 4, which were relatively rare in all populations (Table 3.1). Allele 3 was generally slightly more frequent than allele 4, although population Tuk 7 again proved exceptional in that allele 4 was much more common than allele 3.

b) Williams Lake Populations

Populations of D. magna from Williams L. (WL) were polymorphic at 3 of 9 enzyme loci: GOT, MDH and AMY. GOT phenotypes observed for these populations were similar to those

Figure 3.1.1 Electrophoretic phenotypes of GOT in *D. magna*. From left to right: 13, 11, 12, 22, 23, 33. All phenotypes are Tuk *D. magna*.

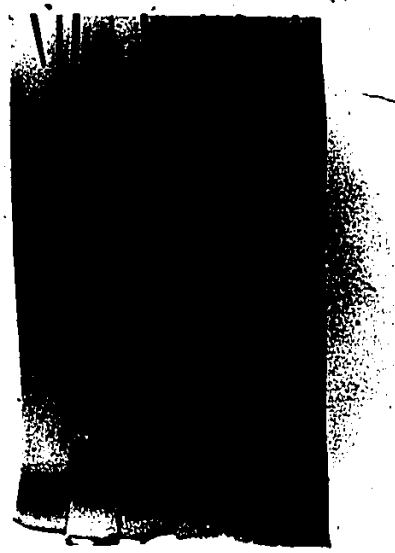


Figure 3.1.2 Electrophoretic phenotypes of LAP in D. magna. From left to right: 22, 23, 23, 24, 44, 24, 13. 13 is a hybrid D. magna with parents from England and Churchill. The remaining phenotypes are Tuk D. magna.

Table 3.1 Gene frequencies at variable loci in *D. magna* populations from Tuk and Williams L.
n denotes electrophoretic sample size.

Enzyme locus	Population	n	Allele 1	2	3	4
GOT	Tuk 1	15	.30	.40	.30	
	Tuk 2	23	.28	.44	.28	
	Tuk 3	111	.29	.41	.30	
	Tuk 4	247	.29	.38	.33	
	Tuk 5	99	.28	.40	.32	
	Tuk 6	118	.24	.46	.30	
	Tuk 7	40	.21	.36	.43	
	Tuk 8	18	.31	.44	.25	
	Tuk 9	85	.34	.39	.27	
	Tuk 10	106	.29	.43	.28	
	WL4	63		.10	.90	
	WL6	22	.07		.93	
LAP	Tuk 1	46		.89	.10	.01
	Tuk 2	89		.92	.08	
	Tuk 3	86		.89	.11	
	Tuk 4	280		.86	.14	
	Tuk 5	126		.88	.09	.03
	Tuk 6	172		.84	.14	.02
	Tuk 7	37		.70	.05	.25
	Tuk 8	105		.96	.04	
	Tuk 9	151		.91	.09	
	Tuk 10	128		.81	.18	.01
MDH	WL4	49	.09	.91		
	WL6	23	.43	.57		
AMY*	WL4	46			1.0	
	WL6	22	.29	.02	.69	

found at Tuk. The same three alleles were detected (1, 2 and 3), but only 3 of the 6 possible phenotypes were observed (13, 23 and 33). The homozygous phenotype (33) was found in both Williams L. 4(WL4) and 6(WL6), but the 13 heterozygote was seen only in WL6 while the 23 heterozygote was seen only in WL4. Allele 3 was by far, the commonest allele in the Williams L. populations, while alleles 2 and 1 were present in low frequencies in WL4 and WL6 respectively (Table 3.1). Two alleles were detected for MDH, but only 2 of the 3 possible phenotypes were observed (12, 22). Allele 2 was more frequent than allele 1 in both populations; however, the frequency of allele 1 was substantially lower in WL4 than in WL6. Only one AMY allele (3) present in the homozygous condition was detected at WL4 (Fig. 3.1.3); however, 3 alleles (1, 2 and 3) were identified at WL6. Of the 3 phenotypes observed in this population, 2 were homozygous (11 and 33) while the third (123) was a rare triple banded phenotype, suggestive of a gene duplication. It is assumed that this phenotype was heritable, although this was not proven. Alleles 1 and 2 were much less common than allele 3 (Table 3.1).

Genotype Frequencies

Table 3.2 shows the results of chi-square analyses carried out on the genotype frequency data of individual Tuk and Williams L. populations. The WL6 AMY locus was polymorphic, but was not included in the analysis due to the

Figure 3.1.3 Electrophoretic phenotypes of AMY. From left to right: 33, 33, 33, 33, 33. The first two phenotypes are Churchill D. magna and the latter three are Williams L. D. magna

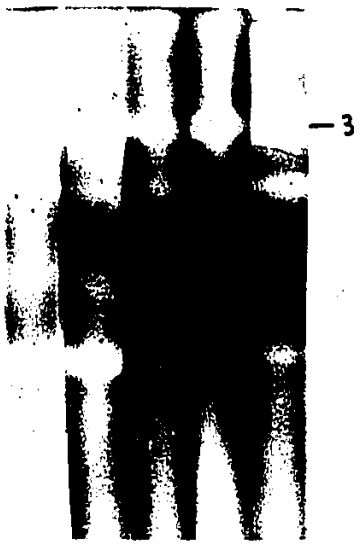


Table 3.2 Population deviations from Hardy-Weinberg equilibrium genotype frequencies.
* P value = 0.001

Population	Polymorphic locus	X^2	Heterozygote excess or deficiency
Tuk 2	LAP	10.58*	deficiency
Tuk 3	GOT	4.48	
	LAP	1.86	
Tuk 4	GOT	2.89	
	LAP	3.16	
Tuk 5	GOT	3.76	
Tuk 6	GOT	14.72*	deficiency
Tuk 7	GOT	6.45	
Tuk 8	LAP	0.17	
Tuk 9	GOT	2.65	
	LAP	1.19	
Tuk 10	GOT	6.42	
	LAP	2.68	
WL4	GOT	0.70	
	MDH	0.50	
WL6	MDH	13.61*	excess

presence of a triple banded phenotype. Two Tuk populations (Tuk 2 and Tuk 6) exhibited significant heterozygote deficiencies and a significant heterozygote excess was found at the MDH locus in WL6. Despite these deviations, the majority of the populations under investigation appeared to be in Hardy-Weinberg equilibrium.

Within population inbreeding coefficients calculated for both enzyme loci showing significant heterozygote deficiencies are presented in Table 3.3. The X^2 value resulting from a second test of departure from Hardy-Weinberg equilibrium incorporating the inbreeding coefficient estimated for the GOT locus at Tuk 6 is also shown. A second chi-square test was not performed for the LAP locus at Tuk 2 since the estimation of the inbreeding coefficient used up the single remaining degree of freedom. In the k allele case, there are $\left[k(k-1)-2\right]/2$ degrees of freedom for the goodness-of-fit test when an estimated inbreeding coefficient is incorporated. Therefore, loci at which only 2 alleles were observed cannot be included in the analysis. The new X^2 value calculated for Tuk 6 was insignificant at the 5% level, indicating that an assumption of inbreeding accounts for the significant heterozygote deficiency observed in this population.

Table 3.4 shows the data on genotypic frequencies in the laboratory population of D. magna. Initially,

Table 3.3 Inbreeding coefficients within Tuk populations having significant deviations from Hardy-Weinberg equilibrium genotype frequencies due to heterozygote deficiencies. n is electrophoretic sample size.

Population	Locus	n	H_o	H_f	F	χ^2	P value
Tuk 2	LAP	89	0.15	0.10	0.31		
Tuk 6	GOT	118	0.64	0.58	0.18	5.61	0.10

Table 3.4 Chi-square analyses of population samples taken from simulated permanent pond. n is electrophoretic sample size. *P value = 0.0001

GOT Time (months)	n	Genotype	Genotype frequency	X^2	LAP Time (months)	n	Genotype	Genotype frequency	X^2
0	247	11	0.11	2.89	0	280	22	0.75	3.16
		12	0.20				23	0.22	
		22	0.14				33	0.03	
		23	0.26						
		33	0.12						
		13	0.17						
2	132	11	0.06	5.97	2	77	22	0.56	2.02
		12	0.17				23	0.42	
		22	0.17				33	0.02	
		23	0.33						
		33	0.18						
		13	0.09						
4	90	23	1.00	90.0*	4	88	22	0.14	50.93*
							23	0.86	

genotype frequencies showed no significant deviation from Hardy-Weinberg expectations. Genotypic frequencies remained in Hardy-Weinberg equilibrium for 2 months, although the incidence of certain heterozygous phenotypes increased at both loci. GOT genotypes carrying allele 1 decreased in frequency while those homozygous for alleles 2 and 3 and those heterozygous for these two alleles increased. At the LAP locus, the 23 heterozygotes underwent a similar increase in frequency, while the proportions represented by either homozygote declined. After approx. 80 days, the population suffered a major decline, followed by a slow increase in density. After 120 days, the population had again achieved high densities, but enormous heterozygote excesses were apparent at the polymorphic loci. All individuals surveyed were found to be 23 heterozygotes at GOT. At the LAP locus, the 23 heterozygote was far more common than the 22 homozygote, and the 33 homozygote was not detected at all. The probability of any GOT heterozygote and any LAP heterozygote reaching fixation at the same time through random events is given by the product of the original frequency of all heterozygotes at both loci:

$$P = (0.20 + 0.26 + 0.17) \times 0.22 = 0.1386 \text{ or approx. } 14\%$$

Heterozygosity

Heterozygosities per individual and the proportion of polymorphic loci are given for each D. magna population from Tuk and Williams L. in Table 3.5.

The 10 Tuk populations had similar levels of genetic

Table 3.5 Summary of genetic variation in D. magna populations from Tuk and Williams L.

Population	Number of loci	Heterozygosity per individual	Proportion of polymorphic loci
TUK			
1	10	.0856	.200
2	10	.0805	.200
3	10	.0859	.200
4	10	.0902	.200
5	10	.0874	.200
6	10	.0911	.200
7	10	.1010	.200
8	10	.0720	.200
9	10	.0825	.200
10	10	.0961	.200
$\bar{x}$	10	.0872	.200
WL			
4	9	.0377	.222
6	9	.1177	.333
$\bar{x}$	9	.0777	.2775

variation. The proportion of polymorphic loci was invariant between populations and the heterozygosities per individual ranged only from 0.07 to 0.10. The mean heterozygosity and proportion of polymorphic loci for the entire metapopulation were 0.09 and 0.20 respectively.

The Williams L. populations of D. magna maintained a higher proportion of polymorphic loci (metapopulation mean = 0.28) than did the Tuk populations. The divergent nature of the 2 Williams L. populations was illustrated by the fact that WL6 had a higher heterozygosity per individual than any Tuk population while WL4 had a lower value than any Tuk population. The mean heterozygosity for the entire metapopulation was 0.08.

Population Subdivision

The results of homogeneity chi-square analyses performed using Tuk allozyme data for 2 loci (GOT and LAP) are presented in Table 3.6. The LAP genotype frequencies showed significant heterogeneity (X^2 = 181.70, P value = .0001), but GOT genotype frequencies were apparently homogenous (X^2 = 48.00, P value = .25). Therefore, there were significant differences in genotype frequencies within the Tuk metapopulation at LAP, but not at GOT. Some populations showed much greater deviation in genotype frequencies from expected values than did others. For example, over 75% of the significant X^2 statistic obtained for the Tuk LAP data was contributed by population Tuk 7.

Table 3.7 shows the within metapopulation (between population) inbreeding coefficient calculated using allozyme

Table 3.6 Homogeneity chi-square analysis of genotypic frequencies at LAP and GOT loci among Tuk populations. Expected numbers are in brackets. n is the electrophoretic sample size.

GOT

Genotype	Population										Row totals
	Tuk 1	Tuk 2	Tuk 3	Tuk 4	Tuk 5	Tuk 6	Tuk 7	Tuk 8	Tuk 9	Tuk 10	
11	1(1.74)	2(2.67)	14(12.86)	26(28.65)	10(11.48)	14(13.69)	4(4.64)	3(2.09)	12(9.86)	14(12.30)	100
12	4(2.89)	8(4.43)	22(21.35)	50(47.57)	17(19.06)	15(22.72)	6(7.70)	4(3.47)	17(16.37)	23(20.41)	166
22	1(2.59)	4(3.98)	19(19.16)	36(42.69)	16(17.11)	30(20.40)	6(6.91)	2(3.11)	15(14.69)	20(18.32)	149
23	6(4.09)	4(6.27)	31(30.23)	64(67.34)	30(26.99)	34(32.17)	11(10.90)	8(4.91)	20(23.17)	27(28.90)	235
33	0(1.55)	4(2.37)	10(11.45)	30(25.50)	7(10.22)	12(12.18)	10(4.13)	0(1.86)	5(8.78)	11(10.94)	89
13	3(2.14)	1(3.28)	15(15.82)	41(35.24)	19(14.13)	13(16.84)	3(5.71)	1(2.57)	16(12.13)	11(15.13)	123
n	15	23	111	247	99	118	40	18	85	106	862

$X^2 = 48.00$ df = 45

LAP

Genotype	Population										Row totals
	Tuk 1	Tuk 2	Tuk 3	Tuk 4	Tuk 5	Tuk 6	Tuk 7	Tuk 8	Tuk 9	Tuk 10	
22	38(35.48)	77(68.65)	64(66.33)	211(215.97)	96(97.19)	130(132.67)	15(28.54)	97(80.99)	126(116.47)	87(98.73)	941
23	5(7.99)	9(15.47)	19(14.94)	60(48.66)	23(21.90)	26(29.89)	4(6.43)	8(18.25)	25(26.24)	33(22.24)	212
33 44 24	3(2.53)	3(4.89)	3(4.72)	9(15.38)	7(6.92)	16(9.45)	18(2.03)	0(5.77)	0(8.29)	8(7.03)	67
n	46	89	86	280	126	172	37	105	151	128	1220

$X^2 = 181.70^*$ df = 18 P value = 0.0001

Table 3.7 Within metapopulation inbreeding coefficient (F_{ST}) for the Tuk area.

Locus	Allele	Mean allele frequency ($\bar{p}_i$)	Variance of allele frequencies (σ^2)	Inbreeding coefficient (F_{ST})	Weighted mean F_{ST} per locus
GOT	1	0.28	1.16×10^{-3}	5.72×10^{-3}	5.92×10^{-3}
	2	0.41	8.69×10^{-4}	3.59×10^{-3}	
	3	0.31	1.97×10^{-3}	9.27×10^{-3}	
LAP	2	0.87	4.55×10^{-3}	3.89×10^{-2}	4.02×10^{-2}
	3	0.10	1.57×10^{-3}	1.73×10^{-2}	
	4	0.03	4.96×10^{-3}	0.17	

data from the two polymorphic loci at Tuk. F_{ST} was calculated to be 0.006 and 0.04 at GOT and LAP respectively.

Metapopulation Comparisons

The extent of genetic differentiation between the Tuk and Williams L. metapopulations and representative clones from Churchill, Man., England and San Diego, are summarized in Tables 3.8-3.9 and Fig. 3.2 and 3.3.

Table 3.8 is a comparison of alleles at each locus. As in the study by Crease and Hebert (1982), three categories of loci are apparent. The first consisted of loci that were monomorphic for the same allele in all populations and clones. TO, XDH and LDH fell into this category. The second category contained those loci which were invariant in North America, but showed substitutions or variation in the English clones. Such loci included PGI, PGM and G6PDH. The remaining loci (MDH, GOT, AMY, LAP) formed the third category, which were those loci showing polymorphism in North American populations. Taking all 5 geographic locations into account, 3 alleles have been detected at MDH. The Tuk populations and the San Diego clones were fixed for allele 2, the Williams L. populations and the Churchill clone were polymorphic for alleles 1 and 2, and the two English clones (SF and MF) were heterozygous for alleles 2 and 3. Allele 1 was present, but uncommon in English populations (Crease 1980). Of the 3 GOT alleles discovered in the Tuk and Williams L. populations, allele 2 was fixed in the English and the San Diego clones,

Table 3.8 Comparison of allelic arrays in populations from 1 English and 4 North American localities.

Locus	Locality				
	Tuk	Williams L.	San Diego	Churchill	England (Cambridge)
PGI	2	2	2	2	1, 2
PGM	2	2	2	2	1
LDH	1	1	1	1	1
MDH	2	1, 2	2	1, 2	2, 3
G6PDH	2	2	2	2	1
XDH	1	1	1	1	1
GOT	1, 2, 3	1, 2, 3	2	3	2
AMY	3	1, 2, 3	3	3	3
LAP	2, 3, 4		2, 3, 4	1	2, 3, 5
TO	1	1	1	1	1

while Churchill D. magna were monomorphic for allele 3. Polymorphism at AMY was detected only at WL6. The remaining populations and clones were monomorphic for allele 3. Finally, a total of 5 alleles were detected at the LAP locus. Of the 3 alleles discovered in the Tuk populations and the San Diego clones (2, 3, 4), two (alleles 2 and 3) were present in the English clones, as was a null allele (5) not seen in North American populations. Churchill D. magna were fixed for a fifth allele (1), which was slightly slower than the most common allele in the Tuk populations.

The average genetic distances and similarities among the investigated metapopulations and/or clones are presented in Table 3.9. The dendrograms in Fig. 3.2 and Fig. 3.3 illustrate the genetic relationships between these groups. Greater distances were observed when data from the LAP enzyme locus was included in the analysis. Within metapopulation genetic similarities were generally quite high, especially for the Tuk metapopulation. Notably, all North American metapopulations were fairly closely related. Tuk D. magna were most closely related to San Diego D. magna, but the similarities of the Tuk metapopulation with those of Williams L. and Churchill were only slightly smaller. The Churchill and Williams L. metapopulations were also very similar to each other, while the comparison of San Diego D. magna to those from Williams L. and Churchill revealed somewhat larger genetic distances. The most striking result of the genetic distance and cluster analyses was the

Table 3.9 Average genetic similarities and distances among populations and/or clones.

15 Populations and/or clones - 10 loci.

Populations and/or clones	Genetic distance	Genetic Similarity
TUK - TUK	2.111×10^{-3}	0.998
TUK - CHURCHILL	0.140	0.870
TUK - ENGLAND	0.455	0.635
TUK - SAN DIEGO	0.085	0.919
CHURCHILL - ENGLAND	0.627	0.535
CHURCHILL - SAN DIEGO	0.211	0.810
ENGLAND - ENGLAND	0.089	0.915
ENGLAND - SAN DIEGO	0.401	0.671
SAN DIEGO - SAN DIEGO	0.080	0.923

17 Populations and/or clones - 9 loci

Populations and/or clones	Genetic distance	Genetic Similarity
TUK - TUK	5.636×10^{-4}	1.000
TUK - CHURCHILL	0.043	0.958
TUK - ENGLAND	0.412	0.662
TUK - SAN DIEGO	0.030	0.971
TUK - WILLIAMS L.	0.054	0.948
CHURCHILL - ENGLAND	0.529	0.589
CHURCHILL - SAN DIEGO	0.118	0.888
CHURCHILL - WILLIAMS L.	0.015	0.985
ENGLAND - ENGLAND	0.000	1.0
ENGLAND - SAN DIEGO	0.347	0.707
ENGLAND - WILLIAMS L.	0.539	0.584
SAN DIEGO - SAN DIEGO	0.025	0.975
SAN DIEGO - WILLIAMS L.	0.126	0.883
WILLIAMS L. - WILLIAMS L.	0.025	0.975

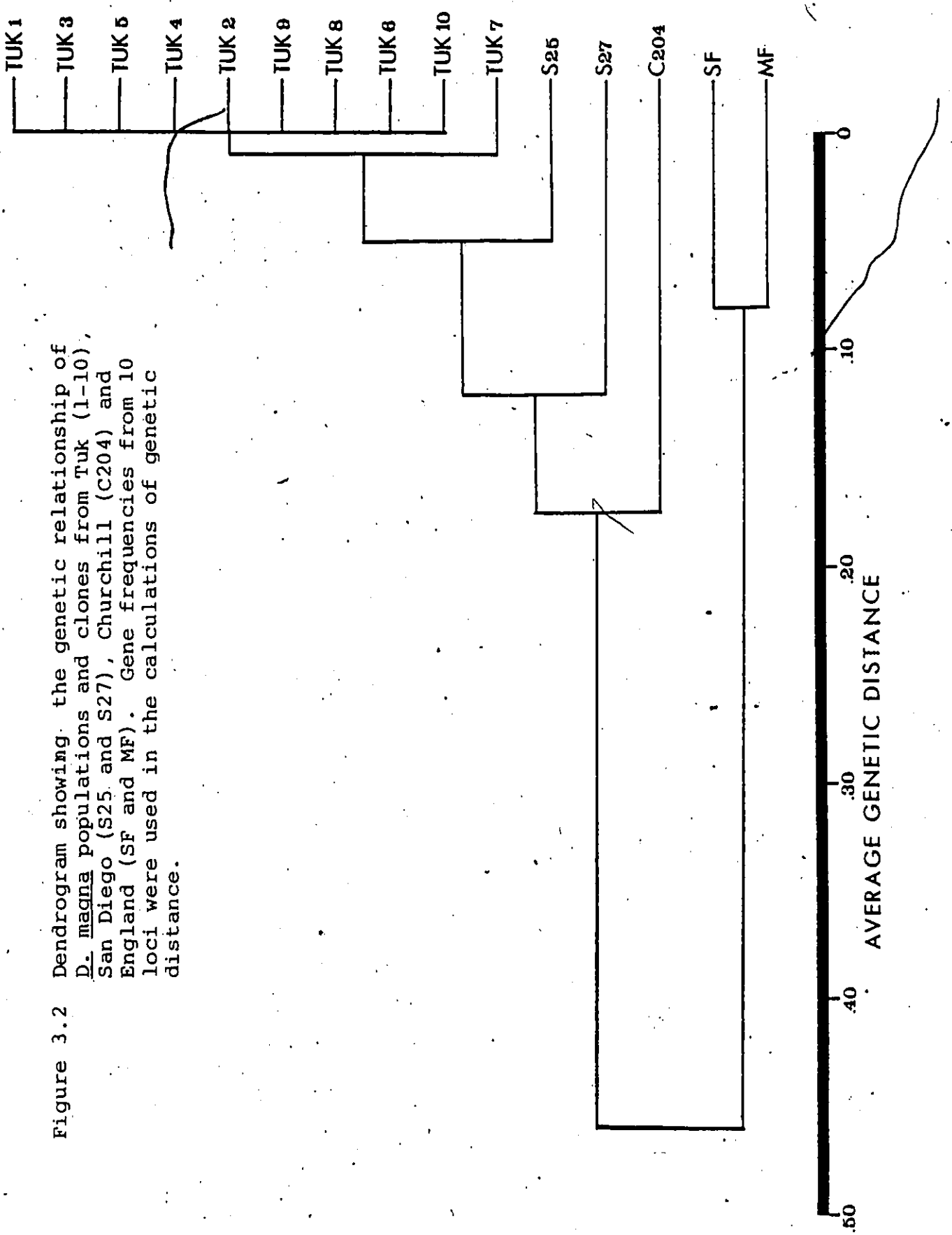
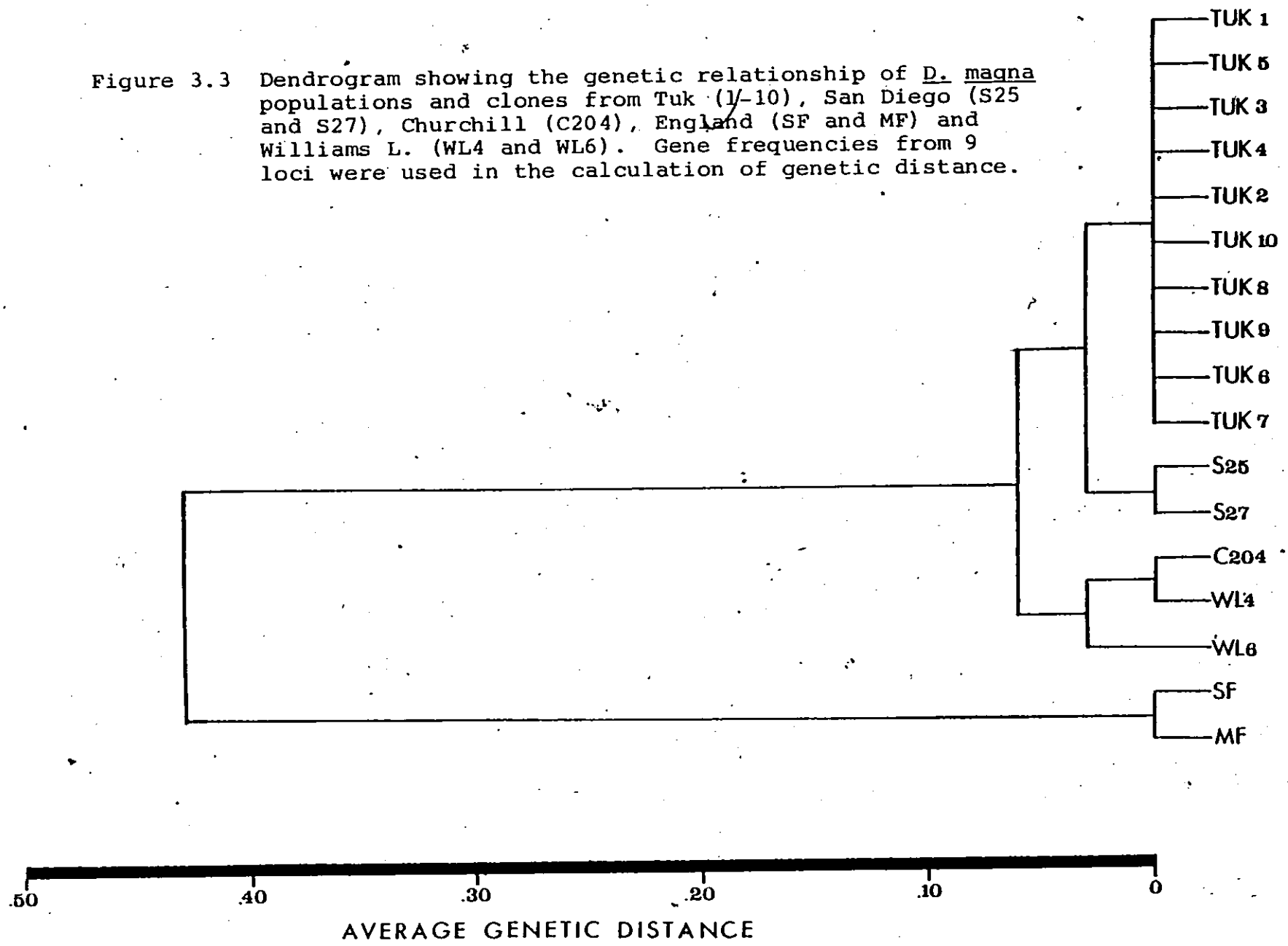


Figure 3.2 Dendrogram showing the genetic relationship of D. magna populations and clones from Tuk (1-10), San Diego (S25 and S27), Churchill (C204) and England (SF and MF). Gene frequencies from 10 loci were used in the calculations of genetic distance.

Figure 3.3 Dendrogram showing the genetic relationship of D. magna populations and clones from Tuk (1-10), San Diego (S25 and S27), Churchill (C204), England (SF and MF) and Williams L. (WL4 and WL6). Gene frequencies from 9 loci were used in the calculation of genetic distance.



vast divergence of N. American D. magna from English D. magna. This divergence is illustrated in both dendrograms which clearly separate the two localities. Of the N. American metapopulations, Tuk and San Diego resembled the English clones to a greater extent than did either Churchill or Williams L.

DISCUSSION

Other workers have shown that populations of cyclic parthenogenetic Daphnia species inhabiting intermittent habitats are generally in good agreement with Hardy-Weinberg expectations (Hebert 1974c; Hebert and Moran 1980; Lynch 1982). The Tuk ponds investigated in the present study froze solid every winter and were therefore classified as intermittent habitats. As expected, the majority of the Tuk D. magna populations surveyed (7 of 9) were in Hardy-Weinberg equilibrium. Apparently, genotypes in these populations were mating randomly at the time of ephippial formation, population sizes were large enough so that inbreeding or genetic drift were relatively unimportant and selective differences between electrophoretically distinguished genotypes were small or absent.

Both of the two Tuk D. magna populations showing significant departures from Hardy-Weinberg expectations (Tuk 2 and Tuk 6) took the form of heterozygote deficiencies. These deficiencies were observed at the LAP locus in Tuk 2 and at the GOT locus in Tuk 6. Zouros et al. (1980) has suggested 5 possible causes of heterozygote deficiencies in natural populations:

(1) the treatment of sex-linked loci as autosomals

(2) the presence of null alleles

(3) the inclusion in the sample of more than one species

(4) the pooling of individuals from several demes into a single population (Wahlund's effect)

(5) the presence of inbreeding

The latter two alternatives were the most likely in the case of the D. magna populations under investigation. Populations of D. magna existing in a particular pond in different years may differ in gene frequencies. Insofar as the ephippial eggs which are produced by these temporally isolated populations may hatch at the same time, a situation leading to the pooling of individuals from different demes may arise. In addition, inbreeding within a population may lead to heterozygote deficiencies. Young (1979b) argued that inbreeding might be enhanced in D. magna populations by the variance in clone sizes and by genotypic differences in the time of sex. Calculation of and subsequent testing of an inbreeding coefficient for population Tuk 6 indicated that inbreeding did provide an acceptable explanation for the observed heterozygote deficiency. The F value of 0.18 calculated for Tuk 6 is similar in magnitude to the mean F value of 0.21 ± 0.02 calculated for populations of the phoronid, Phoronopsis viridis (Ayala, Valentiné, Barr and Zumwalt 1974). Since inbreeding is thought to result from stochastic events such as clone size variation, there is likely to be some diversity in its incidence among natural

populations. Its possible occurrence was indicated infrequently in the D. magna populations considered in the present study.

No heterozygote deficiencies were observed for either of the Williams L. populations, but one population exhibited a significant heterozygote excess at the MDH locus. Although the size of the sample genotyped for this locus was small (23), the heterozygote excess was not thought to be due to sampling error. The high frequency of a single heterozygote and the paucity of homozygotes suggested the presence of heterotic selection. It is not completely certain whether the Williams L. ponds were temporary or permanent, but their large size and their location in mild southern British Columbia suggests the latter alternative. Heterosis has previously been cited as the cause for heterozygote excesses observed in natural, permanent D. magna populations (Hebert 1974b; Young 1979a,b) and populations of other Daphnia species (Mort and Jacobs 1981). Moreover, Hebert et al. (1982) have recently documented the occurrence of heterosis in hybrid strains of Daphnia magna under experimental conditions.

The results of the laboratory experiment suggested that little time may be required to develop large heterozygote excesses in permanent habitats. Clones possessing specific

heterozygous genotypes at both the LAP and the GOT loci dominated the population after only 120 days. Presumably, the fitness differences existing between genotypes at the beginning of the experiment were sufficiently amplified by only a few generations of parthenogenetic reproduction (Berger 1976; Hebert et al. 1982; Hebert 1974a), so that the heterozygous clones were able to exclude other genotypes. It is unlikely that heterotic selection was acting at the loci under investigation; more probably it acted on loci in linkage disequilibrium with them. Selection was apparently most pronounced during the population 'crash' since significant departure from Hardy-Weinberg expectations were not observed prior to this event. Additional evidence that heterozygous genotypes possess greater fitness than homozygotes in stressful environments (e.g. thermal stress and salinity stress) has been obtained by Hebert et al. (1982). In the present study, it is possible that the presumed heterotic genotypes approached fixation simply through chance events, although the probability of this is fairly low (14%). Nevertheless, it is suggested that several replicates of this experiment be carried out in order to ensure that heterosis is indeed the element resulting in the success of these heterozygous genotypes.

In recent years, allozyme studies have revealed great amounts of genetic variation among invertebrate species. In general, invertebrates were found to maintain as much genetic variation as plant populations and substantially

more variation than vertebrates (Hamrick 1979; Selander and Kaufman 1973). However, several exceptions to this regularity have been observed in cyclic parthenogenetic invertebrates. Populations of rotifers (King 1977) and aphids (Wool et al. 1978; Tomiuk and Wohrmann 1980; Suomalainen et al. 1980) are comparatively invariant and are polymorphic at only about 10% of their loci. Natural populations of the pulmonate slug Deroceras laeve which is capable of reproducing through apomictic parthenogenesis as well as through reciprocal outcrossing were found to be uniclonal and contained very little genic heterozygosity (Nicklas and Hoffman 1981). Similarly, Hebert and coworkers have found that the amount of genetic variation in cyclic parthenogenetic Daphnia species, as reflected by individual heterozygosities and the proportion of polymorphic loci, is typically low. Local populations of D. carinata were polymorphic on average at only 6.7% of their loci and individuals were heterozygous at only 2.1% of their loci (Hebert and Moran 1980). Individual heterozygosities and the proportion of polymorphic loci averaged only 1% for Churchill populations of D. magna. English populations of this species were polymorphic on average at only 15% of their loci and individuals were heterozygous at only 7% of their loci (Crease and Hebert 1982). The low amounts of genetic variation observed for the Tuk and Williams L. populations in the present study were consistent with these findings. Individual heterozygosities averaged 8.7% at Tuk and 7.8% at Williams L., and

the proportion of polymorphic loci averaged 20% and 27.8% for these localities. This lack of variation has been attributed to both founder effect (Hebert 1975) and pre-emptive competition (Hebert and Moran 1980).

While the variation at Tuk was low in comparison with other invertebrate groups, it was substantially greater than that of the Churchill populations, and slightly greater than that of the English ones. This may be due to the proximity of Tuk to the large glacial refuge extending across much of Alaska and the Yukon Territories. In fact, there is some speculation as to whether Tuk itself was glaciated, but most workers believe that it was (Mackay 1963; Prest 1970). The nearness of the glacial refuge would permit a larger flow of genetically diverse colonists to the Tuk ponds, thereby increasing the genetic variation of the D. magna populations in this area.

Earlier studies on D. magna (Hebert 1975) and D. carinata (Hebert and Moran 1980) revealed large gene frequency differences among populations only a few meters apart. It was suggested that such microgeographical differences in gene frequencies may be a consequence of founder effects, natural selection and limited gene exchange (Crease and Hebert 1982). Some heterogeneity in gene and genotype frequencies was also noted among the Tuk populations, although the degree of inter-population differentiation as measured by F_{ST} was not as great as that reported for grouped intermittent populations of English D. magna (Hebert 1978) and D. carinata (Hebert

and Moran 1980). This fact was also reflected in the small average genetic distance found among the Tuk populations (0.002 using 10 loci). Average genetic distances among the Williams L. populations (0.025 using 9 loci), and the San Diego clones (0.08 using 10 loci), were also quite low. Similarly, Crease and Hebert (1982) found low genetic distances among the Churchill populations (0.00 using 11 loci) and among the English populations (0.06) using 11 loci). High levels of genetic similarity have been reported for conspecific populations of other invertebrates as well. The mean genetic distance among local populations of different Drosophila species ranged from 0.002 to 0.31 (Zimmerman et al. 1978). Similarly, Ward (1980) reported a mean genetic distance between conspecific populations of ponerine ants of .015. Therefore, the overview of variation at the metapopulation level is one of comparative genetic homogeneity.

The genetic distances between North America metapopulations of D. magna were not large, ranging from 0.015 (using 9 loci) to 0.211 (using 10 loci). However, the genetic distances between any North American metapopulation and the English clones were substantially greater, ranging from 0.347(using 9 loci) to 0.627 (using 10 loci). Therefore, at most, 21% of loci differed between North American metapopulations, but pairs of metapopulations from England and North America were likely to differ at as much as 63%

of their loci. Ayala, Tracey, Hedgecock and Richmond (1974) cited similar distance values between sibling species of the Drosophila willistoni group, while other workers have found values of this order between well differentiated species (range 0.227-0.609)(Zimmerman et al. 1978). Crease and Hebert (1982) also reported a very high genetic distance between Churchill D. magna populations and English ones (0.48 using 11 loci). A similar study by Richardson et al. (1980) revealed that populations of rabbits collected from several areas across the Australian continent were more genetically similar to each other than to populations collected from Tasmania, France, England or Wales. Lakovarra et al. (1972) calculated the genetic relationships between European and American species of the Drosophila obscura group and found the smallest number of amino acid differences among American species, a slightly greater number among European species, and a much greater number between American and European species. Thus, it is apparent that within-continent genetic divergence in many organisms is small in comparison with between-continent divergence.

The genetic relationships between groups of organisms, generated from protein data, have been used to confirm the identity of ancestral stocks (Richardson et al. 1980) and to suggest possible migratory patterns followed by a species in its initial colonization of a land mass (Nixon and Taylor 1977). The great genetic similarities of the North American

metapopulations may be largely due to the way in which D. magna colonized this continent. Brooks (1957) suggested that the ancestral populations of North American D. magna originated in Asia, crossed into North America via the Bering land bridge and persisted in glacial refuges throughout Pleistocene glaciation. The genetic distances between the Tuk, Churchill and English D. magna populations were in agreement with a Bering land bridge colonization of North America. The genetic distances between Churchill and English populations was the largest, followed by the distance between the Tuk and English populations, and finally, the distance between the Tuk and Churchill populations was the smallest. As Cambridge, England, is at the western limit of the Old World range and Churchill is at the eastern limit of the North American range, the genetic divergence between the metapopulations of these two localities is expected to be maximal. After the ice sheets retreated, certain stocks of D. magna may have migrated down the western edge of the continent to colonize the western provinces and states. Other stocks may have migrated eastward along the Arctic Ocean coast to colonize Tuk, and eventually, Churchill. The genetic distances between North American metapopulations may not suggest these particular routes, but it is expected that the relative divergence estimates involving the San Diego and Williams L. localities will change slightly as more populations are included in the sample. Also, different

selective pressures operating in these environmentally distinct localities have undoubtedly led to differing rates of genetic divergence between metapopulations.

SUMMARY

The majority of Daphnia magna populations collected from temporary pools in the area of Tuktoyaktuk, N.W.T. were in Hardy-Weinberg equilibrium. A few instances of heterozygote deficiency were noted and were attributed to the presence of inbreeding or the Wahlund effect. Heterozygote excess was observed in one of two D. magna populations sampled near Williams L., B.C. These populations were presumed to be permanent and the heterozygote excess was attributed to heterotic selection. Laboratory experiments revealed that large heterozygote excesses can develop very rapidly in a permanent habitat. The amount of genetic variation detected in the populations under investigation was lower than that of other invertebrate species, but typical of cyclic parthenogenetic groups. Tuk populations of D. magna were more variable than Churchill, Man. populations due to the proximity of Tuk to a glacial refuge. Heterogeneity in gene frequencies between populations of a locality was noted, but was smaller than that previously recorded for Daphnia populations. North American metapopulations of D. magna were closely related to each other but were quite genetically distinct from English populations. The genetic similarities between the Tuk, Churchill and Cambridge metapopulations are in accord with a Bering Land Bridge colonization of North America by ancestral stocks of D. magna originating in Asia.

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CHAPTER IV

A COMPARATIVE STUDY OF CLONAL DIVERSITY IN *DAPHNIA PULEX* METAPOPULATIONS FROM HABITATS OF DIFFERENT AGES

CHAPTER IV

INTRODUCTION

Recent genetic studies have shown that Daphnia pulex populations in Ontario (Hebert and Crease, 1982), Churchill, Man., and Frobisher Bay, N.W.T. (McWalter and Hebert, 1982) reproduce by obligate parthenogenesis. These populations possess substantial amounts of genetic variation which suggests that asexual forms may not be lacking in evolutionary potential as was originally supposed (Maynard Smith, 1978). Since North American populations of D. pulex reproducing through cyclic parthenogenesis have also been observed (Lynch, 1982; Schwartz, pers. comm.), the need to map the distribution of D. pulex populations capable of sexual reproduction has been recognized (Hebert and Crease, 1982). Accordingly, the present study aimed at determining the extent and nature of genetic variation among populations of D. pulex collected from three sites in the western Canadian arctic: Old Crow, Y.T., Inuvik, N.W.T. and Tuktoyaktuk, N.W.T. The results confirm the absence of sexual reproduction in these populations and provide additional support for the notion that asexual Daphnia species are genetically diverse.

Many hypotheses have been developed in an attempt to explain species (or clonal) richness differences between habitats. These hypotheses range from those which

suggest that species accumulation is asymptotic and depends upon the area occupied by suitable habitat to others which assume that species (or clonal) diversity increases with habitat age. In support of the former model, Strong (1974) showed that insect diversity on host plants is asymptotic; host plant range sets an upper limit to insect diversity. However, the latter model was supported by Southwood's (1961) data which indicated that the number of insect species associated with British trees reflected the time the trees had been in Britain (as measured by the number of Quaternary fossil records of the trees). Similarly, White (1970) has suggested that genetic divergence within and between parthenogenetic populations may be related to the age of the parthenogenetic form. Accordingly, one would expect that an area which has supported a parthenogenetic species for a long time would be more clonally diverse than an area more recently colonized. However, other workers have suggested that clonal diversity may decrease with increasing age of a parthenogenetic form. Jaenike et al. (1982) postulated that if clonal diversity of a species is generated through the multiple origin of parthenogenesis from sexual ancestors, then diversity will drop to an equilibrium level determined by ecological considerations following the extinction of sexual relatives. Accordingly, one objective of the present study was to test the relationship between clonal diversity in D. pulex and habitat age.

The Old Crow area was chosen for study since it lies within an area of 70,000 square miles in the western Yukon which escaped Pleistocene glaciation altogether (Prest, 1970). During the latter part of the Wisconsin glaciation, the environment of the Old Crow area consisted of extensive grassy uplands broken by spruce woodland with lakes, ponds and streams in lower areas (Crossman and Harington, 1970). Much of the Old Crow glacial refuge is too old to be dated accurately through radicarbon methods, but sediment in this region has been dated at $>$41,300 years of age (Prest, 1970). However, the greater part of the Mackenzie Delta region, including the Inuvik and Tuk areas, was evidently covered by Laurentide glacier ice until approx. 8,000 years B.P. (Hughes, 1970). Therefore, the Tuk and Inuvik areas were also chosen as sites for study as they represent habitats which are considerably younger than the Old Crow locality.

In past studies, many workers have measured clonal diversity of parthenogenetic taxa by counting the total number of electrophoretically distinguishable clones in samples (Selander et al., 1978; Parker, 1979; Suomalainen and Saura, 1973; Parker and Selander, 1976; Lokki et al., 1975; Jaenike et al., 1980; Mitter et al., 1979; Vrijenhoek, 1978, 1979; McWalter and Hebert, 1982; Hebert and Crease, 1982). However, it should be realized that clonal diversity, like species diversity, is not only dependent upon clonal richness but also upon clonal relative abundance. Prior

estimates of clonal diversity have failed to take this into consideration. A wide variety of indices have been derived in an attempt to accurately estimate species diversity. One index (Simpson's diversity index) was used by Jaenike et al. (1982) in order to measure clonal diversity of parthenogenetic earthworm populations. Similarly, in the present study, 3 diversity indices (α, Shannon-Weaver and Brillouin) were calculated for the D. pulex clones inhabiting each of the three sites in the western Canadian arctic. The obtained values have not been taken to represent the absolute clonal diversity of a locality, but have been used only as a means of comparing clonal diversity estimates between localities.

MATERIALS AND METHODS

D. pulex was collected from ponds in the Old Crow, Y.T. (67.35N 139.50W), Inuvik, N.W.T. (68.25N 133.03W), and Tuktoyaktuk, N.W.T. (Tuk - 69.27N 133.02W) (Appendix III) areas. The conductivities and salinities of these ponds are listed in Appendix IV. Nine populations were analyzed from Old Crow and Inuvik and eight were analyzed from Tuk. All ponds were sampled in early August 1980 and most contained parthenogenetic females as well as ephippial and nonreproductive females. Males were not observed in any of the samples. Although many ponds containing darkly pigmented D. pulex were observed at Tuk, no collections were made from them. Samples were taken exclusively from those ponds inhabited by unpigmented morphs. In addition to D. pulex , many of the ponds contained other zooplankters including other Daphnia species.

Old Crow and Inuvik are situated just south of the treeline in the taiga, while Tuk in in the tundra zone. Most of the ponds sampled in the first two areas were small, shallow pools surrounded by grasses and other herbaceous vegetation. Six of the Tuk ponds (Tuk 1, 3, 5, 6, 7, 8) were small frost polygon ponds formed when water freezes in spaces within the soil and expands as ice. The ice causes the soil to bulge; then in spring, the ice cracks and melts forming a pond (Ray and McCormic-Ray, 1981). Tuk 2 was located close to the Beaufort Sea, while Tuk 4 was a larger, deeper pond located further inland.

Live samples of each population were air freighted to Windsor where clones were established from 48 individuals from each population. Loss of clones varied between populations and may be attributed to the fact that many of the females used to initiate cultures were not carrying parthenogenetic broods. The clonal genotypes were electrophoretically determined at 10 enzyme loci: glucose-6-phosphate dehydrogenase (G6PDH), xanthine dehydrogenase (XDH), lactate dehydrogenase (LDH), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), glutamate oxaloacetate transaminase (GOT), esterase-1 (EST), amylase-1 (AMY-1), amylase-2 (AMY-2) and malate dehydrogenase (MDH). For details on electrophoretic procedures see Crease (1980). Alleles were numbered in order of increasing mobility, using the same allelic designations as McWalter (1981).

Two different experiments were carried out in order to establish the mode of reproduction of the clones identified in this study. First females from a random sample of clones were grown in small numbers to ensure no males were present. Ephippial egg production was induced and resultant ephippia were checked for egg deposition. Clones reproducing by cyclic parthenogenesis normally fail to release eggs in the absence of males. In addition, ephippial eggs from a number of clones were hatched, in an attempt to determine if segregation occurred during ephippial egg production. Hatching was induced by freezing ephippia from each clone in 100 ml of synthetic pond water (formula

given in Crease, 1980) for a period of 3 weeks. They were then thawed and approx. 2 ml of a mixed algal suspension (primarily Scenedesmus and Kirschneriella) were added to the pond water. Ephippia were maintained at 10°C and 24 hr. light (intensity = 24 microeinsteins $m^{-2}sec^{-1}$) until hatching occurred.

Genotypic frequencies at polymorphic loci in each population were checked for concordance with Hardy-Weinberg expectations using a chi-square goodness-of-fit test. Populations were not included in the analysis if greater than one-third of the expected frequencies were less than 4. The data on clonal genotypes were used to calculate average heterozygosity per locus ($\underline{h}_L$ = number of heterozygous loci / total number of loci) for all the arctic D. pulex clones. Gene frequency data obtained from the genotypes of the individual arctic clones and the Ontario D. pulex clones investigated by Hebert and Crease (1982) were used to calculate Nei's (1975) measures of genetic divergence, I and D, for each pair of Ontario, Inuvik, Old Crow and Tuk clones. Only Ontario clones which had been genotyped at the 10 loci investigated in the present study were included. Thus, seven Windsor clones were omitted from the analysis since their EST genotypes were unknown. Single link cluster analysis was performed on the matrix of genetic distances among the arctic and Ontario clones to construct a dendrogram. This procedure was repeated using only arctic D. pulex clones. The cluster analysis was based on average genetic distance and used the BMD P1M program.

Clonal diversities in each of the three areas (Old Crow, Tuk and Inuvik) were estimated by the Shannon-Weaver ($H' = -\Sigma p_i \ln p_i$, where p_i = the proportion of i^{th} species in the population), Brillouin ($H = \frac{1}{N} \ln \frac{N!}{N_1!N_2!...N_s!}$, where s = the number of species, N = the total number of individuals and N_i = the number of individuals of the i^{th} species) and α diversity indices. These three indices were calculated using a microcomputer (see Appendix V for computer programs). The ponds of each area were ordered in a random fashion. Values of the three indices were calculated for the first pond; then the data from the second pond was pooled with that of the first and new estimates were made. In this manner, all the ponds in a locality were eventually included and diversity estimates were calculated for each newly expanded sample. This procedure was repeated nine times and mean values of each diversity index were calculated for the different values of the number of ponds included in the pooled sample. Successive mean values of H and α were then plotted against the number of ponds accumulated in the pooled sample.

RESULTS

The ten enzymes surveyed in this study fell into two categories. The first category contained those enzymes (XDH and G6PDH) which were invariant in all three areas and all individuals were designated as 11 homozygotes at these loci. The second category were those enzymes (LDH, PGI, PGM, AMY-1, AMY-2, MDH, GOT, EST) which were variable at one or more localities. The population genotypic frequencies at these eight polymorphic loci are shown in Table 4.1 and photographs of the allozyme patterns at all 10 loci appear in Fig. 4.1.1-4.1.10. Two LDH alleles (1,3) were observed but only allele 1 (present in the homozygous condition) was found at Tuk and Old Crow. Two triple banded homozygotes (11, 33) and one fifteen banded heterozygote (13) were seen in the Inuvik populations. Four alleles were observed at the PGI locus (1,4,6,6*). Allele 6* was a null allele with the same mobility as allele 6. Five PGI phenotypes were observed; including 3 single banded homozygotes (11,44;66), one triple banded heterozygote (14) and one double banded heterozygote (46*). Seven phenotypes were present at the PGM locus. Two were single banded homozygotes (22,33) and the remaining five were double banded heterozygotes (12, 13,23,24, 25). AMY-1 was characterized by five phenotypes: three single banded homozygotes (33,44,55), one double banded heterozygote (34) and one triple banded pattern (013)

Table 4.1 Genotypic frequencies at 8 polymorphic loci in populations of D. pulex from Old Crow, Inuvik and Tuk. Alleles designated as 1' are intermediate in mobility between alleles 1 and 2. All null alleles are designated with *.

		LDH			PGI				
Population	n	11	13	33	11	44	14	66	46*
Old Crow 1	48	1.00			0.98	0.02			
Old Crow 2	27	1.00			0.74	0.26			
Old Crow 3	28	1.00			1.00				
Old Crow 4	44	1.00			1.00				
Old Crow 5	12	1.00			0.67	0.33			
Old Crow 6	31	1.00			0.45	0.55			
Old Crow 7	40	1.00			0.25	0.75			
Old Crow 8	28	1.00			0.57	0.43			
Old Crow 9	39	1.00			0.56	0.44			
Inuvik 1	38	0.03	0.97			0.53	0.47		
Inuvik 2	44	0.02	0.98			0.27	0.73		
Inuvik 3	30		0.27	0.73		1.00			
Inuvik 4	48		0.27	0.73		1.00			
Inuvik 5	33		1.00			1.00			
Inuvik 6	43	0.19	0.81			1.00			
Inuvik 7	47		1.00			1.00			
Inuvik 8	47		1.00			1.00			
Inuvik 9	46	0.85	0.15			1.00			
Tuk 1	24	1.00				0.83		0.17	
Tuk 2	38	1.00							1.00
Tuk 3	35	1.00				1.00			
Tuk 4	1	1.00							1.00
Tuk 5	4	1.00							1.00
Tuk 6	8	1.00			0.13				0.87
Tuk 7	18	1.00				1.00			
Tuk 8	27	1.00				1.00			

Table 4.1 Continued

	PGM							MDH			
Population	12	1'3	22	23	24	25	33	11	0'1*	0'3	33
Old Crow 1		0.98					0.02	1.00			
Old Crow 2		0.74					0.26	1.00			
Old Crow 3		1.00						1.00			
Old Crow 4		1.00						1.00			
Old Crow 5		0.67			0.33			1.00			
Old Crow 6		0.10	0.35	0.06			0.49	1.00			
Old Crow 7		0.25		0.05			0.70	1.00			
Old Crow 8		0.57					0.43	1.00			
Old Crow 9		0.56		0.36			0.08	1.00			
Inuvik 1						0.03	0.97	1.00			
Inuvik 2				0.07		0.02	0.91	1.00			
Inuvik 3	0.93						0.07	1.00			
Inuvik 4			0.27				0.73	1.00			
Inuvik 5				1.00				1.00			
Inuvik 6				1.00				1.00			
Inuvik 7				0.32			0.68	1.00			
Inuvik 8				0.81			0.19	1.00			
Inuvik 9				0.15		0.50	0.35	1.00			
Tuk 1							1.00	1.00			
Tuk 2							1.00	1.00			
Tuk 3			0.11	0.89				0.11	0.89		
Tuk 4							1.00	1.00			
Tuk 5							1.00			1.00	
Tuk 6		0.13					0.87	0.13			0.87
Tuk 7				1.00					1.00		
Tuk 8			0.33	0.67				1.00			

Table 4.1 Continued

	AMY-1					AMY-2		
Population	013	33	34	44	55	0*0*	12	22
Old Crow 1		1.00						1.00
Old Crow 2		0.74	0.26			0.26		0.74
Old Crow 3		1.00						1.00
Old Crow 4		1.00						1.00
Old Crow 5		1.00				0.33		0.67
Old Crow 6		0.52	0.42	0.06		0.52		0.48
Old Crow 7		0.97	0.03			0.03		0.97
Old Crow 8		0.64	0.36			0.18		0.82
Old Crow 9		0.79	0.21			0.08		0.92
Inuvik 1		1.00						1.00
Inuvik 2		1.00						1.00
Inuvik 3		1.00						1.00
Inuvik 4		0.73	0.27					1.00
Inuvik 5		1.00						1.00
Inuvik 6		0.81	0.19			0.19		0.81
Inuvik 7		0.32			0.68			1.00
Inuvik 8		0.81			0.19			1.00
Inuvik 9		0.65	0.35					1.00
Tuk 1		1.00				1.00		
Tuk 2	1.00							1.00
Tuk 3			0.89	0.11			1.00	
Tuk 4	1.00							1.00
Tuk 5					1.00			1.00
Tuk 6	0.87	0.13						1.00
Tuk 7				1.00			1.00	
Tuk 8				1.00			1.00	

Table 4.1 Continued

Population	GOT		EST						
	22	23	13	23	24	22	33	34	44
Old Crow 1	1.00						0.02	0.98	
Old Crow 2	1.00						0.26	0.74	
Old Crow 3	1.00							1.00	
Old Crow 4	1.00							0.91	0.09
Old Crow 5	0.67	0.33					0.33	0.67	
Old Crow 6	0.52	0.48					0.58	0.42	
Old Crow 7	0.97	0.03					0.97	0.03	
Old Crow 8	1.00				0.32		0.68		
Old Crow 9	1.00				0.08		0.20	0.72	
Inuvik 1	0.03	0.97		0.89	0.11				
Inuvik 2	0.02	0.98	0.16	0.75			0.09		
Inuvik 3	0.53	0.47	0.07		0.46		0.47		
Inuvik 4	1.00						1.00		
Inuvik 5		1.00					1.00		
Inuvik 6	0.19	0.81					0.81	0.19	
Inuvik 7	0.68	0.32					1.00		
Inuvik 8	0.19	0.81					1.00		
Inuvik 9	0.85	0.15					0.65	0.35	
Tuk 1	0.17	0.83					1.00		
Tuk 2	1.00					1.00			
Tuk 3	1.00						1.00		
Tuk 4	1.00					1.00			
Tuk 5	1.00					1.00			
Tuk 6	1.00					0.87		0.13	
Tuk 7	1.00						1.00		
Tuk 8	1.00						1.00		

Figure 4.1.1 Electrophoretic phenotypes of XDH. From left to right: 00, 00, 00, 11, 11, 11. 00 homozygotes are D. curvirostris and 11 homozygotes are D. pulex from the western arctic.

Figure 4.1.2 Electrophoretic phenotypes of G6PDH. From left to right: 00, 00, 00, 11, 11, 11. 00 homozygotes are *D. curvirostris* and 11 homozygotes are *D. pulex* from the western arctic.

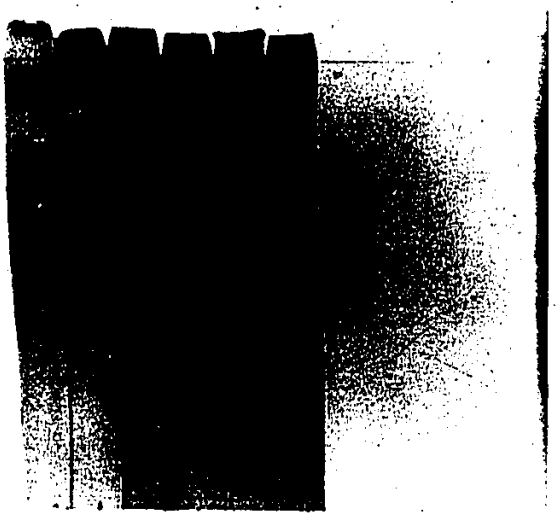


Figure 4.1.3 Electrophoretic phenotypes of LDH. From left to right: 11, 11, 13, 13, 33, 33. All phenotypes are D. pulex from the western arctic.

Figure 4.1.4 Electrophoretic phenotypes of PGI. From left to right: 00*, 0'0', 11, 14, 44, 46*, 66. 00* and 0'0' are D. curvirostris and 11, 14, 44, 46*, 66 are D. pulex from the western arctic.

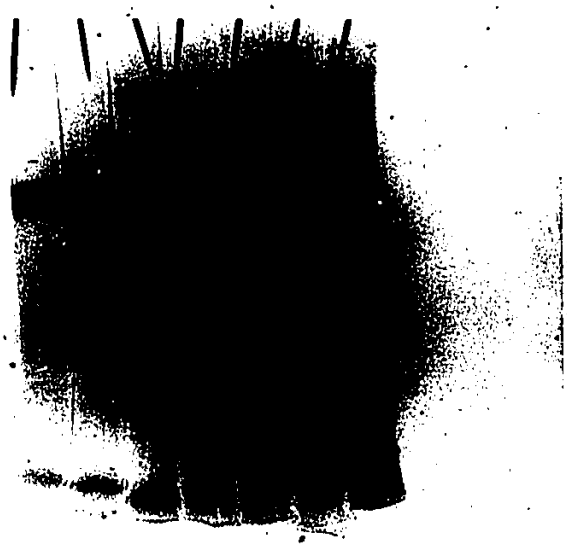


Figure 4.1.5 Electrophoretic phenotypes of PGM. From left to right: 12, 22, 23, 33, 1'3, 14, 24, 25, 35, 34. 34 is D. schodleri, 35 is D. middendorffiana from the Churchill area and 12, 22, 23, 33, 1'3, 24, 25 are D. pulex from the western arctic.

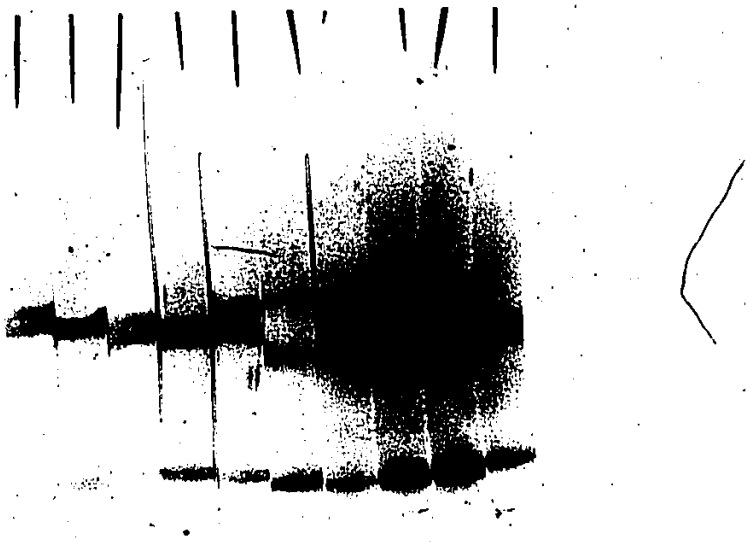
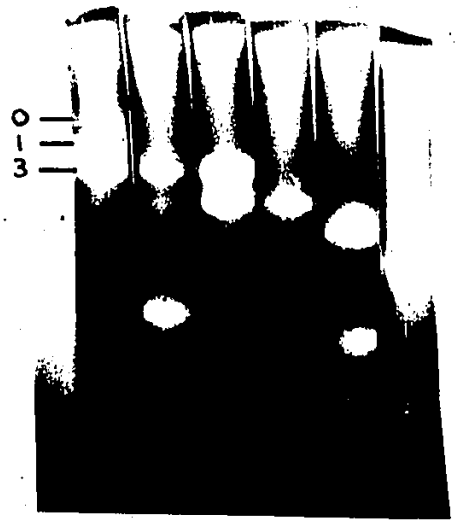


Figure 4.1.6 Electrophoretic phenotypes of AMY-1. From left to right: 013, 33, 34, 44, 55, 77. 77 is D. schodleri and 013, 33, 34, 44 and 55 are D. pulex from the western arctic.



which may have been the result of a gene duplication. The triple banded phenotype was seen only in two of the Tuk clones. AMY-2 phenotypes were explained by the presence of two normal activity alleles (1,2) and one null activity allele (0*). The null allele and allele 2 were detected at all three localities, while allele 1 was present only in three of the Tuk clones as a 12 heterozygote. Three normal activity alleles (0',1,3) and one null activity allele (1*) were detected at the MDH locus. Alleles 1 and 1* appeared to have the same mobility. Four MDH phenotypes were seen at Tuk: 2 double banded homozygotes (11,33), one 4 banded heterozygote (0'3) and one triple banded null heterozygote (0'1*). All Old Crow and Inuvik individuals were 11 homozygotes. Single banded GOT homozygotes (22) and triple banded heterozygotes (23) were observed in clones from all three areas under investigation. The EST locus was characterized by 4 alleles (1,2,3,4) found as 3 single banded homozygotes (22,33,44) and 4 double banded heterozygotes (13,23,24,34).

Clonal Diversity at Old Crow

Genotypic data for 6 polymorphic loci at Old Crow permitted the recognition of 17 genetically distinct clones (Table 4.2-4.3). Based on the 10 loci scored for each clone, clonal heterozygosities ranged from 0-40.0% with an average of 16.5%. There was substantial variation in clonal abundances. 01 was the most common clone, present in 6 of 9 habitats while several other clones (04,05,06,07,08,010,011, 012,013,014,015,016,017) were detected only in a single pond.

Figure 4.1.7 Electrophoretic phenotypes of AMY-2. From left to right: 22, 22, 12, 12, 0*0*, 0*0*. 0*0* homozygotes are D. curvirostris and 22 and 12 are D. pulex from the western arctic.

Figure 4.1.8 Electrophoretic phenotypes of MDH. From left to right: 11, 12, 12, 0'1*,0'3, 00, 11, 33. 00 is D. curvirostris, 12 is a black-back D. pulex from the Churchill area and 11, 0'1*,0'3, 33 are D. pulex from the western arctic.

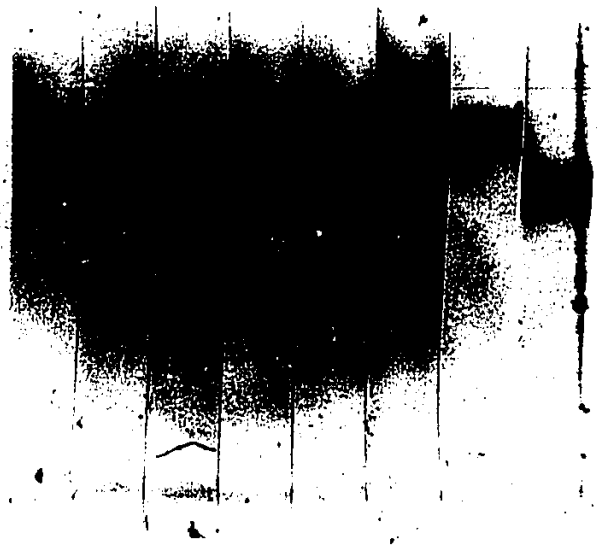


Figure 4.1.9 Electrophoretic phenotypes of GOT. From left to right: 1'1', 13, 22, 23, 33. 1'1' and 33 are *D. curvirostris*, 13 is a *D. middendorffiana* from the Churchill area and 22, 23 are *D. pulex* from the western arctic.

Figure 4.1.10 Electrophoretic phenotypes of EST. From left to right: 22, 23, 33, 13, 24, 34, 44. All phenotypes are *D. pulex* from the western arctic.

Table 4.2 Genotypic characteristics of arctic *D. pulex* at 8 polymorphic loci.

Clone	LDH	PGI	PGM	AMY-1	AMY-2	MDH	GOT	EST	Clonal heterozygosity
01	11	11	1'3	33	22	11	22	34	.200
02	11	44	33	33	22	11	22	33	.000
03	11	44	33	34	0*0*	11	22	33	.100
04	11	11	1'3	33	22	11	22	44	.100
05	11	44	24	33	0*0*	11	23	33	.200
06	11	11	22	34	0*0*	11	23	34	.300
07	11	44	33	44	22	11	23	33	.100
08	11	11	1'3	33	0*0*	11	22	33	.100
09	11	11	1'3	33	22	11	22	33	.100
010	11	44	23	33	22	11	22	33	.100
011	11	44	23	34	0*0*	11	23	34	.400
012	11	44	33	34	22	11	22	24	.200
013	11	44	33	34	22	11	22	33	.100
014	11	44	33	34	0*0*	11	22	24	.200
015	11	44	23	33	22	11	22	34	.200
016	11	44	23	34	22	11	22	33	.200
017	11	11	1'3	33	22	11	22	24	.200
									$\bar{x}$ = .165

Table 4.2 Continued

Clone	LDH	PGI	PGM	AMY-1	AMY-2	MDH	GOT	EST	Clonal heterozygosity
I18	13	44	33	33	22	11	23	24	.300
I19	13	44	33	33	22	11	23	23	.300
I20	13	14	33	33	22	11	23	23	.400
I21	11	44	25	33	22	11	22	23	.200
I22	11	44	25	33	22	11	22	33	.100
I23	13	44	33	33	22	11	23	13	.300
I24	13	44	23	33	22	11	23	33	.300
I25	13	44	12	33	22	11	23	33	.300
I26	33	44	33	33	22	11	22	13	.100
I27	33	44	12	33	22	11	22	24	.200
I28	33	44	12	33	22	11	23	33	.200
I29	11	44	22	34	22	11	22	33	.100
I30	33	44	33	33	22	11	22	33	.000
I31	11	44	23	34	0*0*	11	22	34	.300
I32	13	44	33	55	22	11	22	33	.100
I33	11	44	33	34	22	11	22	34	.200
									$\bar{x}$ = .213

Table 4.2 Continued

Clone	LDH	PGI	PGM	AMY-1	AMY-2	MDH	GOT	EST	Clonal heterozygosity
T34	11	66	33	33	0*0*	11	22	33	.000
T35	11	44	33	33	0*0*	11	23	33	.000
T36	11	46*	33	013	22	11	22	22	.200
T37	11	44	23	34	12	0'1*	22	33	.300
T38	11	44	22	44	12	11	22	33	.100
T39	11	46*	33	55	22	0'3	22	22	.200
T40	11	46*	33	013	22	33	22	22	.200
T41	11	44	23	44	12	0'1*	22	33	.300
T42 = 01	11	11	1'3	33	22	11	22	34	.200
									$\bar{x}$ = .167

Table 4.3 Clonal complements of the habitats. Clonal abundances are in brackets.

Pond	Sample Size	Clonal Abundances
Old Crow 1	48	01(47), 02(1)
Old Crow 2	27	01(20), 03(7)
Old Crow 3	28	01(28)
Old Crow 4	44	01(40), 04(4)
Old Crow 5	12	01(8), 05(4)
Old Crow 6	31	02(13), 06(11), 07(2), 08(3), 011(2)
Old Crow 7	40	02(28), 09(10), 010(1), 011(1)
Old Crow 8	28	09(16), 012(6), 03(2), 014(3), 013(1)
Old Crow 9	39	01(19), 017(3), 03(3), 015(9), 016(5)
Inuvik 1	38	I18(4), I19(15), I20(18), I21(1)
Inuvik 2	44	I19(1), I20(32), I22(1), I23(7), I24(3)
Inuvik 3	30	I25(8), I26(2), I27(14), I28(6)
Inuvik 4	48	I29(13), I30(35)
Inuvik 5	33	I24(33)
Inuvik 6	43	I24(35), I31(8)
Inuvik 7	47	I24(15), I32(32)
Inuvik 8	47	I24(38), I32(9)
Inuvik 9	46	I22(23) I24(7) I33(16)
Tuk 1	24	T34(4), T35(20)
Tuk 2	38	T36(38)
Tuk 3	35	T37(31), T38(4)
Tuk 4	1	T36(1)
Tuk 5	4	T39(4)
Tuk 6	8	T42 = 01(1), T40(7)
Tuk 7	18	T41(18)
Tuk 8	27	T38(9), T41(18)

Between 1 and 5 clones were found in each habitat (Table 4.3).

Chi-square analyses of genotype frequencies at polymorphic loci in each Old Crow population revealed marked deviations from Hardy-Weinberg equilibrium (Table 4.4). Of the 30 X^2 values calculated for the Old Crow metapopulation, 19 were significant at the 5% level. Thirteen of the significant deviations took the form of a heterozygote deficiency while the remaining 6 deviations were the result of heterozygote excesses. Heterozygote excesses and deficiencies frequently existed at different loci in the same population. Linkage disequilibria were quite pronounced between certain loci. For example, with the exception of one clone (06) all PGI 11 homozygotes were also PGM 1'3 heterozygotes.

Values of 3 diversity indices (Shannon Weaver, Brillouin and α) calculated after each of the 9 Old Crow ponds were included in a pooled sample are listed in Table 4.5. The final diversity values calculated when all ponds of the area were pooled were as follows: α index = 3.92, Shannon Weaver index = 2.81 and Brillouin index = 2.66. α and Brillouin diversity indices were plotted against the number of ponds in the pooled sample in Figure 4.2. The curves of both indices tended to level off as the number of ponds in the pooled sample increased.

Clonal Diversity at Inuvik

Seven of ten loci studied were variable at Inuvik and clonal heterozygosities ranged from 0-40.0% with an average of 21.3% (Table 4.2). Sixteen clones were recognized at

Table 4.4 Chi-square analysis of genotype frequency in *D. pulex* populations.
*P value=0.05 **P value=0.001 ***P value=0.0001

Population	Locus	D.F.	X^2	Heterozygote excess (E) or deficiency (D)
Old Crow 2	PGI	1	27.00***	D
	PGM	1	9.34**	E
	AMY-1	1	0.60	
	AMY-2	1	27.00***	D
	EST	1	9.34**	E
Old Crow 3	PGM	1	28.00***	E
	EST	1	28.00***	E
Old Crow 4	EST	1	30.56***	E
Old Crow 5	PGI	1	12.00**	D
	AMY-2	1	12.00**	D
	EST	1	3.00	
Old Crow 6	PGI	1	31.00***	D
	PGM	3	23.43***	D
	AMY-1	1	0.09	
	AMY-2	1	31.00***	D

Table 4.4 Continued

Population	Locus	D.F.	X^2	Heterozygote excess (E) or deficiency (D)
Old Crow 6	GOT	1	3.16	
	EST	1	3.16	
Old Crow 7	PGI	1	40.00^{***}	D
	PGM	3	1.25	
	AMY-1	1	6.40×10^{-3}	
	AMY-2	1	40.00^{***}	D
	GOT	1	6.40×10^{-3}	
	EST	1	6.40×10^{-3}	
Old Crow 8	PGI	1	28.00^{***}	D
	PGM	1	4.48^{*}	E
	AMY-1	1	1.32	
	AMY-2	1	28.00^{***}	D
Old Crow 9	PGI	1	39.00^{***}	D
	AMY-1	1	0.51	
	AMY-2	1	39.00^{***}	D

Table 4.4 Continued

Population	Locus	D.F.	X^2	Heterozygote excess (E) or deficiency (D)
Inuvik 1	LDH	1	34.20***	E
	PGI	1	3.66	
	GOT	1	34.20***	E
Inuvik 2	LDH	1	40.18***	E
	PGI	1	14.37***	E
	GOT	1	40.18***	E
	EST	3	30.56***	E
Inuvik 3	LDH	3	60.00***	D
	GOT	1	2.78	
Inuvik 4	LDH	3	96.00***	D
	PGM	1	48.00***	D
	AMY-1	1	1.18	
Inuvik 5	LDH	1	33.00***	E
	PGM	1	33.00***	E
	GOT	1	33.00***	E

Table 4.4 Continued

Population	Locus	D.F.	X^2	Heterozygote excess (E) or deficiency (D)
Inuvik 6	LDH	1	20.25***	E
	AMY-1	1	0.45	
	AMY-2	1	43.00***	D
	GOT	1	20.25***	E
	EST	1	0.45	
Inuvik 7	PGM	1	1.69	
	AMY-1	1	47.00***	D
	GOT	1	1.69	
Inuvik 8	PGM	1	21.64***	E
	AMY-1	1	47.00***	D
	GOT	1	21.64***	E
Inuvik 9	LDH	1	0.32	
	PGM	3	59.36***	E
	AMY-1	1	2.04	

Table 4.4 Continued

Population	Locus	D.F.	X^2	Heterozygote excess (E) or deficiency (D)
Inuvik 9	GOT	1	0.32	
	EST	1	2.04	
Tuk 1	PGI	1	24.00***	D
	GOT	1	12.25**	E
Tuk 2	PGI	1	38.00***	E
Tuk 3	PGM	1	22.11***	E
	AMY-1	1	22.11***	E
	AMY-2	1	22.11***	E
	MDH	1	22.11***	E

Table 4.4 Continued

Population	Locus	D.F.	X^2	Heterozygote excess (E) or deficiency (D)
Tuk 7	PGM	1	18.00**	E
	MDH	1	18.00**	E
	AMY-2	1	18.00**	E
Tuk 8	PGM	1	6.13*	E
	AMY-2	1	16.88**	E

Table 4.5 Successive mean diversity indices (± S.E.) calculated from 10 different random orderings of ponds in each arctic locality. The final diversity estimate after all ponds from a locality are included in the sample cannot vary.

OLD CROW

No. of Ponds in Pooled Sample	Shannon-Weaver Index	Brillouin Index	α Index
1	0.63 ± 0.15	0.53 ± 0.13	1.03 ± 0.19
2	1.32 ± 0.15	1.19 ± 0.13	1.68 ± 0.37
3	1.71 ± 0.08	1.57 ± 0.06	2.20 ± 0.30
4	2.01 ± 0.06	1.84 ± 0.04	2.44 ± 0.19
5	2.30 ± 0.05	2.13 ± 0.03	3.19 ± 0.18
6	2.44 ± 0.02	2.27 ± 0.02	3.35 ± 0.13
7	2.56 ± 0.02	2.40 ± 0.03	3.51 ± 0.16
8	2.71 ± 0.01	2.55 ± 0.01	3.82 ± 0.09
9	2.81	2.66	3.92

INUVIK

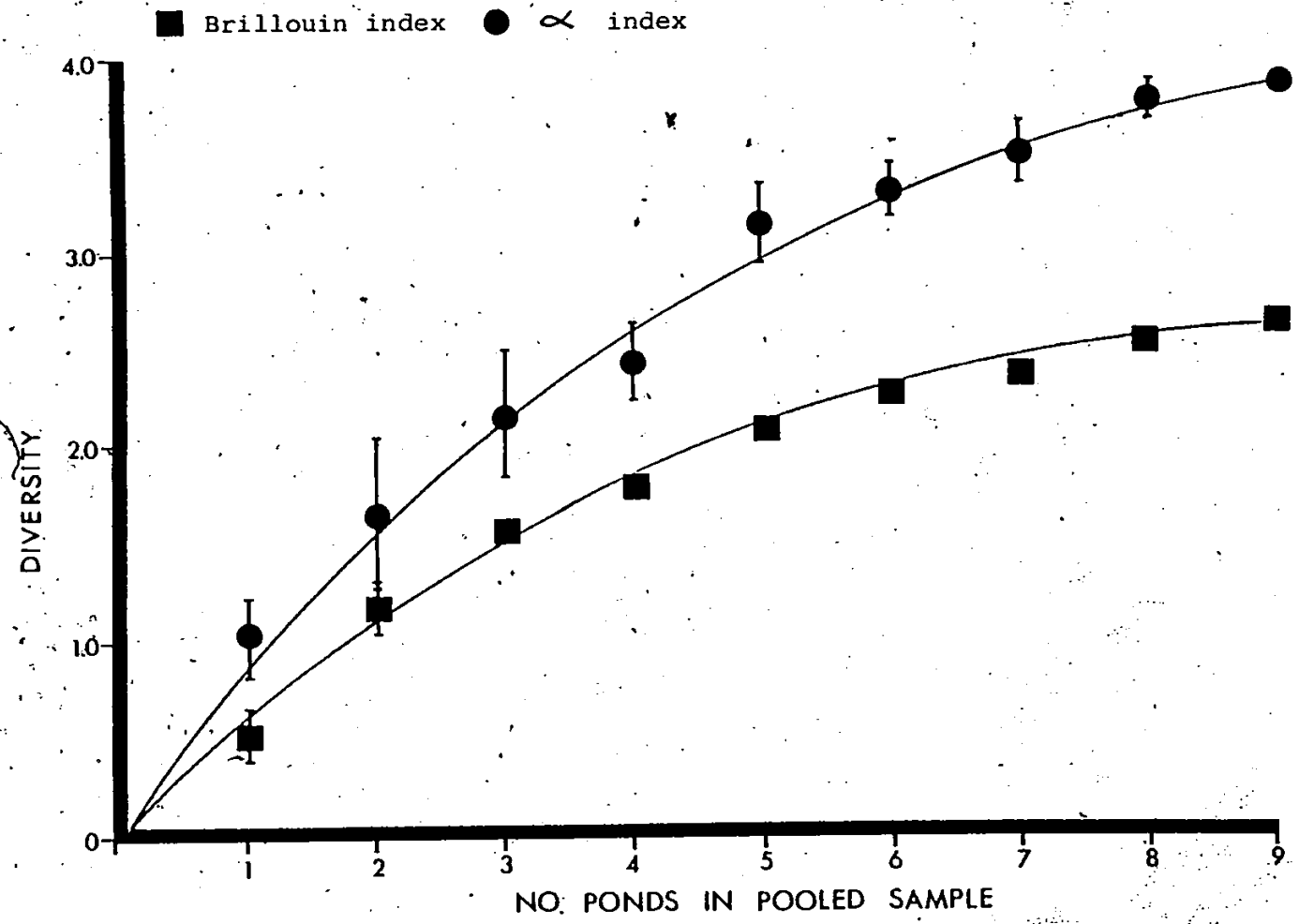
No. of Ponds in Pooled Sample	Shannon-Weaver Index	Brillouin Index	α Index
1	0.97 ± 0.03	0.85 ± 0.03	1.29 ± 0.05
2	1.44 ± 0.03	1.34 ± 0.02	1.53 ± 0.04
3	1.77 ± 0.04	1.69 ± 0.03	1.82 ± 0.10
4	2.11 ± 0.04	1.99 ± 0.03	2.16 ± 0.15
5	2.32 ± 0.04	2.26 ± 0.07	2.55 ± 0.19
6	2.47 ± 0.03	2.34 ± 0.03	2.62 ± 0.18
7	2.66 ± 0.03	2.48 ± 0.03	2.92 ± 0.14
8	2.78 ± 0.01	2.65 ± 0.01	3.33 ± 0.08
9	2.88	2.76	3.39

Table 4.5 Continued

TUK

No. of Ponds in Pooled Sample	Shannon-Weaver Index	Brillouin Index	α Index
1	0.38 ± 0.06	0.26 ± 0.05	0.86 ± 0.05
2	0.93 ± 0.06	0.80 ± 0.05	1.09 ± 0.06
3	1.29 ± 0.08	1.12 ± 0.08	1.35 ± 0.09
4	1.50 ± 0.04	1.37 ± 0.03	1.58 ± 0.10
5	1.67 ± 0.03	1.54 ± 0.03	1.69 ± 0.07
6	1.78 ± 0.05	1.68 ± 0.02	1.87 ± 0.07
7	1.99 ± 0.02	1.86 ± 0.02	2.01 ± 0.07
8	2.08	1.96	2.08

Figure 4.2 A plot of successive estimates (±S.E.) of the mean Brillouin and mean α diversity indices versus the number of ponds included in the pooled sample at Old Crow. S.E. not shown are enclosed by the dot.



Inuvik, with as few as 1 and as many as 5 being detected in the same pond (Table 4.3). I24 was the most common clone, representing 131 of 376 individuals surveyed and inhabiting 6 of 9 ponds. Eleven other clones (I18,I21,I23,I25,I26,I27, I28,I29,I30,I31,I33) were found in only a single habitat.

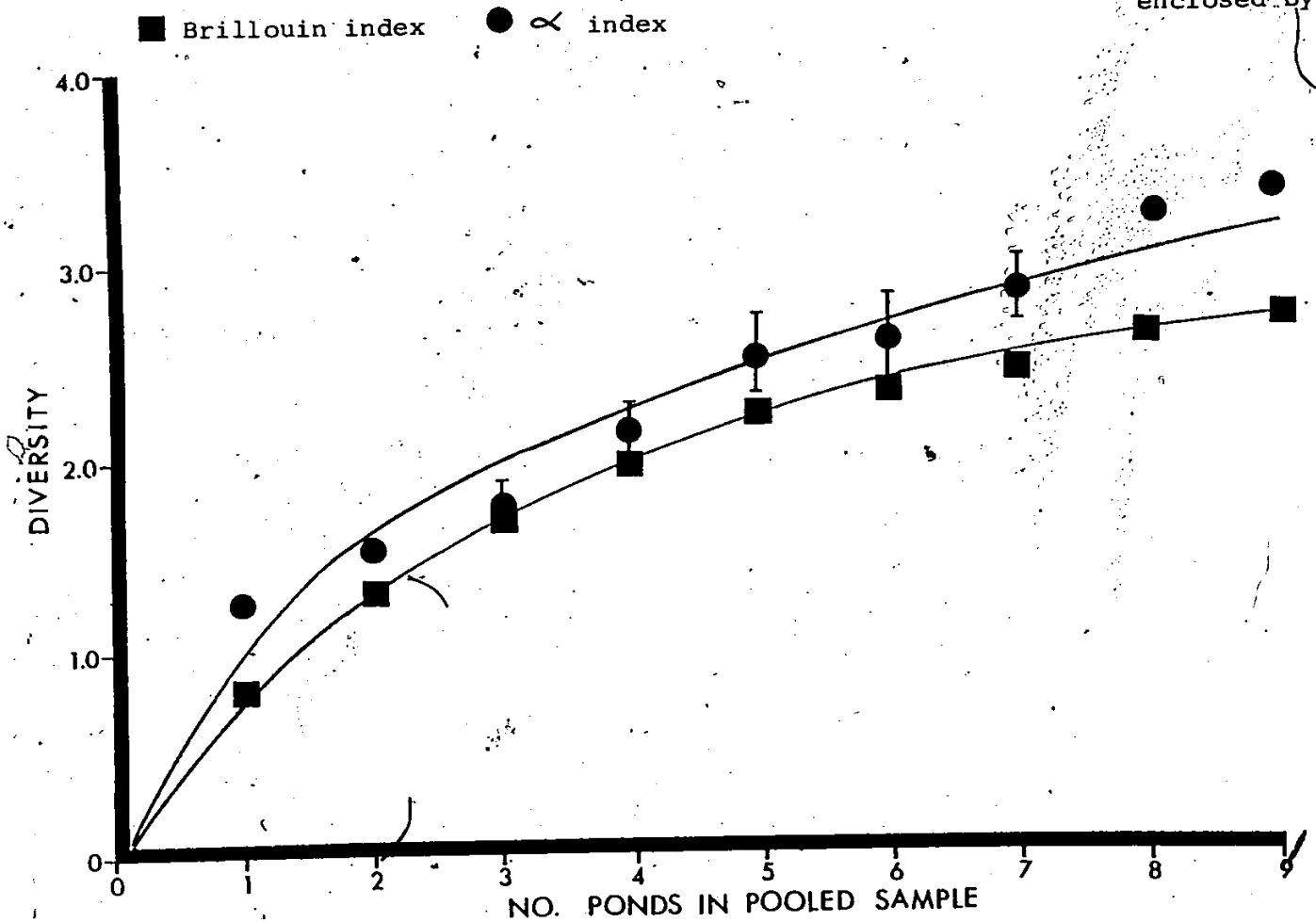
As noted at Old Crow, marked deviations from Hardy-Weinberg proportions were observed in each Inuvik population (Table 4.4). Of 31 X^2 values calculated, 6 represented significant (at the 5% level) heterozygote deficiencies and 14 represented significant heterozygote excesses. Significant heterozygote deficiencies and excesses commonly existed in the same population and linkage disequilibria were observed between certain loci. For example, with the exception of 2 clones (I28,I32), all LDH 13 heterozygotes were also GOT 23 heterozygotes and all LDH homozygotes (11 or 33) were GOT 22 homozygotes.

Values of the diversity indices calculated for the Inuvik metapopulation (α index = 3.39, Shannon Weaver index = 2.88 and Brillouin index = 2.76) were similar to those estimated for Old Crow (Table 4.5). The rate of increase of α and Brillouin indices slowed appreciably as the number of ponds in the pooled sample increased (Fig. 4.3).

Clonal Diversity at Tuktoyaktuk

Genotypic data from the 7 polymorphic loci at Tuk permitted the recognition of 9 clones. Clonal heterozygosities ranged from 0-30.0% with an average of 16.7% (Table 4.2).

Figure 4.3 A plot of successive estimates (±S.E.) of mean Brillouin and mean ∝ diversity indices versus the number of ponds included in the pooled sample at Inuvik. S.E. not shown are enclosed by the dot.



No more than 2 clones were present in a single habitat and 4 of the 8 ponds surveyed were uniclonal. Only 3 of the 9 clones (T36,T38,T41) were found in more than one pond and no clone was present in more than 2 habitats (Table 4.3).

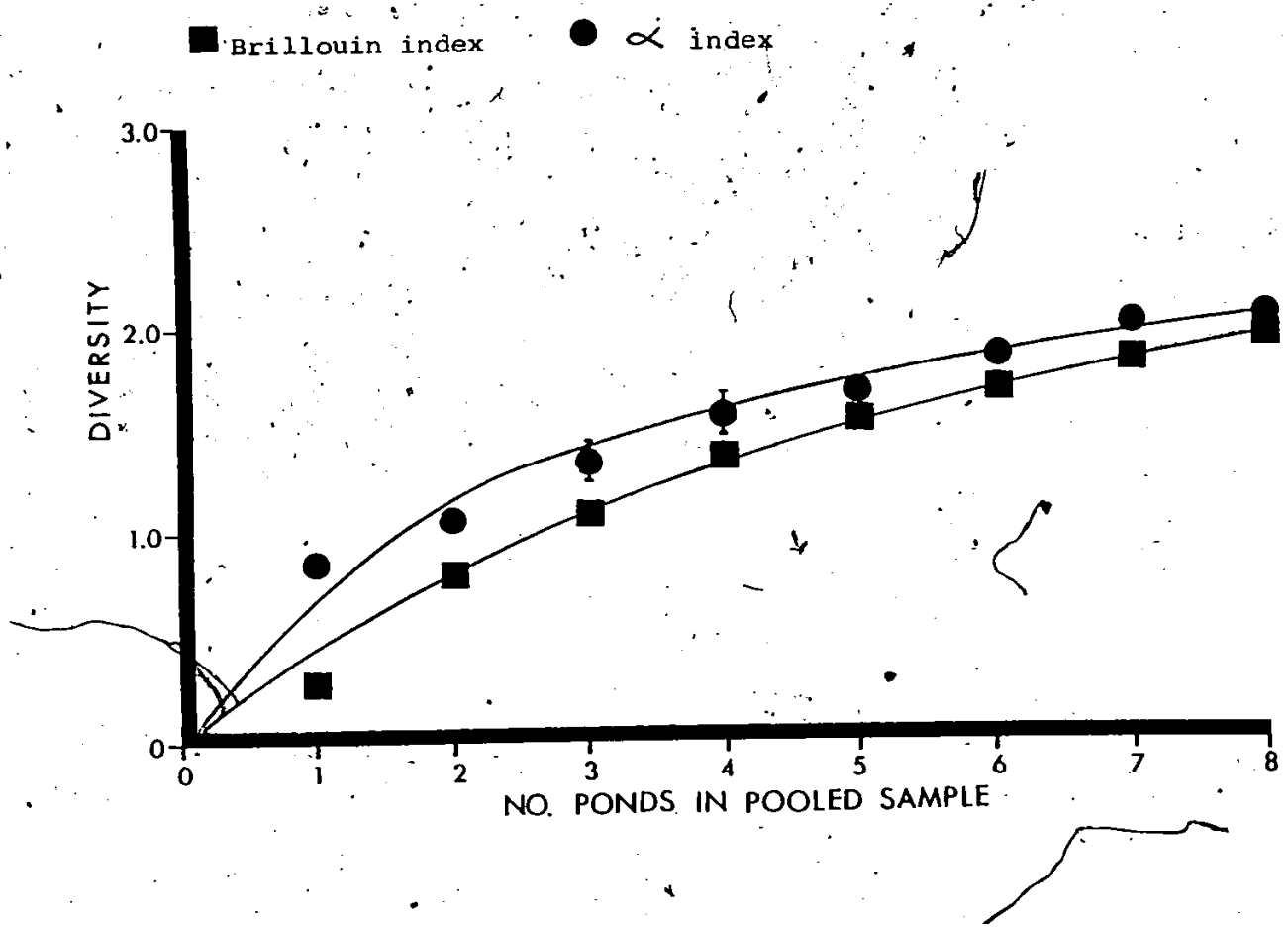
Significant (at the 5% level) deviations from Hardy-Weinberg equilibrium were observed at all polymorphic loci in the Tuk populations that were subjected to a chi-square goodness-of-fit test (Table 4.4). Chi-square values were not calculated for the AMY-1 locus at Tuk 2 or Tuk 6 due to the presence of a triple-banded phenotype. Heterozygote excesses and deficiencies were present in the same populations, although heterozygote excesses were more common than heterozygote deficiencies at the Tuk polymorphic loci.

Values of the diversity indices calculated for the Tuk metapopulation (α index = 2.08, Shannon Weaver index = 2.08 and Brillouin index = 1.96) were somewhat smaller than those of the Old Crow or Inuvik metapopulations. This is likely due at least in part to the small sample size at Tuk. For the same reason, the curves representing the trends of the α and Brillouin diversity indices with increasing sample size (Fig. 4.4) do not level off to the same extent as those for the Inuvik and Old Crow metapopulations

Evidence of Obligate Parthenogenesis

Marked Hardy-Weinberg deviations and the non-random associations of genotypes at different loci suggested that

Figure 4.4 A plot of successive estimates (±S.E.) of mean Brillouin and mean α diversity indices versus the number of ponds included in the pooled sample at Tuk. S.E. not shown are enclosed by the dot.



the arctic D. pulex clones reproduced by obligate parthenogenesis. No males were ever observed in the natural population samples or in the lab cultures of clones from the three sites. Each of 13 randomly selected clones released ephippial eggs into the brood pouch in the absence of males (Table 4.6). Perhaps as a result of inadequate food levels, individuals of all 13 clones produced some ephippia lacking eggs. In addition, no segregation was observed in 237 hatchlings of 10 different clones scored at a total of 693 potentially segregational situations $\left[\sum_{i=1}^{10}(\text{no. of ephippial hatchlings for clone } i)(\text{no. of heterozygous loci for clone } i)\right]$ (Table 4.7).

Genetic Relationships Among the Clones

The mean genetic distances ($\pm$ S.E.) among D. pulex clones from each of the three arctic sites and Ontario are listed in Table 4.8. The dendrograms in Fig. 4.5-4.6 were constructed on the basis of the average genetic distances among arctic D. pulex clones (Fig. 4.5) and among the arctic and Ontario D. pulex clones (Fig. 4.6). Five major clusters are recognizable in Fig. 4.5. The first cluster consists of 4 Old Crow clones; the second and fourth clusters are a mixture of Old Crow, Inuvik and Tuk clones; the third cluster contains 10 Inuvik clones and the fifth cluster consists of 3 Tuk clones. With the addition of Ontario clones in Fig. 4.6, it was apparent that many of the Ontario and arctic D. pulex clones were closely related.

Table 4.6 Number of ephippia containing eggs.

Clone	n	Proportion of total number of ephippia containing		
		0 eggs	1 egg	2 eggs
01	17	0.12	0.29	0.59
04	28	0.43		0.57
07	24	0.46	0.50	0.04
011	21	0.48		0.52
013	20	0.60	0.10	0.30
015	12	0.17	0.25	0.58
I24	31	0.52	0.35	0.13
I29	36	0.31	0.33	0.36
I32	30	0.20	0.20	0.60
T34	35	0.89	0.09	0.02
T37	3	0.67	0.33	
T39	12		1.00	
T41	18	0.22	0.28	0.50
T42 = 01	21	0.48		0.52

Table 4.7 Results of genotyping ephippial hatchlings of clones at heterozygous loci.

Clone	Number of ephippial hatchlings	Heterozygous loci
01	21	PGM, EST
04	1	PGM
010	16	PGM
014	5	EST, AMY-1
015	7	PGM, EST
017	1	PGM, EST
I31	7	PGM, EST, AMY-1
I32	6	LDH
T37	62	PGM, MDH, AMY-1, AMY-2
T41	111	PGM, MDH, AMY-2

Table 4.8 Mean genetic distances (±S.E.) among clones of D. pulex from Old Crow, Inuvik, Tuk and Ontario.

	Old Crow	Inuvik	Tuk	Ontario
Old Crow	0.23 ± 0.21	0.26 ± 0.26	0.28 ± 0.04	0.28 ± 0.37
Inuvik		0.18 ± 0.12	0.33 ± 0.18	0.33 ± 0.09
Tuk			0.35 ± 0.11	0.40 ± 0.05
Ontario				0.19 ± 0.40

Figure 4.5 Dendrogram showing the genetic relationship of D. pulex clones from Old Crow (O), Inuvik (I) and Tuk (T).

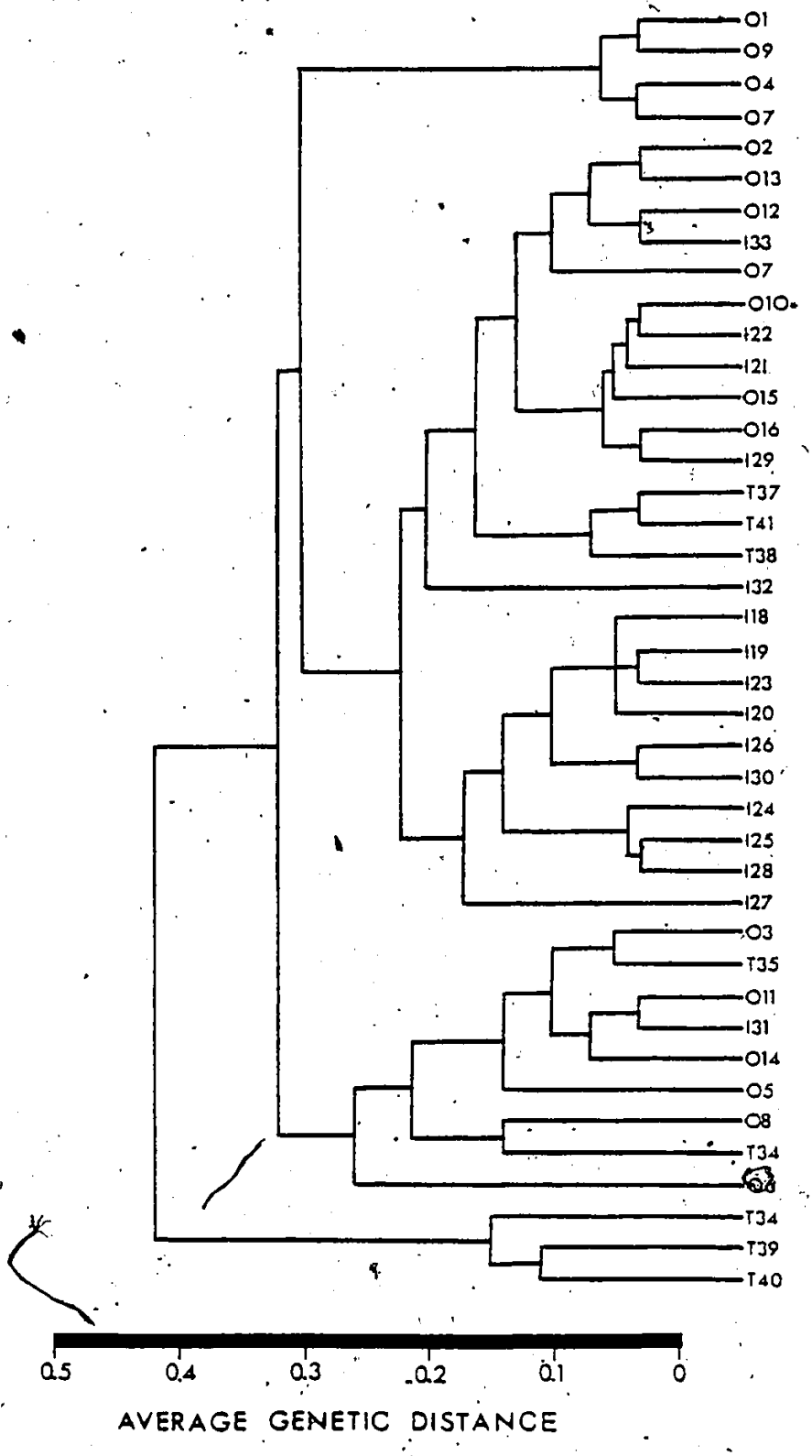
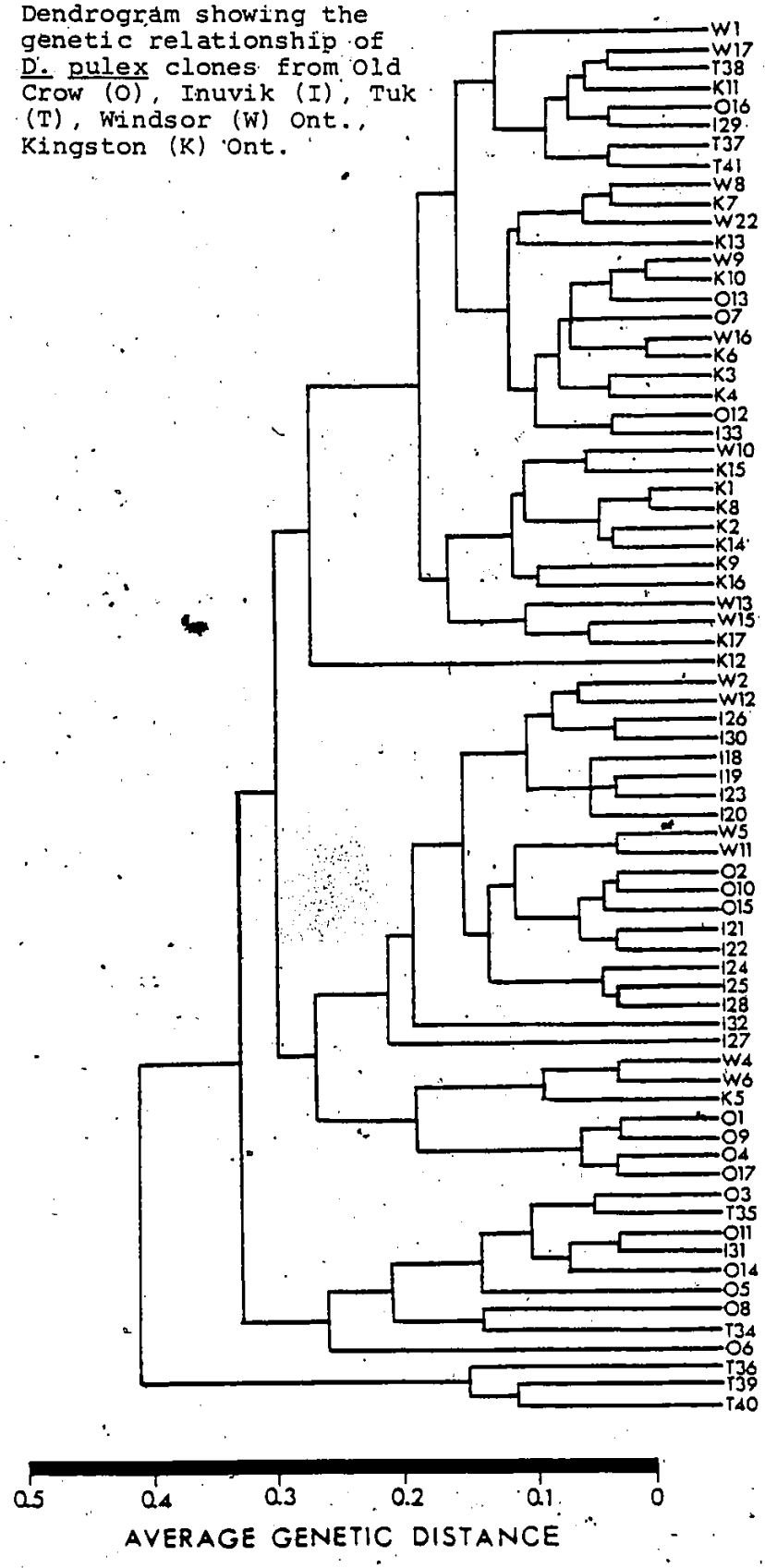


Figure 4.6 Dendrogram showing the genetic relationship of D. pulex clones from Old Crow (O), Inuvik (I), Tuk (T), Windsor (W) Ont., Kingston (K) Ont.



DISCUSSION

The results of the present study indicated that Daphnia pulex inhabiting ponds of the northwestern Canadian arctic are obligately parthenogenetic, like their Ontario conspecifics (Hebert and Crease, 1982). Marked deviations from Hardy-Weinberg expectations were observed at polymorphic loci in all populations from each of the 3 areas surveyed (Old Crow, Inuvik and Tuk) and linkage disequilibria between loci were evident. The high frequency of heterozygote deficiencies observed in these populations can be attributed to the loss of sexual reproduction rather than any short-term selection against heterozygotes (Hebert et al., 1982). Similar genotypic characteristics are common in other species reproducing by obligate parthenogenesis (Suomalainen and Saura, 1973; McWalter, 1981; Mitter et al., 1979) while genotypic frequencies in cyclic parthenogenetic Daphnia species inhabiting temporary ponds are generally in agreement with Hardy-Weinberg proportions and disequilibria between loci are absent (Loaring and Hebert, in prep., Hebert, 1974; Hebert and Moran, 1980; Lynch, 1982). Since the ponds sampled in the present study freeze solidly to the bottom each winter, all populations are regenerated each spring from ephippial eggs. Therefore, the deviations from Hardy-Weinberg equilibrium observed in these populations suggest the apomictic production of ephippial eggs.

Several additional lines of evidence supported the notion that these D. pulex populations do not reproduce

sexually. Males were never observed in either natural population samples or in laboratory cultures. Contrary to this observation, Meijering (1975) identified D. pulex males in three out of four ponds sampled from the Tuk area. An explanation for this discrepancy may lie in the sampling of different D. pulex populations between the two studies. Meijering identified individuals possessing several D. middendorffiana-like characteristics (including brown pigmentation on the dorsal part of the head) as D. pulex. However, for the present study, only unpigmented individuals were collected. These populations were generally found in the inland frost-polygon ponds and not in the ponds on the sea coast where Meijering sampled. Consequently, it is possible that the different morphs of D. pulex present in the Tuk area vary in their capacity to produce males. Moreover, Meijering collected his samples in early Sept. while the samples in this study were collected in early Aug. Accordingly, the populations sampled in the present study may have been capable of male production but not until later in the year; and laboratory conditions may not have induced male production. For example, black morphs of D. pulex inhabiting ponds in the Churchill area normally do not engage in male production before the month of Sept. (Hebert, pers. comm.). However, it is important to bear in mind that Daphnia clones reproducing by obligate parthenogenesis often retain male production (Hebert and Crease, 1982). In addition to the

apparent lack of males, females of a random sample of D. pulex clones released ephippia containing eggs while in the absence of males. Females of species reproducing by cyclical parthenogenesis normally resorb their eggs in the absence of males and shed empty ephippia (Agar, 1920; Ojima, 1958; Crease and Hebert, 1982). Finally, the ephippial offspring of 10 clones showed no segregation at variable loci. The existence of obligate asexuality in several Daphnia species, including D. pulex (Hebert and Crease, 1982), D. middendorffiana (McWalter and Hebert, 1982) and D. cephalata (Hebert, 1981) has been previously documented.

Previous studies have provided evidence that differences in the amount of intraspecific genetic variation exist between asexual taxa. Several workers have documented an absence of variation within parthenogenetic aphid species and have attributed this lack to the action of directional selection operating upon the aphid clones (Suomalainen et al., 1980; Tomiuk and Wohrmann, 1980; Wool et al., 1978). Similarly, parthenogenetic populations of Daphnia cephalata (Hebert, 1981) and Octolasion tyrtaeum (Jaenike et al., 1980) exhibited little clonal diversity. On the other hand, many studies have shown that parthenogenetic species are quite clonally diverse and are presumably capable of evolving (Hebert and Crease, 1982; McWalter and Hebert, 1982; Mitter et al., 1979; Suomalainen and Saura, 1973; Parker, 1979; Selander et al., 1978). Populations of D. pulex investigated in the present study were also found to be clonally diverse.

Most habitats contained more than one clone and up to five clones were detected in a single pond. As in the case of the Ontario D. pulex populations, (Hebert and Crease, 1982), most habitats are thought to have been colonized by several clones since the genetic distances between coexisting clones were often too large to have arisen through in situ mutation.

Although clonal diversity was high in the D. pulex populations under investigation, few clones were widespread and most inhabited only a single pond. Similar situations have been noted in parthenogenetic moth (Mitter et al., 1979) and earthworm populations (Jaenike et al., 1980) and have led to the suggestion that rare clones are specialists, while common clones represent 'general purpose genotypes'.

In their study of obligate parthenogenetic Daphnia species, McWalter and Hebert (1982) listed several factors which suggested that there had been substantial clonal diversification after the adoption of asexuality:

1. high heterozygosity levels
2. high incidence of null alleles at central metabolic loci
3. a possible increase in the frequency of gene duplications
4. the extent of genetic divergence at peripheral loci
5. existence of morphological and ecological differences between clones.

In the present study, the average clonal heterozygosities were 16.5%, 21.3% and 16.7% for the Old Crow, Inuvik and Tuk clones respectively. As such, the levels of heterozygosity

in these D. pulex clones are substantially higher than those seen in cyclic parthenogenetic species (Crease and Hebert, 1982; Hebert and Moran, 1980; Hebert, 1975; Hann and Hebert, in prep.) or in sexually reproducing invertebrates (Selander and Kaufman, 1973; Beck and Price, 1981; Hamrick, 1979). Obligate parthenogenetic clones of D. pulex from Churchill and Frobisher Bay were also found to have high heterozygosity levels (32.4%) (McWalter and Hebert, 1982). In the present survey, null alleles were detected at two loci (AMY-2 and MDH) and a possible gene duplication (as revealed by a 3-banded phenotype for the monomeric enzyme AMY-1) was observed in 2 Tuk clones. Neither of these phenomena were as prevalent as in the clones studied by McWalter and Hebert (1982), but their failure to be documented for other cladocerans reproducing by cyclic parthenogenesis suggests origins subsequent to the adoption of obligate parthenogenesis. Moreover, the EST locus proved to be highly variable among clones of D. pulex from the western arctic. Unfortunately, it is not known whether the clones differed ecologically, but differences in head shape and tail spine length were apparent between genetically distant clones (Hebert and Loaring, in prep.). Therefore, according to the criteria given by McWalter and Hebert (1982), the present study has provided additional evidence that genetic diversification is possible after the adoption of obligate parthenogenesis.

As a result of multi-locus electrophoretic studies, Avise (1974) noted that levels of genic similarity between

conspecific populations appear very high. Other workers have found that local populations of sexual species generally differ at less than 5% of their loci (Beck and Price, 1981; Zimmerman et al., 1978). For example, Ward (1980) found that the mean genetic distance between populations of several ponerine ant species was only 0.015. However, the mean genetic distances among the D. pulex clones within a specific area ranged from 0.18 ± 0.12 at Inuvik to 0.35 ± 0.11 at Tuk. Earlier studies have also documented the occurrence of large genetic distances between clones. McWalter (1981) and Hebert and Crease (1982) found substantial genetic distances between clones of D. middendorffiana and D. pulex and the 8 clones of Octolasion tyrtaeum detected by Jaenike et al. (1980) were found to differ at 30% of their loci, on average.

Only a single clone (clone 01 = T42), isolated from the Tuk and Old Crow populations, was common to two areas. The mean genetic distances among clones within an area (range: 0.18 ± 0.42 to 0.35 ± 0.11) were slightly smaller than the mean genetic distances among clones from different areas (range: 0.26 ± 0.26 to 0.40 ± 0.05). Similarly, Tilley et al. (1978) found that levels of genetic divergence were usually highest among populations of salamanders from different mountain ranges. In fact, it has been suggested that intraspecific genetic distance increases with geographical distance (Nicklas and Hoffman, 1981). The results of the present study did not support this conclusion. The

mean genetic distances between clones from any two of the western arctic areas (range: 0.26 ± 0.26 to 0.33 ± 0.18) were roughly the same size as the mean genetic distances between clones from Ontario and those from the Northwest (range: 0.28 ± 0.37 to 0.40 ± 0.55). In a similar study, Richardson et al. (1980) found no relationship between genetic distances and geographical distances separating rabbit populations in Australia.

A single index which characterizes the pattern of the abundances of different species or clones is of great practical use and several such measures have been formulated. Two of the most commonly used indices are the Shannon-Weaver diversity index and α, which is a parameter of the log series distribution. Taylor et al. (1976) have shown that although the log series model is by no means always an ideal description of population structure, diversity as measured by α generally behaves more predictably and consistently than diversity predicted by the Shannon-Weaver index. In particular, α is unaffected by sample size once n exceeds 1000, it is much less sensitive than the Shannon-Weaver index to the abundance of the commonest species and replicate collections invariably have similar α values. Despite these persuasive arguments, many ecologists have employed the Shannon-Weaver index or the closely related Brillouin index as convenient measures of diversity. In fact, Pielou (1966) suggested the use of the Brillouin index to describe the diversity of a collection

that is small enough for all its members to be identified and counted. In the present study, the clonal diversities of D. pulex collections made from each of the three western arctic areas (Old Crow, Inuvik and Tuk) were estimated and compared using the Brillouin, Shannon-Weaver and α diversity indices. Although the estimates for α were usually larger than those of the other 2 indices, all three exhibited similar trends within a locality as populations were added to the pooled sample.

Since glacial refuges have been available for colonization and habitation longer than areas that were ice-covered during the last glacial advance, it was thought that the clonal diversity of D. pulex populations collected from a refuge (Old Crow) would differ from the diverstiy present in glaciated areas. This prediction was not supported by the data of the present study. With a similar sampling effort, the number of clones (17) detected at Old Crow did not differ greatly from the number of clones at Inuvik (16), Windsor (22) or Kingston (17), all of which were covered by glacial ice. The Tuk area, which was also glaciated, had fewer clones (9) than any of the other areas surveyed, but this undoubtedly reflected smaller sample size and the failure to collect pigmented D. pulex morphs observed in this locality. In addition, clonal diversity as measured by the Brillouin, Shannon-Weaver and α diversity indices were no higher at Old Crow than at Inuvik. Pielou's (1966) method of plotting successive estimates of the Brillouin index against the number of ponds (quadrats) included in the estimate resulted in

curves which increased rapidly at first and then levelled off for both the Old Crow and Inuvik areas. Once diversity reached a certain level, the addition of new ponds to the sample had two opposing effects: common clones were added more rapidly than rare clones previously encountered, thereby reducing diversity; and at the same time, previously unrecorded clones were brought into the sample, thereby increasing diversity. When the two effects balanced, the curve levelled off (Pielou, 1966). The tendency of the curves to level off would undoubtedly be expressed to a lesser or greater extent with different random orderings of pond accumulation. The near-absence of the levelling trend in clonal diversity for the Tuk area indicated that many Tuk clones went undetected in the analysis due to small sample size. Workers using the method of Pielou are able to estimate the Shannon-Weaver diversity index and its standard error from successive increments in the Brillouin index taken after the curve has levelled. Such estimates have generally entailed the analysis of greater than 100 different quadrats (Pielou, 1966,1974; Lloyd et al., 1968). As such, the failure to sample adequate pond numbers and collect larger samples from each pond prevented the calculation of a Shannon-Weaver index for the areas under investigation. Instead, the final Brillouin index calculated when all ponds were included in the sample was taken to represent the diversity of the pooled collections of an area with unknown sampling variance. These values are not

representative of, but hopefully are correlated with, the true clonal diversities of each area. The observation of a levelling trend in diversity for both Old Crow and Inuvik suggests that with larger sample sizes, an estimate of the clonal diversity of an area could feasibly be made.

The absence of a clear difference in clonal diversity levels between glaciated areas and glacial refuges can be explained if one assumes that the rate of clonal acquisition in an area is described by a rapid asymptotic approach to a fixed value. This line of reasoning is quite similar to Strong's (1974) asymptotic species accumulation model. He argued that variation in diversity between habitats is accounted for by a species-area phenomenon with a richness asymptote reached within a short period of time. The asymptote is set by the structural properties of the environment, independently of age for all but the youngest habitats. Thus, by the time of the glacial retreat, the Old Crow area may have attained the maximum number of clones it was capable of supporting. Diversity levels at Old Crow undoubtedly exceeded those at Inuvik or Tuk at this time, but if the latter two areas were rapidly colonized by several clones that subsequently underwent further genetic diversification in situ, then clonal diversity levels would soon approach those of glacial refuge areas. If such was the case, then clonal diversity levels would not reflect the length of time that an area has been suitable for habitation.

In a number of cases, it has been shown that bisexual relatives of asexual taxa survive in glacial refuges (Lokki et al., 1975; Suomalainen and Saura, 1973). The present work revealed no evidence of the presence of sexually reproducing D. pulex in the Old Crow area. Therefore, it is possible that this species may have adopted an asexual mode of reproduction prior to the Wisconsin glaciation or that the sexual forms may have been excluded from the area by competitively superior asexual descendents. Present data, while admittedly scanty, suggest that sexually reproducing populations of D. pulex occur in the midwestern U.S.A. and perhaps extend northward to Alberta.

SUMMARY

Populations of Daphnia pulex in both glacial refuges and glaciated areas of the western Canadian arctic reproduce by obligate parthenogenesis. The great extent of genetic diversification observed in these populations weakens the argument that asexual taxa are evolutionary dead ends. On average, clones from the same locality were more closely related than clones from different localities, but genetic distances between arctic clones and Ontario clones were no higher than the distances between clones from two different arctic localities. The results of the present study do not support the hypothesis that clonal diversity is related to habitat age, for diversity values were similar in refuge and glaciated areas. The lack of variation in clonal diversity levels between glaciated areas and glacial refuges can be explained by assuming a rapid, asymptotic approach to an equilibrium diversity.

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C

CHAPTER V

THE *DAPHNIA PULEX* GROUP: AGAMIC COMPLEXES AND SIBLING SEXUALS

INTRODUCTION

The systematics of the genus Daphnia have been and remain in a state of disorder. Although forms collected within a small geographic area may fall into distinct morphological groups, populations from outside the study area or even new collections from within the study area often contain intermediate forms (Dodson 1981). Daphnia pulex, probably the commonest species in pond habitats throughout the Holarctic region, is a case in point. Until the late 1940's, all European forms with a prominent medial pecten were identified as D. pulex. Based on a study of English specimans, Scourfield (1942) and Johnson (1952) pointed out that at least two additional taxa, D. curvirostris and D. obtusa, merited recognition. Another species of the "pulex group", D. pulicaria has been recognized in central Europe (Hrbacek 1959), but the validity of this species remains unclear. Only D. pulicaria and D. pulex have been reported from North America; old records for D. curvirostris and D. obtusa have been discounted (Brooks 1957). In his monograph of North American Daphnia, Brooks (1957) recognized that individuals with a morphology intermediate between two described species were frequent. As an explanation, he suggested that introgressive hybridization between species was common. He felt that North American populations of

Daphnia pulex hybridized with D. middendorffiana, D. schodleri and D. rosea. Hebert and McWalter (1982) have proposed an alternate explanation for such intergradation between 'species'. They argued that these intergrades reflect not hybridization, but the absence of sexual reproduction. Taxonomic difficulties have arisen from a misguided attempt to impose species boundaries on an agamic complex. There is no doubt that many, if not all, populations of D. pulex and D. middendorffiana reproduce by obligate parthenogenesis. Study of populations in the eastern and central arctic indicates that these two forms are closely related genetically and may comprise a single agamic complex. The present study aimed to extend this survey of genetic diversity to D. pulex in the western arctic.

In the course of this work, three clones were encountered with genetic characteristics markedly different from those of D. pulex clones studied in the present and previous analyses. Morphological examination indicated that these clones were, in fact, Daphnia curvirostris. In addition to the collections of D. pulex and D. curvirostris, two clones of D. schodleri were also examined electrophoretically. The results indicated that D. schodleri reproduced by obligate parthenogenesis.

The present study has pointed out the value of combining morphological observations with allozyme investigations in taxonomic work. The suspicion that D. schodleri,

as well as D. pulex and D. middendorffiana, may be part of an agamic complex has been confirmed, but the likely occurrence of unrecognized species in the D. pulex group has also been indicated. Moreover, this work has revealed the presence of D. curvirostris in North America, while other workers have suggested that North American populations of D. obtusa may also exist (Hebert and Schwartz pers. comm.).

MATERIALS AND METHODS

Live samples were collected from 28 Daphnia populations (composed of several species, but predominantly D. pulex) located near Old Crow, Y.T. (67.35N 139.50W), Inuvik, N.W.T. (68.25N 133.30W) and Tuktoyaktuk (Tuk), N.W.T. (69.27N 133.02W) (Appendix III). The populations were sampled in Aug. 1980 and air freighted to Windsor where 48 individuals were isolated from each population in an attempt to establish clones. Some of these individuals failed to establish clones; this loss varied among populations. The clonal genotypes were electrophoretically determined at 10 enzyme loci: glucose-6-phosphate dehydrogenase (G6PDH), xanthine dehydrogenase (XDH), lactate dehydrogenase (LDH), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), glutamate oxaloacetate transaminase (GOT), esterase-1 (EST), amylase-1 (AMY-1), amylase-2 (AMY-2) and malate dehydrogenase (MDH). Alleles were numbered in order of increasing migration from the anode and the allelic designations are those of McWalter and Hebert (1982). For details of the electrophoretic procedures see Crease (1980). Gene frequencies calculated for each clone were used to determine Nei's (1975) measures of genetic divergence, I and D. A dendrogram was constructed using single link cluster analysis performed on the matrices of genetic distances among clones.

The clustering procedure, performed with the assistance of an IBM 3031 computer was based on average genetic distance and used the BMD-P1M program. The genetic distance between two clones is defined as $D = -\ln I$ where I is the mean genetic identity between clones. Since two clones (I28 and C3) shared no alleles at any of the 10 loci examined, I equalled 0 and D was an undefined quantity. As a result, clone I28 was omitted in the construction of a dendrogram.

Based on the results of the cluster analysis, 8 D. pulex clones (03,I18,I22,I24,I28,T34,T37,T39), 1 D. schodleri clone (S1) and 1 arctic D. curvirostris clone (C1) selected to represent most of the major clusters, were subjected to morphological examination using Leitz and Nikon photomicroscopes. In addition, scanning electron micrographs of 2 D. pulex clones (I28,T39), 1 D. schodleri clone (S1), 1 arctic D. curvirostris clone (C1) and 1 English D. curvirostris clone were taken using a Semco Nanolab 7 scanning electron microscope with 15 KV accelerating voltage. Specmans for use in SEM were air dried on double sided cellulose tape and coated by the evaporation of a gold palladium alloy for 2-3 sec. to obtain a coat thickness of approximately 150-200 angstroms.

Ephippia shed by females of two D. schodleri clones (S1 and S2) and two D. curvirostris clones (C1 and C3) maintained in the absence of males were examined in order to determine if these clones released ephippial eggs. The results were compared with those of the D. pulex clones

studied by Loaring and Hebert (in prep.). Four ephippial hatchlings of D. schodleri clones S1 and S2 were each scored at several heterozygous loci. The conditions under which ephippial hatching took place are described in Loaring and Hebert (in prep.).

RESULTS

Morphology

Morphological examination of the clones under study revealed that 3 of the 47 clones were Daphnia curvirostris, 2 were D. schodleri and the remaining 42 were D. pulex.

The morphological characteristics of clones C1, C2, C3 and the English clone were consistent with those listed by Johnson (1952) as being diagnostic of D. curvirostris. The head shape was very similar to that of D. pulex, having a rounded anterior margin and a concave ventral margin (Fig. 5.1.1). However, the antennular mound (Fig. 5.1.11-5.1.12) was low and not nearly as well developed as that observed in D. pulex (Fig. 5.1.13-5.1.14). The tail spine (Fig. 5.1.36-5.1.37) was quite short and the dorsal ridge of the ephippium (Fig. 5.1.26-5.1.27) was smooth and devoid of spinules (Fig. 5.1.31-5.1.32). In contrast, the ephippia shed by D. pulex (Fig. 5.1.28-5.1.29) possessed numerous distinct spinules on its dorsal ridge (Fig. 5.1.33-5.1.34). The pectens (Fig. 5.1.21-5.1.22) on the postabdominal claw (Fig. 5.1.16-5.1.17) of D. curvirostris had more numerous, finer teeth (medial 8 - 11; proximal 10-11) than the pectens of D. pulex (Fig. 5.1.23-5.1.24) which had comparatively few coarse teeth (medial 5-7; proximal 6-8). Of the three D. curvirostris clones identified, one (C1) was found in a single Old Crow habitat (Old Crow 4), one (C2) was found

in a single Tuk pond (Tuk 7) and C3 inhabited 2 Tuk ponds (Tuk 7 and Tuk 10).

The taxonomic features described in Brooks (1957) were used to identify clones S1 and S2 as D. schodleri. The morphological distinction between clones of this species and D. pulex clones was based primarily upon the relative size and shape of the head, and the length of the tail spine. The ventral margin of the head of D. schodleri was nearly straight (Fig. 5.1.10) whereas that of D. pulex was concave (Fig.5.1.2,.4,.6,.8). The antennular mounds were well developed (Fig. 5.1.15) as in the D. pulex clones. The tail spine of S1 and S2 was stout and long (Fig. 5.1.40), whereas the tail spine of D. pulex was shorter and thinner (Fig. 5.1.38). The postabdominal claw (Fig. 5.1.20) was similar to that of D. pulex in that it possessed 5 - 6 teeth in the medial pecten (Fig.5.1.25) and 6 - 7 teeth in the proximal pecten. Clone S1 was found in 2 Tuk ponds (Tuk 4 and Tuk 9) while clone S2 was isolated only from Tuk 9. Of the 28 habitats investigated in this study, Tuk 9 and Tuk 10 were the only ones in which D. pulex was not identified.

The remaining 42 clones under study were identified as D. pulex but substantial variation was noted in their morphologies. Many clones (03, I22, T34,T39) possessed the concave head shape (Fig. 5.1.2,.4,.6,.8) and short tail spine (Fig. 5.1.38) characteristic of D. pulex while others (I28) had heads with straighter ventral

Figure 5.1.1 D. curvirostris clone C1 female head (100x)

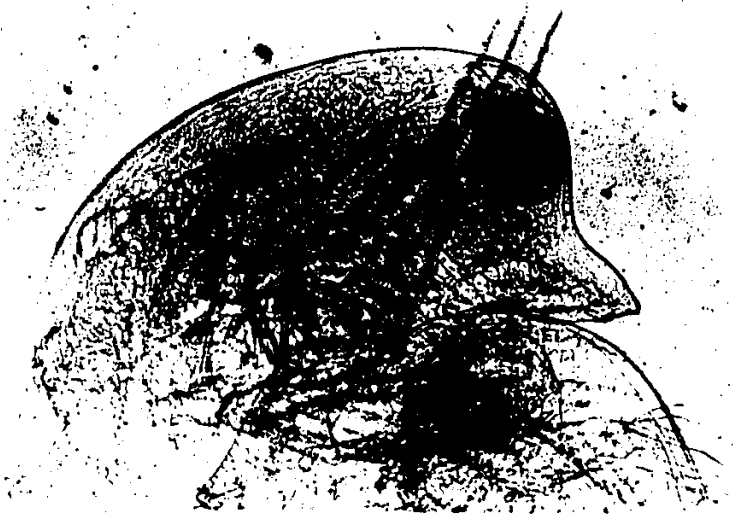

Figure 5.1.2 D. pulex clone 03 female head (100x)

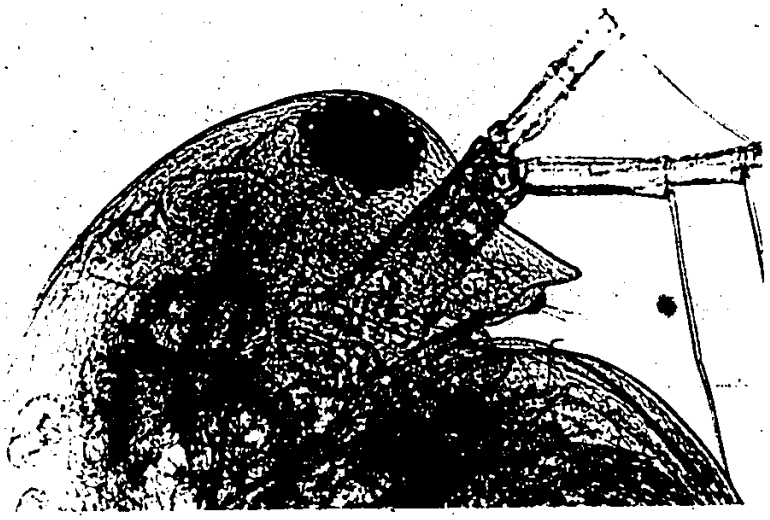

Figure 5.1.3 D. pulex clone I18 female head (100x)

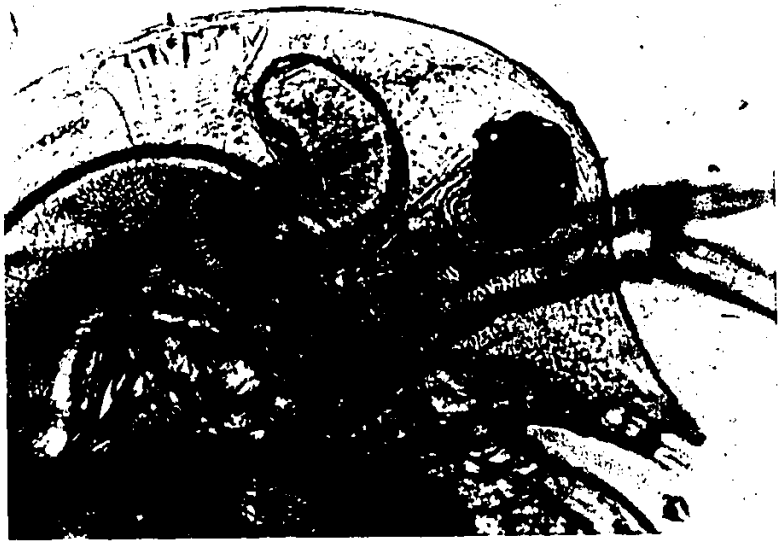

Figure 5.1.4 D.pulex clone I22 female head (100x)

Figure 5.1.5 D. pulex clone I24 female head (100X)

Figure 5.1.6 D. pulex clone T34 female head (100X)

Figure 5.1.7 D. pulex clone T37 female head (100X)

Figure 5.1.8 D. pulex clone T39 female head (100X).

Figure 5.1.9 D. pulex clone I28 female head (100X)

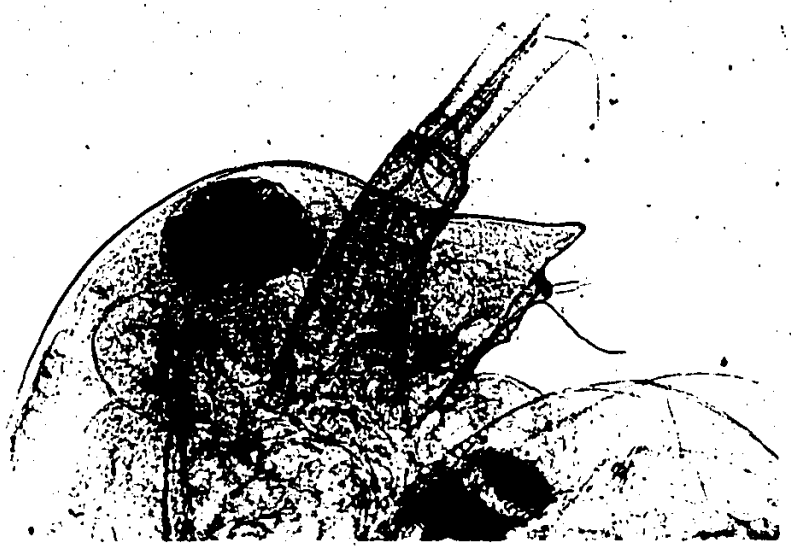

Figure 5.1.10 D. schodleri clone S1 female head (100X)

Figure 5.1.11 SEM of D. curvirostris clone C1 antennular mounds (500X)

Figure 5.1.12 SEM of English D. curvirostris clone antennular mounds (500X)

Figure 5.1.13 SEM of D. pulex clone T39 antennular mounds (500X)

Figure 5.1.14 SEM of D. pulex clone I28 antennular mounds (500X)

Figure 5.1.15 SEM of D. schodleri clone S1 antennular mounds (500X)

Figure 5.1.16 SEM of D. curvirostris clone C1 post-abdominal claw (400X)

Figure 5.1.17 SEM of English D. curvirostris clone postabdominal claw (400X)

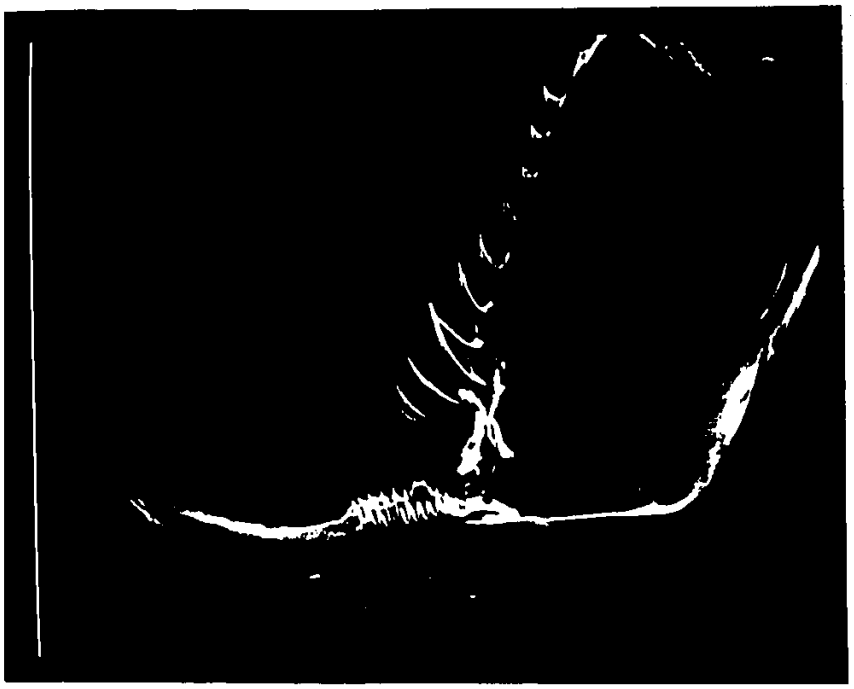

Figure 5.1.18 SEM of D. pulex clone T39 postabdominal claw (260X)

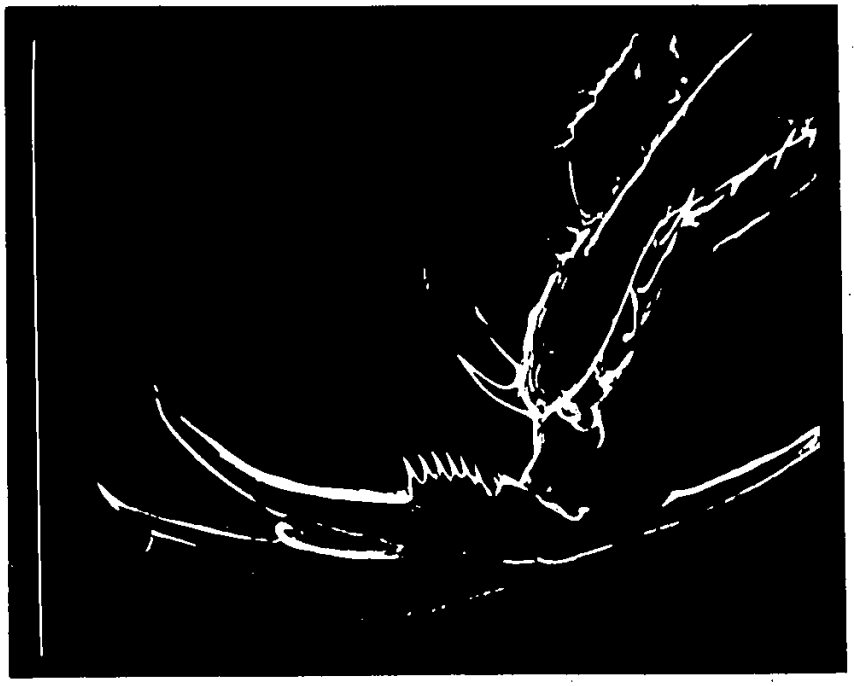

Figure 5.1.19 SEM of D. pulex clone I28 postabdominal claw (200X)

Figure 5.1.20 SEM of D. schodleri S1 postabdominal claw (250X)

Figure 5.1.21 SEM of D. curvirostris clone C1 medial pecten (1500X)

Figure 5.1.22 SEM of English D. curvirostris clone medial pecten (1100X)

Figure 5.1.23 SEM of D. pulex clone T39 medial pecten (1300X)

Figure 5.1.24 SEM of D. pulex clone I28 medial pecten (1200X)

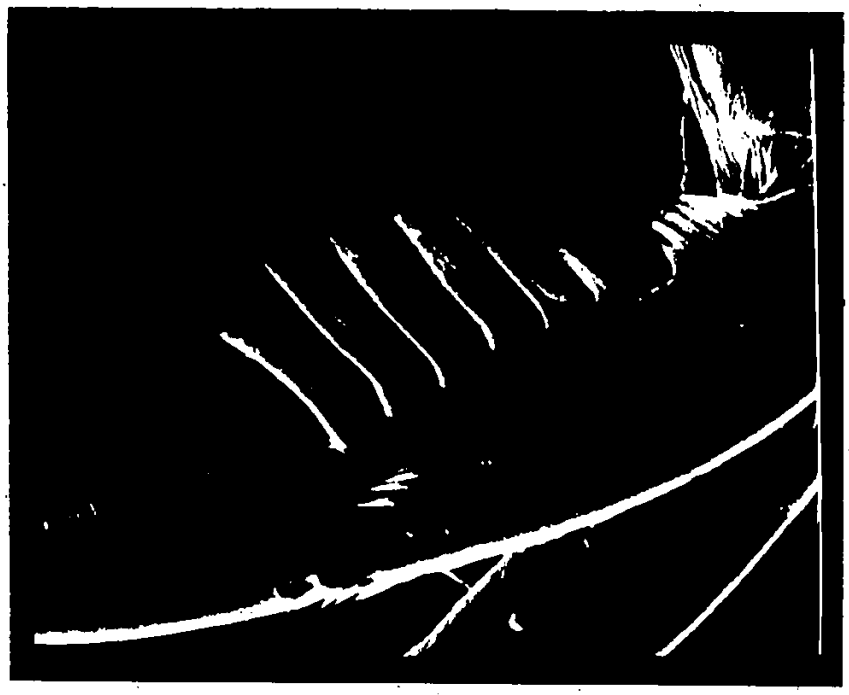

Figure 5.1.25 SEM of D. schodleri clone S1 medial pecten (1300X)

Figure 5.1.26 SEM of D. curvirostris clone C1 ephippium (110X)

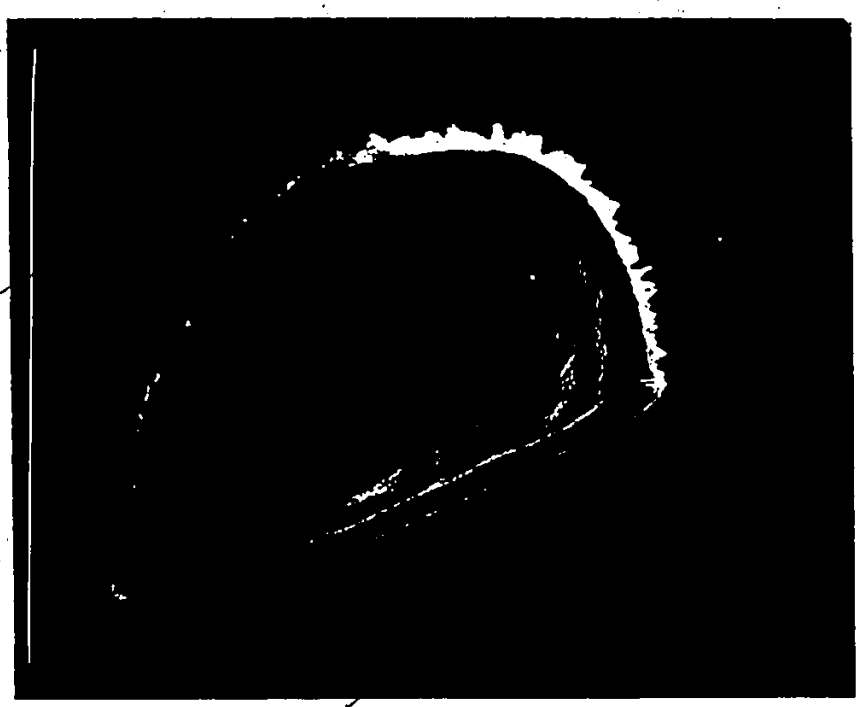

Figure 5.1.27 SEM of English D. curvirostris clone ephippium (110X)

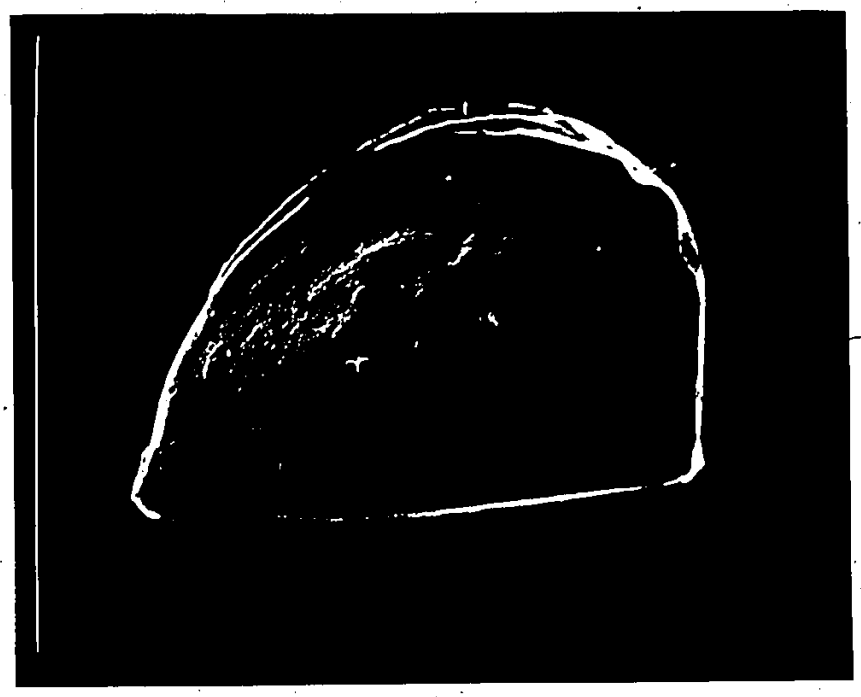

Figure 5.1.28 SEM of D. pulex clone T39 ephippium (85X)

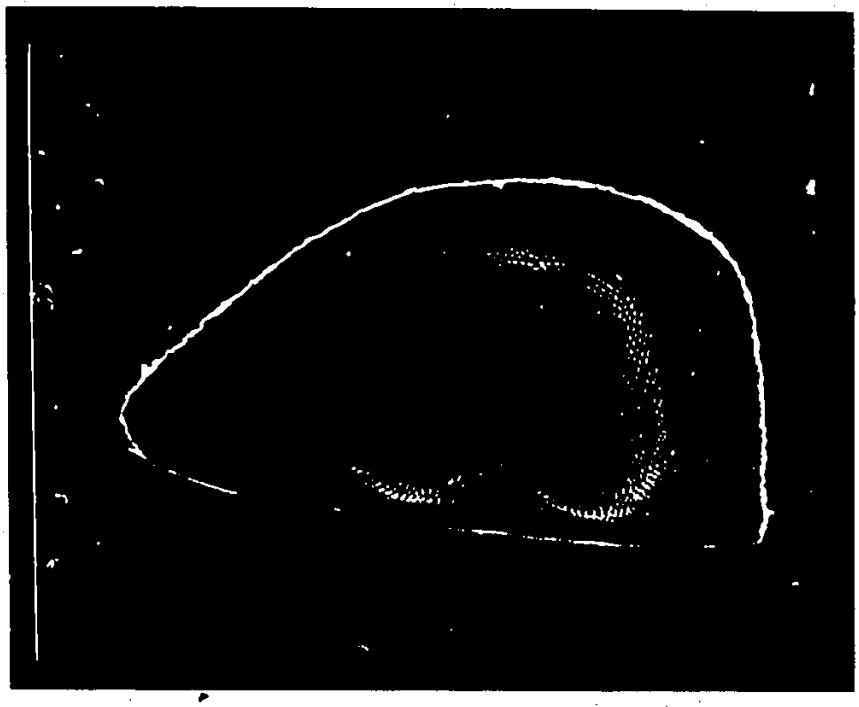

Figure 5.1.29 SEM of D. pulex clone I28 ephippium (85X)

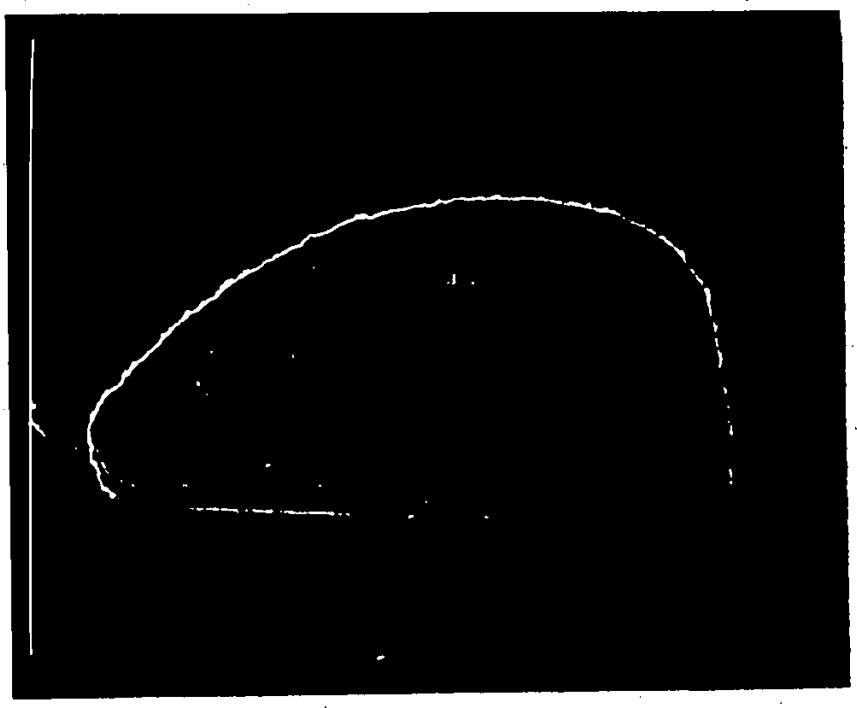

Figure 5.1.30 SEM of D. schodleri clone S1 ephippium (85X)

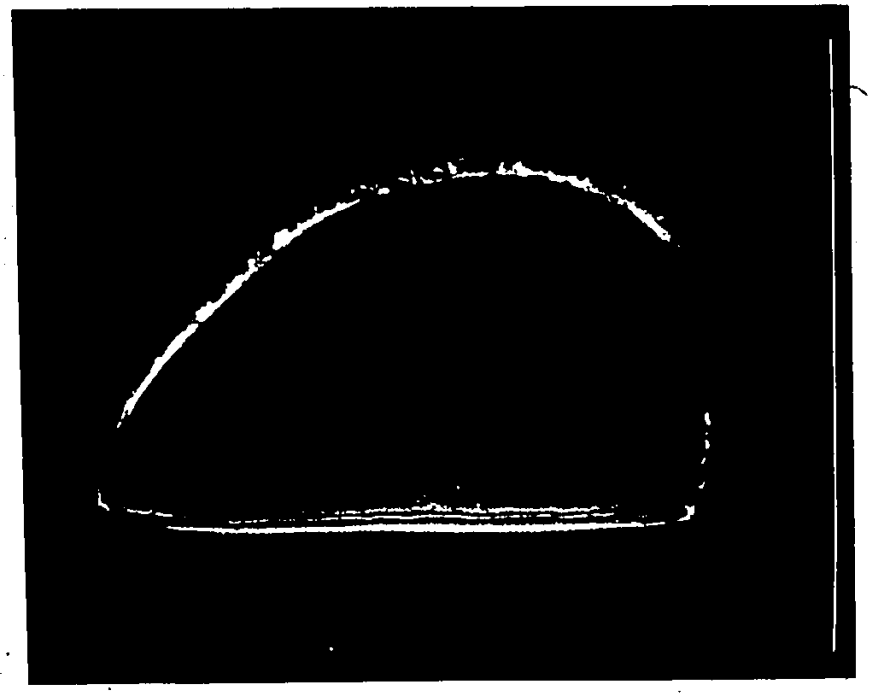

Figure 5.1.31 SEM of D. curvirostris clone C1 ephippial spines (1000X)

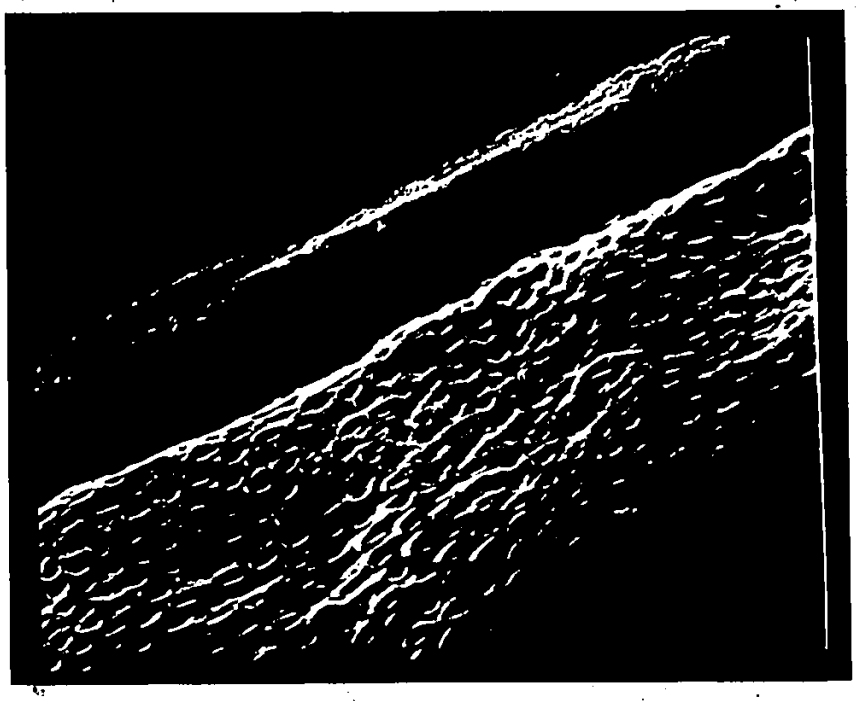

Figure 5.1.32 SEM of English D. curvirostris clone ephippial spines (1000X)

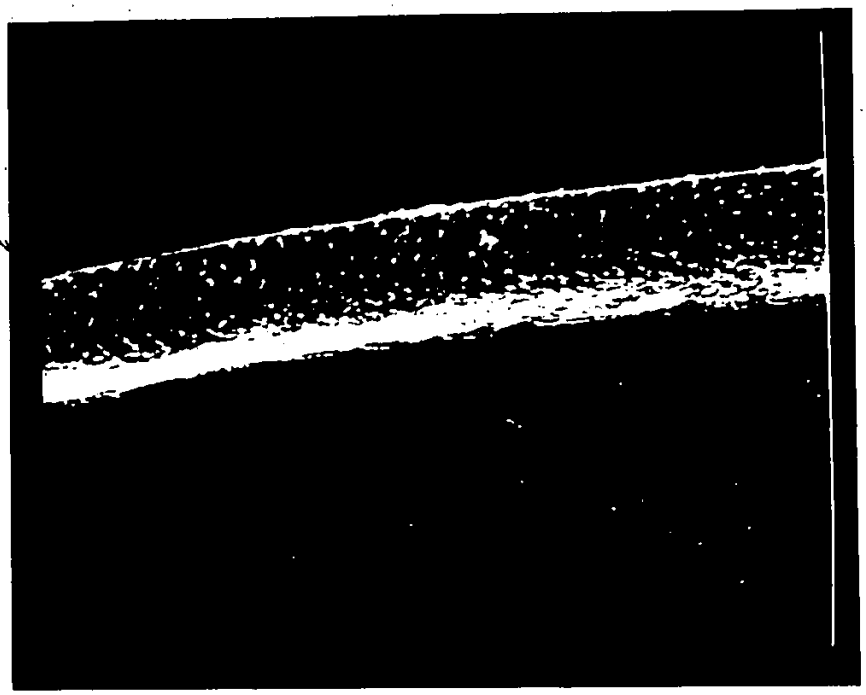

Figure 5.1.33 SEM of D. pulex clone T39 ephippial spines (1000X)

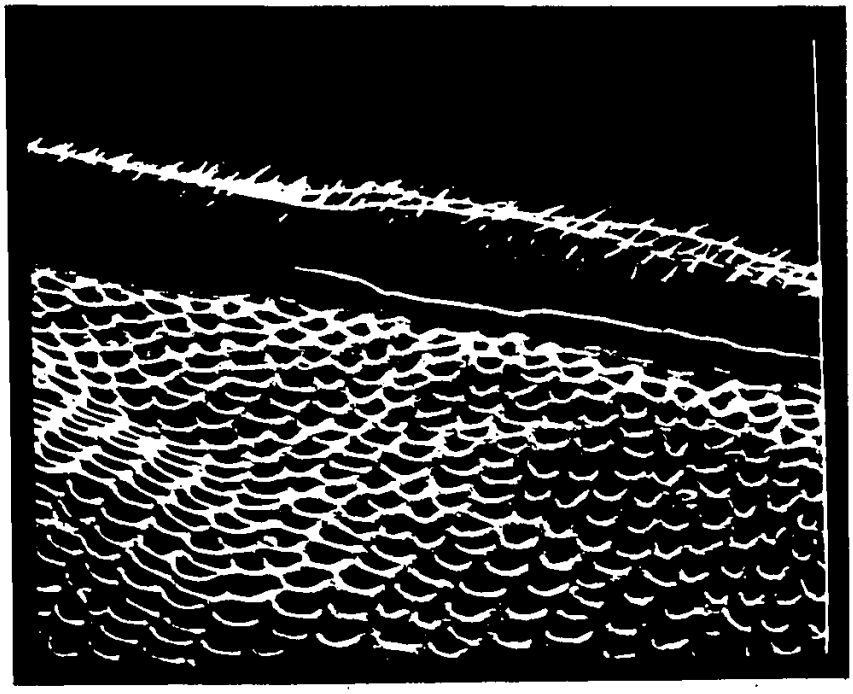

Figure 5.1.34 SEM of D. pulex clone I28 ephippial spines (1000X)

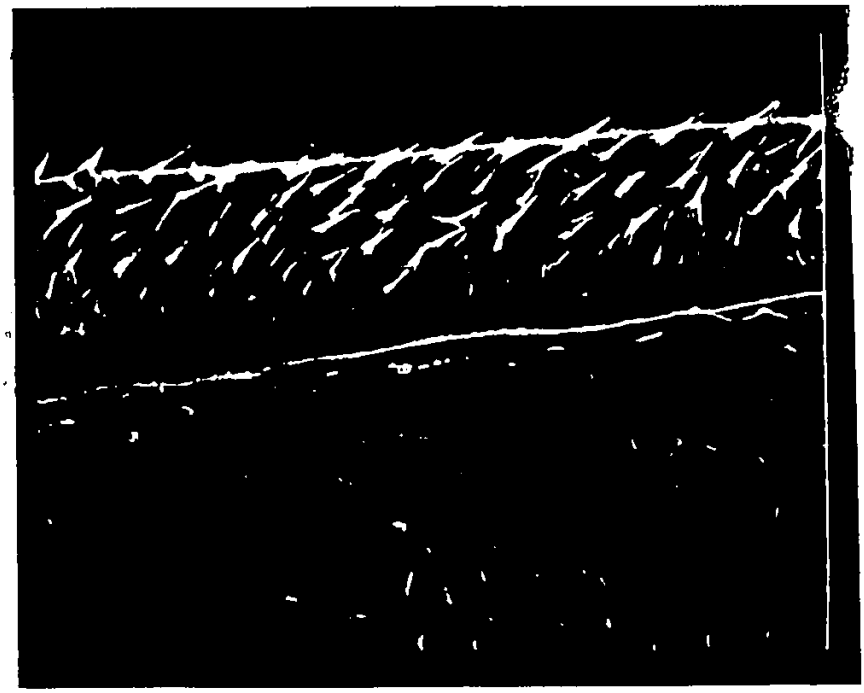

Figure 5.1.35 SEM of D. schodleri clone S1 ephippial spines (1000X)

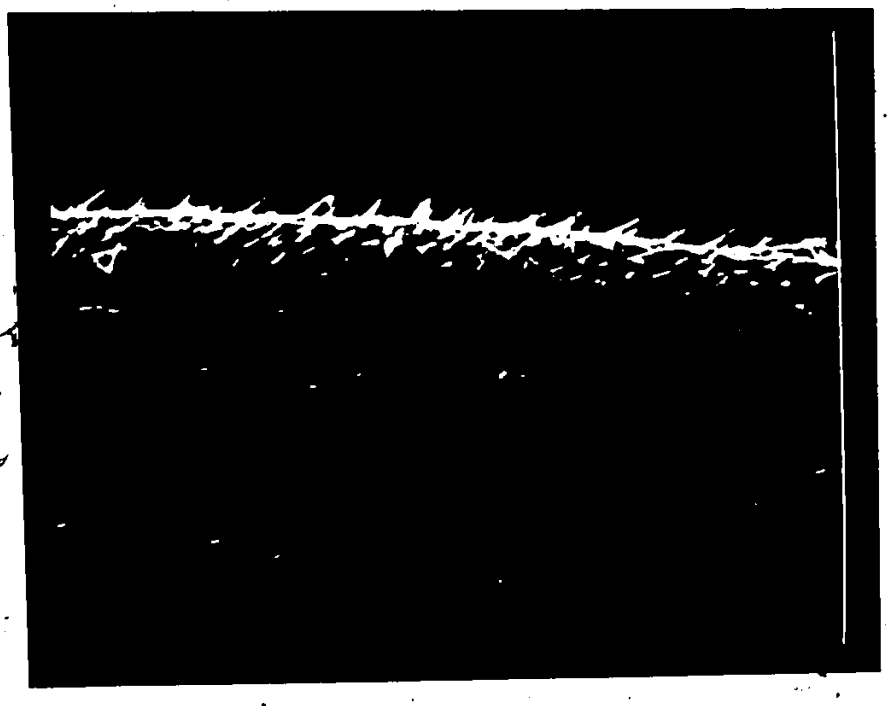

Figure 5.1.36 D. curvirostris clone C1 female

Figure 5.1.37 English D. curvirostris clone female

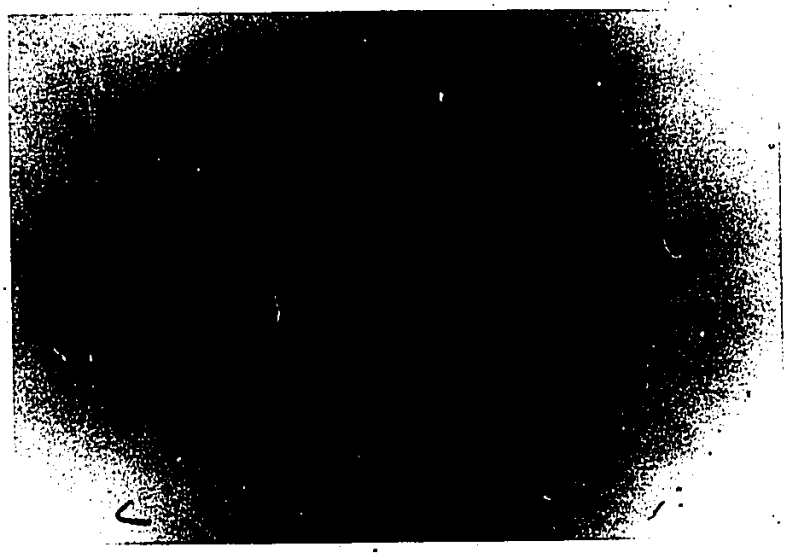

Figure 5.1.38 D. pulex clone T39 female

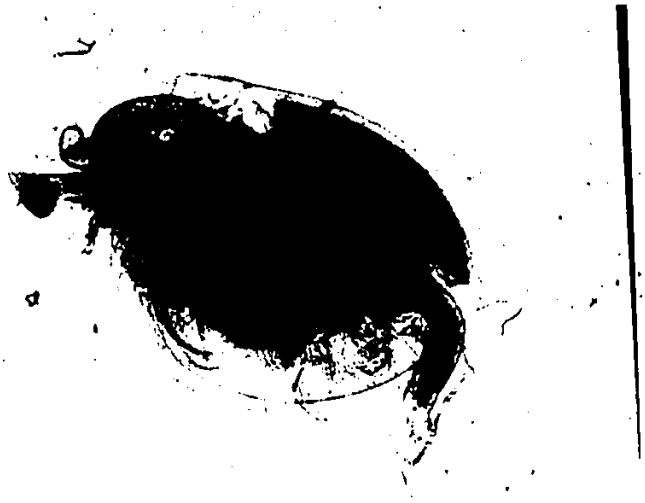

Figure 5.1.39 D. pulex clone I28 female

Figure 5.1.40 D. schodleri clone S1 female

margins (Fig. 5.1.9) and longer tail spines (Fig. 5.1.39) like D. schodleri. It was interesting to note that these clones were quite genetically similar to those clones identified as D. schodleri (Fig. 5.2).

Enzyme Phenotypes

The three clones of D. curvirostris showed marked genetic differentiation from the 42 clones of D. pulex. They were substituted at 6 of the 10 loci examined (Table 5.1). The genotypic data for the D. pulex clones and photographs of the allozyme phenotypes found in D. curvirostris, D. pulex and D. schodleri are in Loaring and Hebert (in prep.). The two normal activity alleles (0,0') and the one null allele (0*) observed at the PGI locus in D. curvirostris were slower than any found in either D.pulex or D.schodleri. Alleles 0' and 0* appeared to have the same mobility. Clones C1 and C2 were single banded homozygotes (0'0'), while clone C3 was a double banded null heterozygote (00*). Three alleles were observed at the GOT locus (1', 2, 3). Alleles 2 and 3 were commonly observed in D. pulex clones, but the slowest allele (1') was unique to clone C3 in which it existed in the homozygous condition. Clone C1 was homozygous for allele 3 and clone C2 possessed a triple banded heterozygous phenotype (23). At three loci (MDH, XDH, G6PDH), clones C1, C2 and C3 were homozygous for an allele (0) which was slower than any allele present in D.pulex or D.schodleri. Similarly, the D. curvirostris clones were homozygous at

Table 5.1 Genotypic characteristics of D. curvirostris clones and D. schodleri clones. Alleles designated as 0' are intermediate in mobility between alleles 0 and 1.

Clone	LDH	PGI	PGM	AMY-1	AMY-2	MDH	GOT	EST	XDH	G6PDH	Average Heterozygosity
C1	11	0'0'	33	77	00**	00	33	44	00	00	0.0
C2	11	0'0'	33	77	00**	00	23	44	00	00	0.10
C3	11	00*	33	77	00**	00	1'1'	44	00	00	0.10
										$\bar{x}$ =	0.07
S1	13	14	34	33	22	11	23	34	11	11	0.50
S2	13	14	34	33	22	11	23	24	11	11	0.50
										$\bar{x}$ =	0.50

AMY-1 for an allele (7) which was faster than any allele noted in D. pulex or D. schodleri. Homozygous patterns of common D. pulex alleles were observed in all D. curvirostris clones at PGM (33), LDH (11), EST (44) and AMY-2 (0*0*). As in the case of the D. pulex clones, AMY-2 allele 0* was a null allele with no activity in the homozygous condition.

The D. schodleri clones (S1 and S2) were characterized by a double banded heterozygous PGM phenotype (34) which was not observed in any clones identified as D. pulex (Table 5.1). Phenotypes observed at the other 9 loci were commonly noted in D. pulex clones (Loaring and Hebert, in prep.).

Genetic Divergence

The heterozygosities calculated for each D.curvirostris and D. schodleri clone are listed in Table 5.1. The mean genetic distances ($\pm$ S.E.) among clones of D. curvirostris, D. schodleri and D. pulex are shown in Table 5.2. The dendrogram in Fig. 5.2 illustrates the genetic relationships among the clones of the three species. Six major clusters were distinguished. The first cluster included the 3 D. curvirostris clones, which were quite genetically distinct from all other clones in the study. Cluster 2 contained the 2 D. schodleri clones and several D. pulex clones from the Inuvik locality. Clone I28 fell into this cluster (adjacent to clone I27) when it was included in the cluster analysis. The third and fifth clusters were a mixture of D. pulex clones isolated

Table 5.2 Mean genetic distances (±S.E.) among clones of *D. pulex*, *D. schodleri* and *D. curvirostris*.

	D. pulex	*D. schodleri*	*D. curvirostris*
D. pulex	0.27 ± 0.06	0.25 ± 0.16	1.47 ± 0.03
D. schodleri		0.03	1.54 ± 0.05
D. curvirostris			0.09 ± 0.06

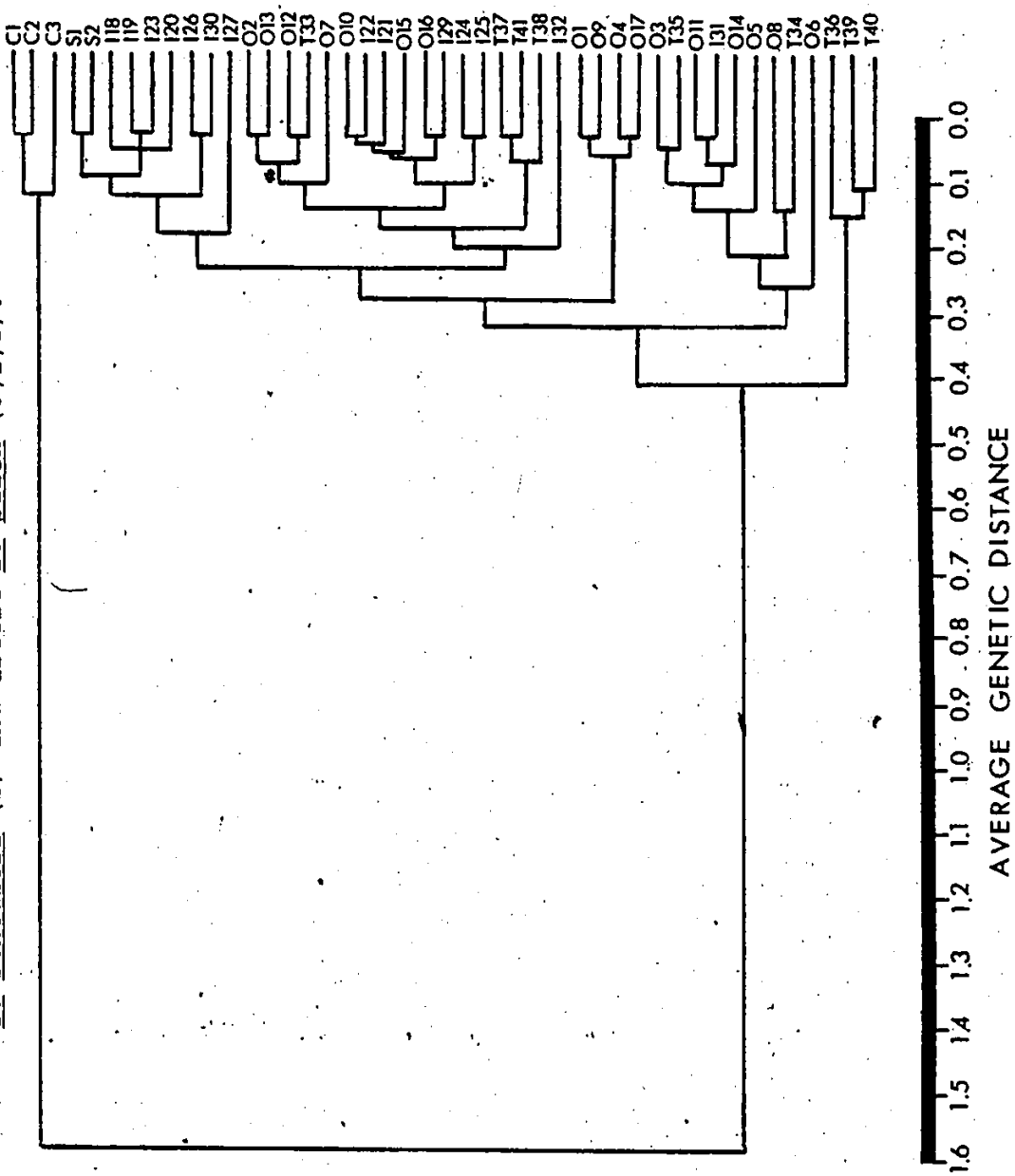

Figure 5.2 Dendrogram showing genetic relationship of D. curvirostris (C), D. schddleri (S) and arctic D. pulex (O,I,T).

from all three arctic localities. The fourth cluster included 4 D. pulex clones from Old Crow and the sixth contained 3 D. pulex clones from Tuk.

Evidence Pertaining to Mode of Reproduction

Two ephippial hatchlings from each of the two D. schodleri clones (S1 and S2) were examined for segregation at the five loci (LDH,PGI,PGM,GOT,EST) heterozygous in their maternal parent. Attempts to hatch ephippia produced by D. curvirostris clones heterozygous at any locus, failed.

The studies on ephippial egg production in the absence of males indicated that D. curvirostris produced only empty ephippia, while D. schodleri , like D. pulex, frequently produced ephippial eggs amictically. Of the 64 ephippia produced by clone S1 females in the absence of males, 42 contained at least one egg. Similarly, 1 or 2 eggs were found in 23 of 28 ephippia shed by females of clone S2 in the absence of males. Under similar conditions, 13 randomly selected obligate parthenogenetic clones of D. pulex all released ephippia containing eggs. In contrast, the 22 ephippia cast by clone C1 and the 6 ephippia produced by females of clone C3 were all empty. Moreover, no males of D. schodleri were observed in the laboratory cultures, but at least one D. curvirostris clone (C2) regularly produced males.

DISCUSSION

One of the most interesting findings of this study was the discovery of Daphnia curvirostris inhabiting ponds of the western Canadian arctic. Little information is available regarding the distribution of this species, but Johnson (1952) reported its existence in shallow pools throughout the south and east of England as well as in Europe and Corfu (Stephanides 1948). Although the species was recorded in North America (Birge 1918; Kiser 1950; Fordyce 1901; Johnson 1952), Brooks (1957) has since discounted these records.

Unlike the D. pulex clones from the western arctic (Loaring and Hebert in prep.) or those studied throughout Canada (McWalter and Hebert 1982; Hebert and Crease 1982), the clones of D. curvirostris analyzed in this study did not appear to reproduce by obligate parthenogenesis. The presence of males in laboratory cultures and the lack of eggs in ephippia shed by females maintained in the absence of males indicated that, in all likelihood, D. curvirostris is a cyclic parthenogen. The low heterozygosity levels (mean = 6.7%) observed in clones of this species were consistent with the relative lack of genetic variation observed in several other cyclic parthenogenetic Daphnia species (Hebert 1974; Hebert and Moran 1980). More direct evidence, such as the demonstration of segregation in the ephippial hatchlings of a heterozygous

D. curvirostris clone, or an assessment of genotypic frequencies in natural populations, is needed to confirm that Canadian populations of D. curvirostris are cyclically parthenogenetic.

Clones of D. curvirostris and D. pulex are deceptively similar and only a few taxonomic characteristics serve to distinguish them. Despite this morphological similarity, the average genetic distance between clones of these two species (1.47 ± 0.03) was substantially higher than distances between well differentiated species, genera and even families of organisms studied by other workers (Nei, 1975). There have been previous instances in which high levels of genetic divergence within or between species have been associated with a lack of morphological variation (Nixon and Taylor 1977; Ayala et al. 1974; Tilley et al. 1978; Crease and Hebert 1982). As pointed out in other studies (Crease and Hebert 1982; Turner 1974; Nixon and Taylor 1977), the lack of agreement between morphological and allozyme variation may imply different rates of evolution at these two levels. Since arctic D. curvirostris has diverged so dramatically from other Daphnia species inhabiting the same locality, it would be interesting to determine the extent to which these populations have diverged from European D. curvirostris.

Populations of D. pulex reproducing by obligate parthenogenesis have been reported in several areas of

Canada (Loaring and Hebert in prep.; McWalter and Hebert 1982; Hebert and Crease 1982). However, other workers (Lynch 1982) have recently reported the existence of cyclic parthenogenetic populations of D. pulex in North America. The results of the present study suggest that these populations may have been incorrectly identified. Indeed, cyclic parthenogenetic populations from Nebraska, identified as D. pulex, were revealed to be D. obtusa upon closer study (Hebert and Schwartz pers. comm.). Thus, there is a definite need for the careful examination of all cyclic parthenogenetic populations presumed to be D. pulex in order to rule out the possibility that they are a morphologically similar species, such as D. curvirostris or D. obtusa.

The Daphnia schodleri clones were genetically similar to the D. pulex clones investigated in this study. In fact, the average genetic distance between clones of these two "species" was no larger than the average genetic distance among the D. pulex clones. It was interesting to note that the degree of genetic differentiation among the D. pulex and D. schodleri clones was related to the degree of clonal morphological differentiation. The 2 D. schodleri clones fell at one end of the genetic distance dendrogram, while the opposite extreme was occupied by "true" D. pulex clones which conformed quite closely to the description of D. pulex given by Brooks (1957). Intermediate forms, which were genetically similar to both the D. pulex and

D. schodleri groups and which possessed morphological features characteristic of both groups, occupied the central portion of the dendrogram. These results suggest that D. schodleri and D. pulex belong to an agamic complex which includes an enormous number of clones which vary in terms of morphology and allozyme phenotype. In an earlier study, Hebert and McWalter (1982) indicated that arctic D. middendorffiana and D. pulex may form such a complex. The addition of D. schodleri to this apomictic complex emphasizes the need for a survey of the genetics and mode of reproduction of Daphnia populations. As pointed out by Hebert and McWalter (1982), the difficulties encountered by taxonomists in identifying a group of characters which distinguish D. pulex from several of its close relatives stems from their attempt to place species boundaries on an apomictic clonal complex. Dodson (1981) has suggested that the D. pulex species group is comprised of a larger number of species than are presently recognized or that the D. pulex species group is one widespread and variable "species". The present study shows that both hypotheses may be true.

CHAPTER V

SUMMARY

The present study has involved a study of genotypic diversity in populations of Daphnia pulex and its close relative D. schodleri from localities in the western arctic. It has been shown that both species reproduce by obligate parthenogenesis. There is a close genetic relationship between the species and morphological studies indicate the existence of a continuum ranging from classical D. pulex forms (as described by Brooks 1957) to classical D. schodleri forms. The difficulties which taxonomists have faced in distinguishing these species are evidently the result of attempts to place species boundaries on an apomictic complex.

This study has also documented the existence of D. curvirostris in North America. Despite its morphological similarity to D. pulex, genetic studies indicate that the two species are only distantly related and that D. curvirostris appears to reproduce by cyclic parthenogenesis. The presence of such sibling species makes clear the need to confirm that cyclic parthenogenetic populations identified as D. pulex do, in fact, belong to this species.

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APPENDICES

APPENDIX I

Linear regression analyses of :

i) weekly maximum water temperature on weekly high mean air temperature and

ii) weekly minimum water temperature on weekly low mean air temperature.

Data from weeks 9 - 25 were used in the analyses.

Dependent Variable	Regressor Variable	Intercept	Regression Coefficient	F	P value	R^2
Max.water temp.	High mean air temp.	-1.88	1.20	73.08	0.0001	.8490
Min.water temp.	Low mean air temp.	-0.36	0.83	76.75	0.0001	.8550

APPENDIX II

Weekly measurement of mean water temperature, algae density (group 1, group 2, total), *Daphnia* density ±.S.E. (juvenile, adult, total), reproductive phenotype proportion (parthenogenetic females, ephippial females, non-reproductive females, males) and genotype frequency (with 95% confidence limits) in the 4 aquaria are given in the following pages. The variables are as follows:

WK = week number

AQ = aquarium number

MT = mean water temperature

G1 = group 1 algae density

G2 = group 2 algae density

TOAL = total algae density

JU = juvenile *Daphnia* density

AD = adult *Daphnia* density

TODA = total *Daphnia* density

PF = proportion of adult population represented by parthenogenetic females

EP = proportion of adult population represented by ephippial females

NF = proportion of adult population represented by non-reproductive females

ML = proportion of adult population represented by males

C1 = genotype frequency of clone 1

C4 = genotype frequency of clone 4

C6 = genotype frequency of clone 6

C13 = genotype frequency of clone 13.

WK	MT	AQ	G1	G2	TOAL	JU	AD	TODA
2		1	21494		21494			
		2	542544		542544			
		3	57152		57152			
		4	182838		182838			
3		1	93616		93616			
		2	159392		159392			
		3	67952		67952			
		4	622112		622112			
4	11.0	1	98896		98896	15.86±2.54	1.97±0.52	17.83±2.84
		2	96896		96896	27.77±1.86	8.26±1.56	36.02±3.11
		3	62480		62480	15.78±0.56	6.97±0.80	22.75±0.59
		4	66160		66160	25.27±3.58	1.50±0.67	26.77±4.24
5	11.2	1	79352		79352	34.69±3.17	10.23±2.57	44.92±5.71
		2	69264		69264	218.27±9.09	42.28±15.04	260.55±24.12
		3	31024		31024	15.26±5.24	18.33±2.41	33.59±7.63
		4	56816		56816	19.45±1.82	20.53±4.19	39.99±5.33
6	17.2	1	271200		271200	138.25±25.95	40.06±4.90	178.32±22.11
		2	8720		8720	305.17±1.92	44.66±7.26	349.84±13.74
		3	108388		108388	13.11±2.35	6.99±.64	20.09±2.93
		4	81312		81312	632.28±37.67	32.41±9.26	664.28±37.67
7	15.55	1	3040		3040	224.23±23.19	110.11±20.90	334.34±43.69
		2	576		576	208.42±41.23	124.65±31.98	333.06±73.20
		3	3376		3376	2.27±0.41	9.42±0.72	11.68±1.08
		4	928		928	595.38±6.48	95.72±7.17	691.11±5.80
8	20.25	1	2448		2448	46.78±12.27	115.99±35.97	162.77±27.74
		2	1728		1728	90.73±7.45	53.45±8.55	144.18±15.94
		3	2074		2074	17.85±2.83	22.86±3.58	40.71±6.40
		4	1568	768	2336	39.47±5.55	157.75±1.53	157.75±1.53
9	23.0	1	1168	90	1258	21.71±10.80	41.38±7.97	63.09±11.45
		2	1328	112	1440	5.90±3.62	42.04±4.37	47.94±7.67
		3	4912	240	5152	13.01±3.03	1.13±1.13	14.13±3.21
		4	1488		1488	20.17±3.52	97.17±21.77	117.34±23.84

WK	MT	AQ	G1	G2	TOAL	JU	AD	TODA
10	20.5	1	7696		7696	11.95±2.38	9.56±2.20	21.51±4.50
		2	432		432	15.89±6.24	67.11±14.14	83.0±20.34
		3	7536	224	7760	14.94±3.71	9.99±1.98	24.99±5.68
		4	928	224	1152	16.94±1.59	78.83±2.22	95.76±1.49
11	21.0	1	6620	288	6908	10.46±4.29	14.16±3.18	24.62±6.14
		2	15200		15200	1.24±0.29	61.91±11.23	63.16±11.40
		3	3168	32	3200	94.46±13.66	62.60±11.35	157.06±21.91
		4	1600	160	1760	7.39±1.37	54.69±8.96	62.08±9.35
12	20.0	1	13920		13920	20.32±2.87	8.01±1.27	28.33±2.50
		3	16352		16352	34.52±5.09	46.83±8.97	81.35±13.72
		4	2304	416	2720	5.93±1.81	55.21±10.19	61.14±11.99
13	23.75	1	215520	640	216160	3.68±0.68	0.42±0.08	4.09±0.71
		3	251360	960	252320	15.03±2.23	10.05±1.72	25.09±2.09
		4	25616	2176	27792	0.18±0.09	0.35±0.35	0.52±0.40
14	22.0	1	54688	304	54992	28.39±4.86	12.43±1.36	40.82±6.04
		3	14272	80	14352	55.97±7.23	66.31±8.22	122.27±6.59
		4	5232	48	5280	18.24±2.86	19.85±3.02	38.09±5.80
15	20.5	1				24.74±4.15	19.43±0.78	44.17±4.93
		3	15344	64	15408	11.43±1.89	89.81±12.62	108.18±14.51
		4	1600	12928	14528	15.40±4.92	24.82±3.48	40.23±8.05
16	22.0	1	54240	108800	163040	0.74±0.29	0.81±0.39	1.55±0.25
		3	52480	49120	101600	5.96±1.14	30.02±6.73	35.97±7.85
		4	2992	11488	14480	11.29±1.09	11.68±2.16	22.97±1.18
17	21.65	1	52736	38240	90976	7.35±0.41	1.02±0.41	8.37±0.63
		3	9600	3840	13440	0.88±0.16	22.65±5.22	23.52±5.10
		4	13068	5024	18092	2.53±0.57	1.63±0.57	4.16±0.25
18	20.0	1	85120	157440	242560	15.52±3.79	2.86±0.60	18.37±4.38
		3	12448	2016	14464	15.1±0.42	0.42±0.17	15.52±0.53
		4	4640	7872	12512	2.51±0.47	0.52±0.16	3.04±0.63
19	20.5	1	72320	181760	254080	11.65±1.43	3.94±1.99	15.59±3.10
		3	24144	15312	39456	2.58±0.66	0.74±0.46	3.32±0.20
		4	3776	27248	31024	9.66±1.15	5.02±0.62	14.68±0.83

WK	MT	AQ	G1	G2	TOAL	JU	AD	TODA
20	21.0	1	874480	1966720	2841200	7.02±1.19	9.36±2.99	16.38±4.04
		3	3676	1296	4972	14.63±2.42	10.64±0.87	25.27±3.10
		4	1536	2832	4368	25.11±4.33	8.23±2.39	29.13±2.40
21	15.0	1	25600	33280	58880	13.38±1.12	23.5±1.94	36.88±1.75
		4	2240	2848	5088	34.59±8.18	25.18±1.70	59.76±9.47
22	17.5	1	5088	1184	6272	6.46±0.15	10.14±0.34	16.59±0.49
		4	831	352	1184	17.06±4.34	45.58±5.86	62.64±2.0
23	13.5	1	8480	16160	24640	2.58±0.37	5.72±0.52	8.30±0.88
		4	1536	4272	5808	17.34±3.73	54.66±8.90	72.0±12.51
25	12.5	1	432	592	1024	34.42±1.03	2.43±0.13	36.90±0.99
		4	2720	2784	5504	3.97±1.32	24.65±1.19	28.62±1.20

WK	AQ	PF	EP	NF	ML	C1	C4	C6	C13
4	1	1.00				0.43±0.10			0.57±0.10
	2	1.00				0.70±0.09			0.30±0.09
	3	1.00					0.35±0.10	0.65±0.10	
	4	1.00					0.54±0.15	0.46±0.15	
5	1	1.00				0.57±0.10			0.43±0.10
	2	1.00				0.85±0.07			0.15±0.07
	3	0.95	0.05				0.39±0.10	0.61±0.10	
	4	1.00					0.34±0.10	0.66±0.10	
6	1	0.22	0.20	0.58		0.70±0.07			0.30±0.09
	2	0.13	0.11	0.76		0.89±0.05			0.11±0.05
	3	0.25	0.06	0.69			0.35±0.10	0.65±0.10	
	4	0.58	0.16	0.26			0.44±0.10	0.56±0.10	
7	1	0.14	0.19	0.67		0.61±0.10			0.39±0.10
	2	0.15	0.05	0.80		0.94±0.05			0.06±0.05
	3	0.57	0.01	0.42			0.77±0.09	0.23±0.09	
	4	0.28	0.12	0.60			0.38±0.10	0.62±0.10	
8	1	0.20	0.31	0.49		0.37±0.10			0.63±0.10
	2			1.00		0.91±0.06			0.09±0.06
	3	0.20	0.02	0.69	0.09		0.77±0.09	0.23±0.09	
	4			1.00			0.41±0.11	0.59±0.11	
9	1	0.03	0.21	0.76		0.56±0.10			0.44±0.10
	2	0.05	0.21	0.74		1.00			
	3				1.00		0.89±0.06	0.11±0.06	
	4	0.04	0.20	0.76			0.17±0.08	0.83±0.08	
10	1		0.34	0.66		0.76±0.09			0.24±0.09
	2	0.03	0.49	0.48		0.98±0.05			0.02±0.05
	3	0.51		0.36	0.13		0.92±0.06	0.08±0.06	
	4	0.09	0.38	0.53			0.19±0.08	0.81±0.08	
11	1	0.58	0.15	0.27		0.62±0.10			0.38±0.10
	2	0.02	0.50	0.48		0.99±0.02			0.01±0.02
	3	0.24	0.31	0.44	0.01		0.91±0.06	0.09±0.06	
	4	0.06	0.26	0.61	0.07		0.18±0.08	0.82±0.08	

WK	AQ	PF	EP	NF	ML	C1	C4	C6	C13
									0.35±0.10
12	1	0.22	0.10	0.68		0.65±0.10			
	3	0.01	0.25	0.59	0.15		0.86±0.09	0.14±0.09	
	4	0.20	0.13	0.56	0.11		0.28±0.10	0.72±0.10	0.35±0.25
13	1	0.59		0.41		0.65±0.25			
	3	0.39	0.03	0.58			0.97±0.05	0.03±0.05	
	4	0.50	0.04	0.46			0.17±0.17	0.83±0.17	0.06±0.06
14	1	0.82		0.18		0.94±0.06			
	3	0.14	0.16	0.70			0.99±0.03	0.01±0.03	
	4	0.63		0.36	0.01		0.20±0.08	0.80±0.08	0.04±0.04
15	1	0.02	0.20	0.78		0.96±0.04			
	3	0.08	0.28	0.64			0.97±0.03	0.03±0.03	
	4	0.25	0.32	0.43			0.13±0.07	0.87±0.07	
16	1	0.29	0.14	0.57		1.00			
	3	0.05	0.37	0.58			0.95±0.05	0.05±0.05	
	4	0.02	0.30	0.66	0.02		0.08±0.06	0.92±0.06	
17	1	1.00				1.00			
	3	0.31	0.13	0.56			0.98±0.04	0.02±0.04	
	4	0.81		0.19			0.94±0.12	0.06±0.12	0.13±0.08
18	1	0.21	0.36	0.43		0.87±0.08			
	3	0.08		0.20			1.00		
	4	0.67	0.11	0.22			0.89±0.20	0.11±0.20	0.02±0.03
19	1	0.02	0.27	0.71		0.98±0.03			
	3	0.50		0.50			1.00		
	4	0.44		0.56			0.63±0.12	0.37±0.12	0.01±0.02
20	1	0.10	0.29	0.61		0.99±0.02			
	3	0.77		0.23			1.00		
	4	0.42	0.15	0.43			0.72±0.09	0.28±0.09	0.04±0.04
21	1	0.07	0.45	0.48		0.96±0.04			
	4	0.08	0.34	0.58			0.69±0.10	0.31±0.10	

WK	AQ	PF	EP	NF	ML	C1	C4	C6	C13
22	1	0.07	0.28	0.65		1.00			
	4	0.06	0.39	0.55			0.74±0.09	0.26±0.09	
23	1	0.35	0.06	0.59		0.99±0.03			0.01±0.03
	4	0.08	0.30	0.56	0.06		0.71±0.09	0.29±0.09	
25	1	1.00				1.00			
	4	0.06	0.42	0.52			0.89±0.06	0.11±0.06	

APPENDIX III

Locations of the Tuk, Old Crow, Inuvik, Williams L. and San Diego ponds are described in the following pages. The symbols are as follows:

TUK 1 M - TUK 10 M = 10 Tuk ponds from which *D. magna* was collected

TUK 1 P - TUK 10 P = 10 Tuk ponds from which *D. pulex*, *D. curvirostris* or *D. schodleri* were collected

OC 1 - OC 9 = 9 Old Crow ponds from which *D. pulex* or *D. curvirostris* were collected

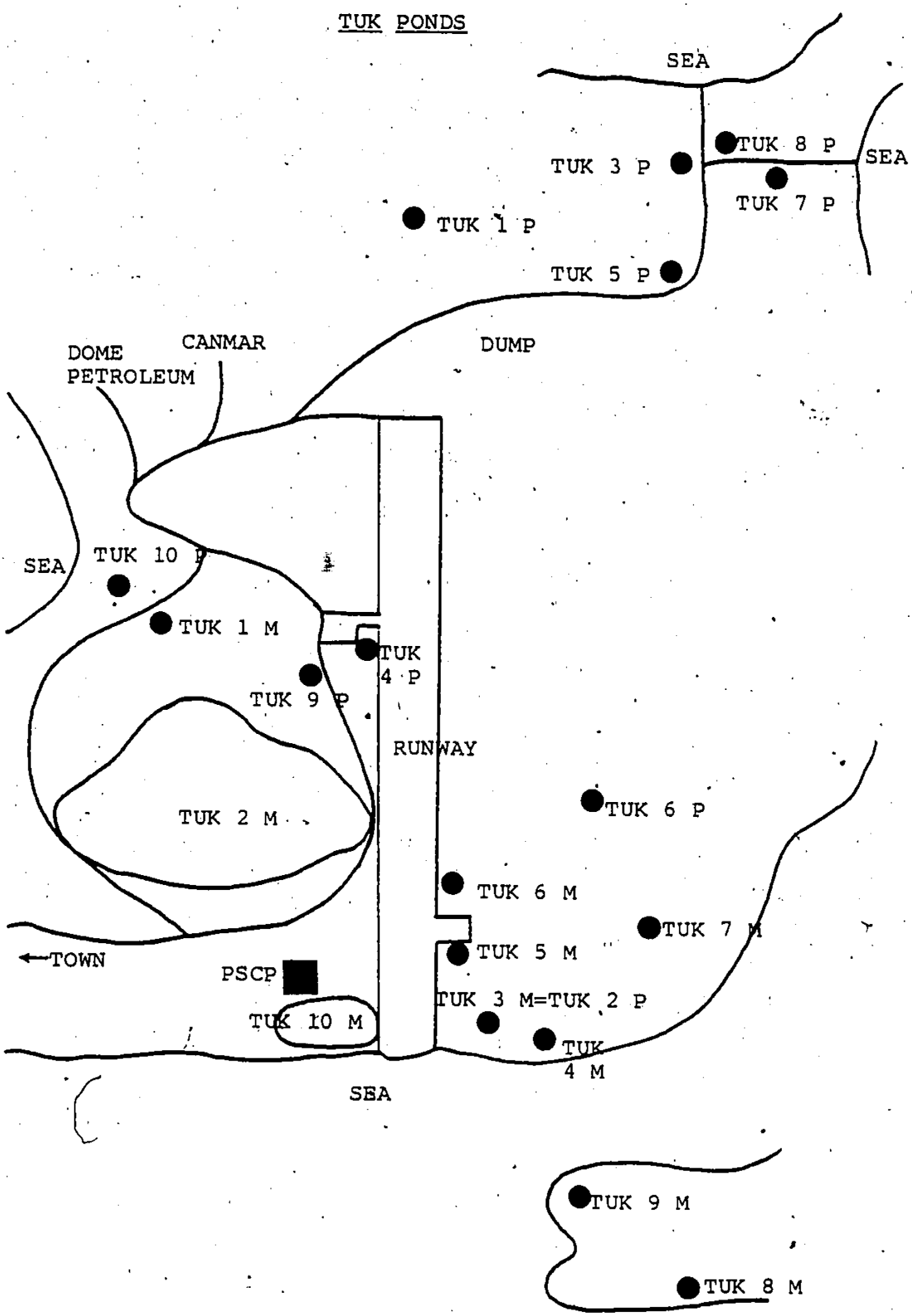
IN 1 - IN 9 = 9 Inuvik ponds from which *D. pulex* was collected

WL4 and WL6 = 2 Williams L. ponds from which *D. magna* was collected.

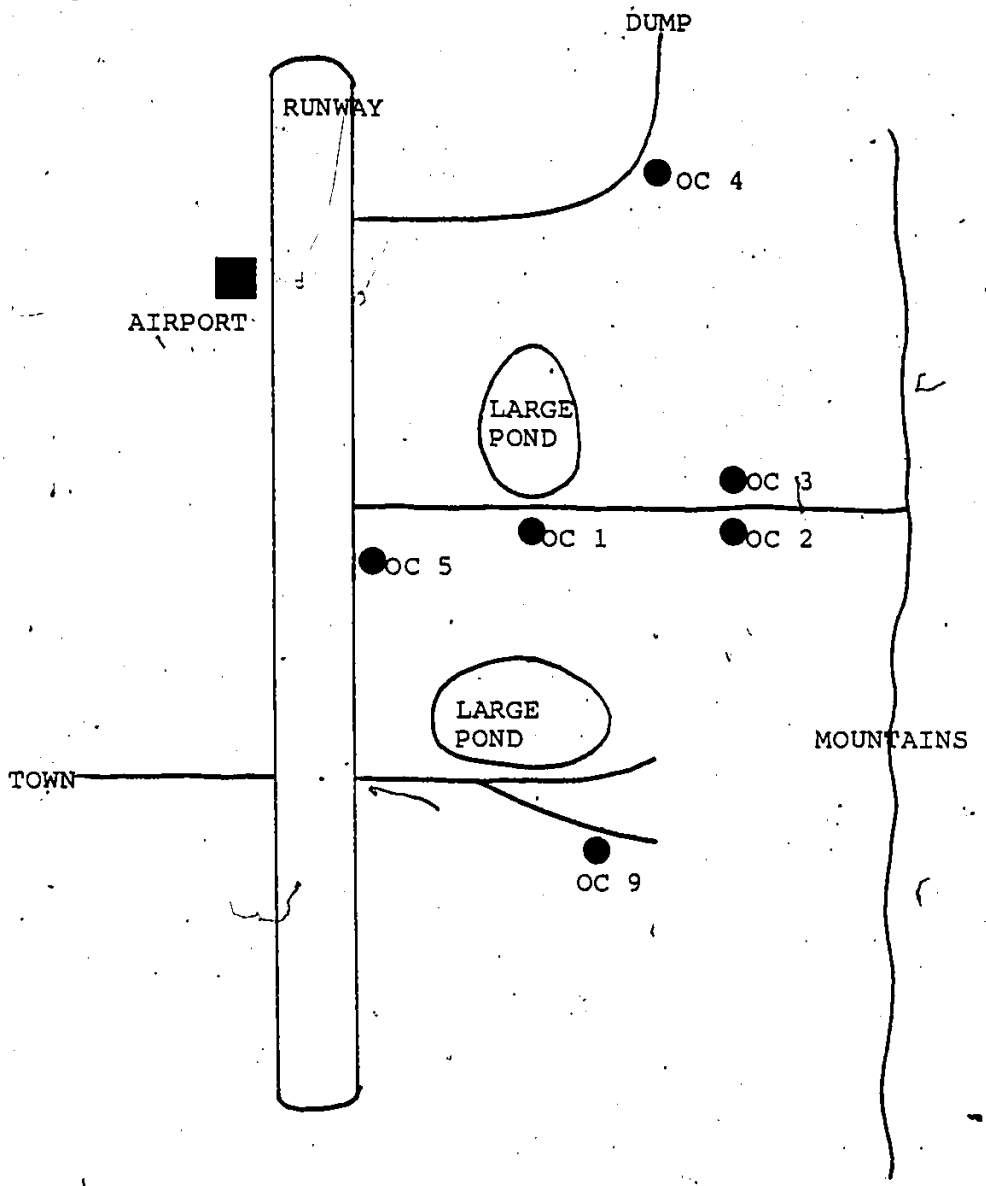
PSCP = Polar Continental Shelf Project

WASRC = Western Arctic Scientific Resource Centre

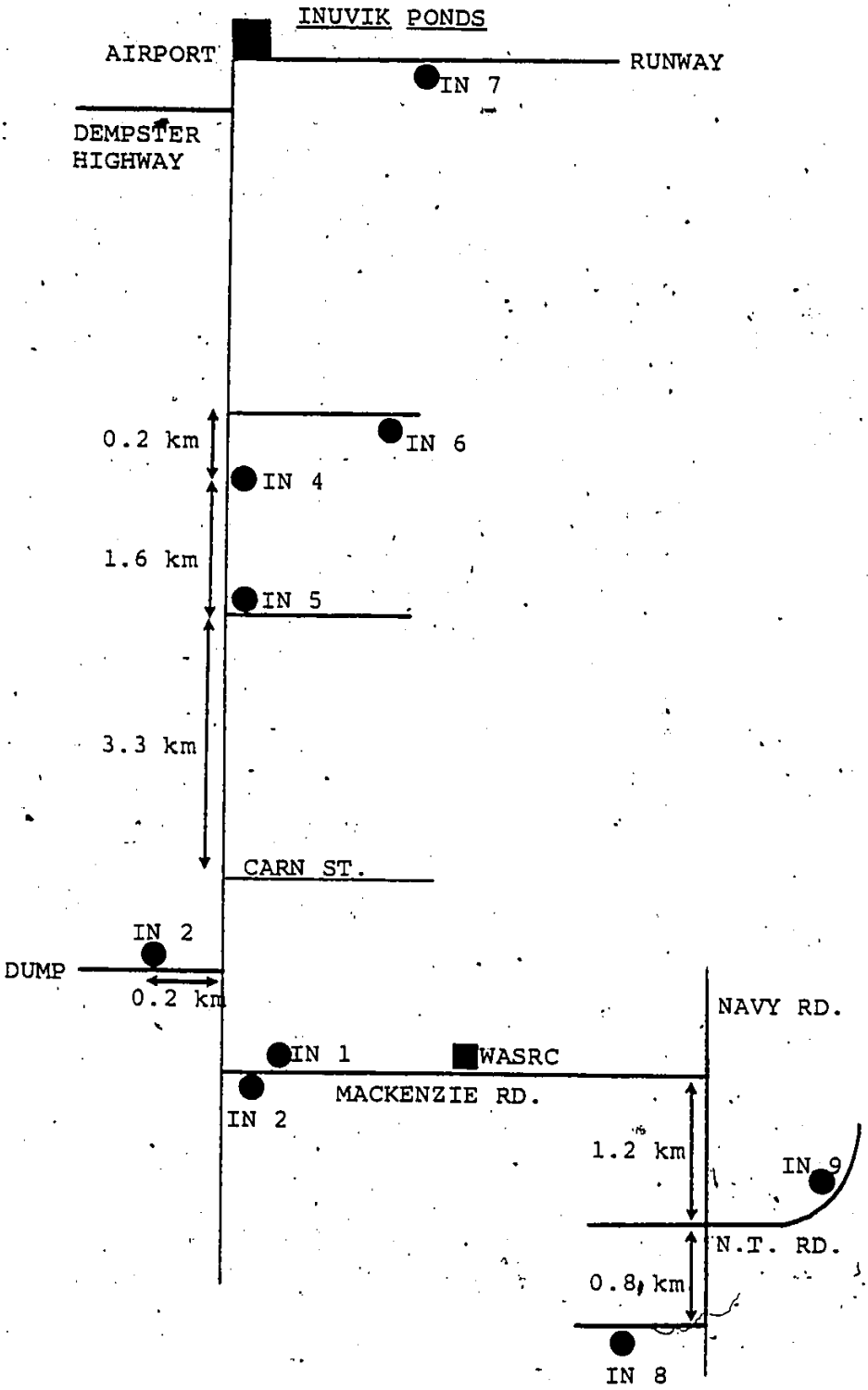
SANTEE LAKES = Connected ponds from which the 2 San Diego *D. magna* clones (S25 and S27) were collected


TUK PONDS
SEA
TUK 8 P
SEA
TUK 3 P
TUK 7 P
TUK 1 P
TUK 5 P
DOME
PETROLEUM
CANMAR
DUMP
SEA
TUK 10 P
TUK 1 M
TUK
4 P
TUK 9 P
RUNWAY
TUK 2 M
TUK 6 P
TUK 6 M
TUK 7 M
TUK 5 M
TOWN
PSCP
TUK 3 M=TUK 2 P
TUK 10 M
TUK
4 M
SEA
TUK 9 M
TUK 8 M

OLD CROW PONDS

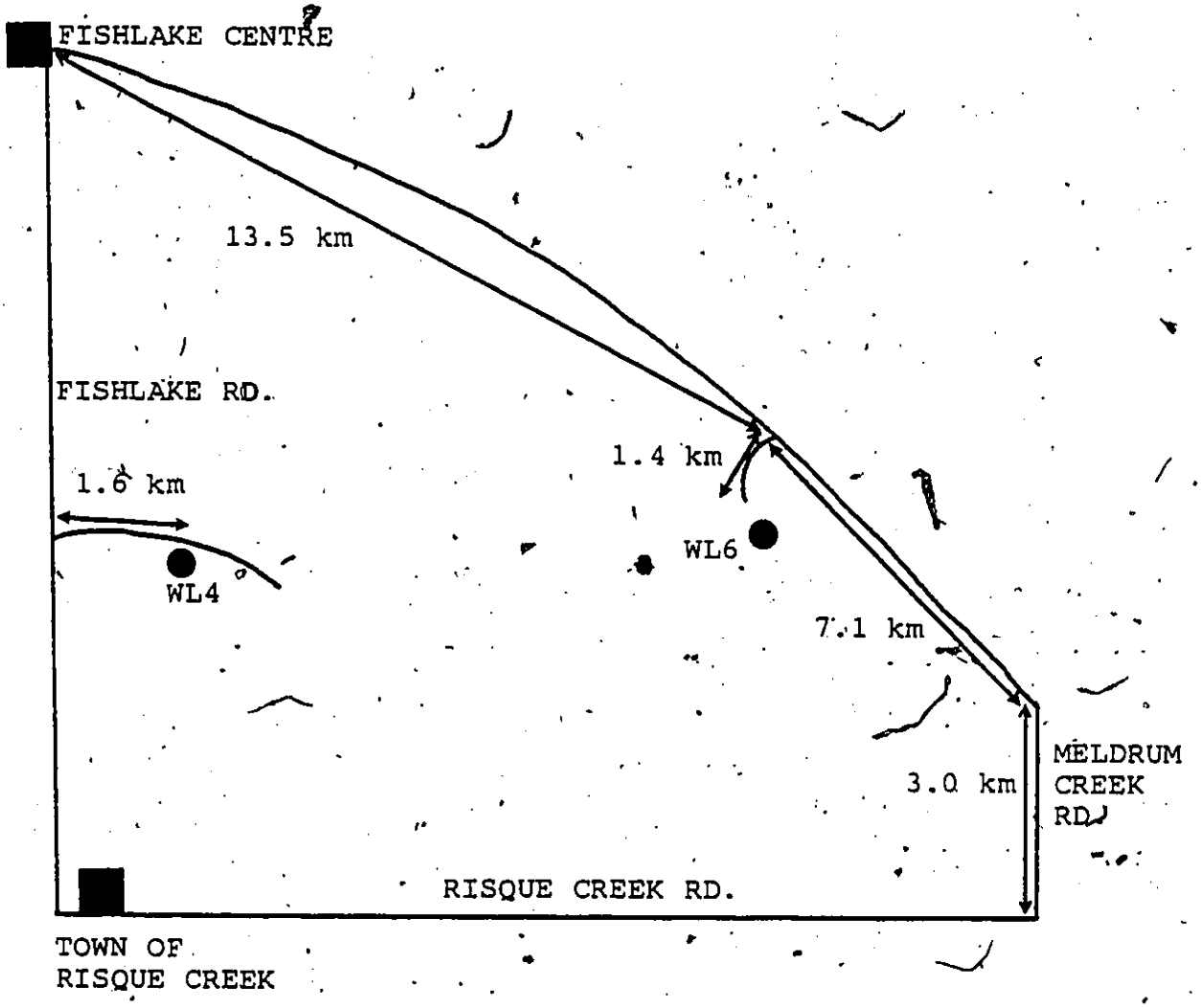


OC 6 - OC 8 LOCATED APPROX. 6 MILES WEST OF OLD CROW ON THE SOUTH BANK OF THE PORCUPINE RIVER

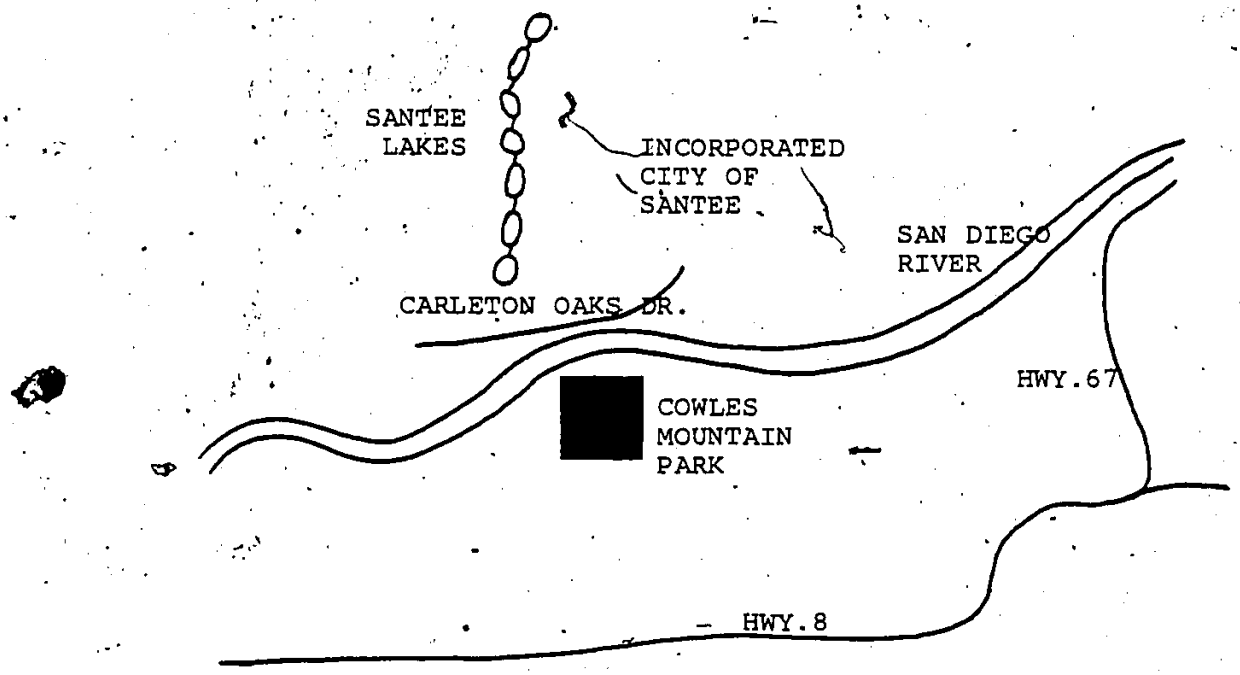

INUVIK PONDS
AIRPORT
RUNWAY
IN 7
DEMPSTER HIGHWAY
0.2 km
IN 6
IN 4
1.6 km
IN 5
3.3 km
CARN ST.
IN 2
DUMP
0.2 km
NAVY RD.
IN 1
WASRC
MACKENZIE RD.
IN 2
1.2 km
IN 9
N.T. RD.
0.8 km
IN 8

WILLIAMS LAKE PONDS

LOCATED APPROX. 30 MILES WEST OF WILLIAMS LAKE, WEST OF THE FRASER RIVER, NEAR THE TOWN OF RISQUE CREEK



SAN DIEGO PONDS



APPENDIX IV

The salinities and conductivites of several of Tuk, Old Crow, Inuvik and Williams L. ponds are listed in the following pages. The Tuk ponds from which *D. magna* were collected are designated as Tuk 1 M - Tuk 10 M and the Tuk ponds from which *D. pulex*, *D. curvirostris* or *D. schodleri* were collected are designated as Tuk 1 P - Tuk 10 P.

Locality	Pond	Conductivity (μhm/cm^2)	Salinity (%)
Tuk	Tuk 1 M	5100	4.5
	Tuk 3 M	2900	2.4
	Tuk 4 M	3000	2.5
	Tuk 5 M	2800	2.2
	Tuk 7 M	3150	2.8
	Tuk 8 M	4400	3.9
	Tuk 9 M	2750	2.2
	Tuk 1 P	185	0.1
	Tuk 2 P	2900	2.4
	Tuk 3 P	260	0.2
	Tuk 4 P	590	0.2
	Tuk 5 P	230	0.2
	Tuk 6 P	1680	1.2
	Tuk 7 P	550	0.8
	Tuk 8 P	320	0.4
	Tuk 9 P	990	0.5
	Tuk 10 P	6500	5.5
Williams Lake	WL 4	1150	1.5
	WL 6	4500	4.0
Old Crow	OC 1	380	0.1
	OC 2	230	0.1
	OC 3	120	0
	OC 4	90	0
	OC 5	450	0.1

Locality	Pond	Conductivity (μmhm/cm^2)	Salinity (%)
Old Crow	OC 6	240	0.1
	OC 7	310	0.1
	OC 8	300	0.1
	OC 9	100	0.05
Inuvik	IN 1	2300	1.5
	IN 2	700	0.4
	IN 3	700	0.2
	IN 4	180	0
	IN 5	550	0.2
	IN 6	720	0.4
	IN 7	710	0.4
	IN 8	270	0
	IN 9	750	0.4

APPENDIX V

The PET microcomputer programs (in BASIC) for the calculation of the Shannon-Weaver, Brillouin and α diversity indices are listed in the following pages.

```
& Diversity Index

5 PRINT"3"
10 PRINT"INPUT # OF SPECIES REPRESENTED BY 1 SPECIMEN"
15 INPUT F
20 PRINT"ENTER THE NUMBER OF SPECIES IN THE COLLECTION"
25 INPUT S
30 PRINT"ENTER THE NUMBER OF SPECIMENS IN THE COLLECTION"
35 INPUT N
40 PO=F/N
45 XE=1.00-PO
50 PRINT"THE APPROXIMATION OF X IS";XE
60 GOTO 100
70 LET XE=XE-.00001
75 GOTO 100
80 LET XE=XE+.00001
85 GOTO 100
100 Y=N/S
110 R=1-XE
115 P=(-LOG(R))/2.3026
120 QU=2.3026*Y-XE/(R*P)
130 PRINT"CURRENT VALUEOF X IS";XE
140 IF QU>.10 THEN 80
150 IF QU<-.10 THEN 70
151 IF QU>.001 THEN157
152 IF QU<-.001 THEN 155
154 GOTO160
155 XE=XE-.0000005
156 GOTO100
157 XE=XE+.0000005
158 GOTO100
160 AL=(R*N)/XE
165 PRINT"3"
170 PRINT"THE MAXIMUM LIKELIHOOD ESTIMATE OF X IS";XE
180 PRINT"THE MAXIMUM LIKELIHOOD ESTIMATE OF ALPHA IS";AL
190 A1=AL 3
200 B1=(N+AL) 2
210 C1=(2*N+AL)/(N+AL)
220 D1=LOG(C1)
230 E1=AL*N
240 F1=((S*N)+(S*AL)-(N*AL)) 2
250 G1=A1*(B1*D1-E1)
260 V1=G1/F1
270 S1=SQR(V1)
280 PRINT"THE STANDARD ERROR OF ALPHA IS";S1
290 END
```

Shannon- Weaver and Brillouin Diversity Indices

```
5 PRINT"3"
6 PRINT
8 H=0
9 PRINT
10 PRINT"ENTER THE NUMBER OF INDIVIDUALS IN THE  COLLECTION"
20 INPUT N
25 FOR A=1 TO N
30 S=S+LOG(A)
40 NEXT A
41 PRINT"3"
60 FOR Y=1 TO 30
65 PRINT"ENTER THE NUMBER OF SPECIES REPRESENTED BY";
66 PRINTY;"SPECIMENS"
67 PRINT"IF THERE IS NO MORE DATA INPUT -1"
70 INPUT C
74 IF C<0 GOTO 134
75 PRINT
76 PRINT
90 FOR B=1 TO Y
100 P=P+LOG(B)
110 NEXT B
120 BT=BT+C*P
125 P=0
128 H=C*((-Y/N)*(LOG(Y/N)))+H
130 NEXT Y
134 PRINT"3"
135 PRINT"ENTER THE ABUNDANCE OF ALL SPECIES WITH N>30";
136 PRINT" ONE BY ONE"
137 PRINT"WHEN ALL THE DATA HAS BEEN ENTERED INPUT 0"
140 INPUT Z
141 PRINT
142 PRINT
155 IF Z=0 GOTO 220
160 FOR C=1 TO Z
170 D=D+LOG(C)
180 NEXT C
190 BT=BT+D
195 H=H+(-Z/N)*(LOG(Z/N))
200 D=0
210 GOTO 135
220 BR=(1/N)*(S-BT)
240 PRINT"3"
260 PRINT"THE VALUE OF THE BRILLOUIN INDEX FOR";
261 PRINT"THIS COLLECTION IS";BR
262 PRINT
263 PRINT
270 PRINT"THE VALUE OF THE SHANNON±WEINER INDEX";
271 PRINT"FOR THIS COLLECTION IS";H
280 END
```

VITA AUCTORIS

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