GENOTYPIC AND PHENOTYPIC VARIATION

IN POPULATIONS OF <u>DAPHNIA PULEX</u>

By

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Abstract

Because of their reproductive pattern, parthenogenetic
organisms may have limited genetic variation and may rely on
alternative mechanisms other than genetic diversity for
maintaining phenotypic variability and adapting to the
environment. This hypothesis was tested by measuring genotypic
and phenotypic variation in several populations of Daphnia pulex
, an apomictic, parthenogenetic cladoceran.

Genotypic variation measured by starch gel electrophoresis
indicated 0% variable loci in 3 species of Daphnia in the lower
mainland around Vancouver, B.C. and 38% polymorphic loci in Near
Roundup, a pond in the Interior of the province near Williams
Lake. Differences in environmental conditions and
electrophoretic patterns provide a rationale for comparing
phenotypic variation in 3 electrophoretically, physically and
geographically similar ponds, P2, P4, and P5, and in an
electrophoretically polymorphic population (NR) which are
physically and geographically distinct.

Means and variances of 5 morphological and 1 to 6
reproductive characters were compared within and among clones in
each population and among populations and indicated the
following: 1) There were significant differences in means for
most characters among clones and among populations regardless of
electrophoretic similarity or dissimilarity among clones or
populations, 2) There was greater intraclonal variation than

interclonal variation in all populations for all characters, 3) there was significantly greater total variance, intraclonal variance, and interclonal variance in P2 than in NR, and 4) variances were partitioned equally within and among clones in P2 whereas the greatest % variation in NR was within clones.

These data suggest an inverse relation of genetic and phenetic variability in these populations of Daphnia and suggest that P2 and NR are examples of adaptation by individual and populational homeostasis. P2 individuals which are electrophoretically monomorphic may rely on extreme phenotypic plasticity in order to adapt to the environment. NR Daphnia may also rely on phenotypic plasticity to a lesser extent as demonstrated by the large % variation within clones, however, the relatively small absolute variance and the electrophoretic variation may indicate adaptation by genetic changes in the population. These possible strategies have been further interpreted relative to selection and temporal stability of the environment.

Phenotypic plasticity and lack of electrophoretic variation in Daphnia and in other parthenogenetic and inbreeding organisms suggest that these organisms are not dependent on genetic changes in the population to survive. There is however evidence of genetic and phenetic variation in parthenogenetic organisms comparable to variation in sexually reproducing organisms and this suggests that genetic variation is not necessarily constrained by the mode of reproduction.

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INTRODUCTION

"The basis of individuality is variation. Variation is the material of science and variation among the members of a species is the material of genetics" (Clarke, 1974).

Classically phenotypic variation has been measured as morphological and physiological variability within and among populations. Phenotypic variation has been studied in a large number of species and for a few species polymorphisms of some characters, such as shell color in snails and wing color in moths, have been demonstrated to be selectively important (Ford, 1976).

Genetic variation has been determined by measurements of qualitative and quantitative variation in characters whose genetic basis is known, and by chromosomal variability among populations or species. More recently genetic variation has been measured by enzyme polymorphisms detected by electrophoresis and the degree of electrophoretic variation of protein in both vertebrates and invertebrates is considerable (Selander, 1976). The maintenance of this variation has been interpreted by a number of explanations based on selection (Levins, 1968); the organisms perception of the environment as being coarse- or fine-grained associated with the mobility and the homeostasis of the organism, (Selander and Kaufman, 1973); reproductive strategies; rate of mutation (Crow and Kimura, 1965) and rate of gene flow. This study deals with genotypic and phenotypic

variation associated with a unique reproductive strategy, parthenogenesis. Phenotypic variation is classified by measures of morphological and physiological variability and genotypic variation is measured by electrophoresis and by comparisons of inter- and intraclonal variation.

One might expect less variation in parthenogens or in asexually reproducing organisms than in sexually reproducing organisms due to 1) lack of random assortment and recombination of chromosomes, and 2) directional selection eliminating most genotypes. Data both supporting and contradicting this hypothesis have been reported for both morphological and for electrophoretic variability. Reduced phenotypic variation has been demonstrated in parthenogenetic _Daphnia longispina_ and in another cladoceran, _Moina spp_ in comparisons of sexually reproduced and parthenogenetically reproduced individuals from the same clones (Banta, 1939). Banta and Wood in an earlier study, however, reported similar amounts of phenotypic variation in _Daphnia_ reproduced sexually and asexually (1927). Other apomictic organisms such as dandelions (Solbrig, 1971), lizards (Wright and Lowe, 1967) and weevils (Suomalainen, 1969) have also been shown to be extremely variable. A total lack of electrophoretic variation has been observed in three species of bees which are haplo-diploid (Snyder, 1974), in _Rumina decollata_ a European land snail which is a facultative selfer (Selander and Kaufman, 1973), and in a triploid lizard, _Cnemidophorus tesselatus_ which reproduces parthenogenetically (Parker and Selander, 1976). On the other hand, large amounts of electrophoretic variation have been reported for parthenogenetic

populations of lizards (Parker and Selander, 1976), weevils (Suomalainen and Saura, 1973), cladocerans (Hebert, 1974; Young, unpublished data; and Smith and Fraser, 1976), and in self-pollinating wild oats (Marshall and Allard, 1970). Data from the literature on electrophoretic variation in parthenogenetically reproducing organisms are summarized in Table 1. Proposed explanations for maintenance of variation in parthenogens include increased incorporation of mutations, stabilizing selection, heterosis, and large amounts of immigration.

Daphnia reproduce parthenogenetically during large parts of the year. Parthenogenesis in Daphnia is thought to be ameiotic based on cytological (Mortimer, 1936) and electrophoretic studies (Hebert and Ward, 1974), eliminating any variation due to recombination in the offspring. Bacci, et al. (1961 and 1965), however, argue that parthenogenesis is endomeiotic and therefore assume that recombination can give rise to genetic variability within single parthenogenetic lines of Daphnia . The genetic similarity of siblings in this study is recognized by electrophoretic similarity and, since no variation was observed among sibs electrophoretically, I have assumed endomeiosis is not occuring in these organisms.

Daphnia are also capable of producing males and subsequent sexual reproduction in response to environmental and/or demographic stimuli associated with decreasing light, temperature, or food, and increasing population density (Stross, 1969). Females usually produce two ephippial eggs which

Table 1 : Electrophoretic variation in parthenogenetic and inbreeding organisms reported in the literature.

organism	mode of reproduction	variable loci / total loci	(%)	reference
Rumina decollata	facultative self-fertilization	0/25	(0%)	Selander and Kaufman (1973)
Augochiora pura	haplo-diploidy	0/13	(0%)	Snyder (1974)
Lasioglossum zephyrum		0/24	(0%)	
Bombus americanorum		0/12	(0%)	
Drosophila mercatorum	parthenogenesis	5/10	(50%) males	Templeton, Carson, and Sing (1976)
		7/12	(58%) females	
Otiorrhynchus scaber (3N)	parthenogenesis	16/26	(62%)	Suomalainen and Saura (1973)
O. scaber (4N)		16/26	(62%)	
O. singularis (3N)		16/23	(70%)	
Strophosomus melanogrammus (3N)		9/20	(45%)	
Cnemidophorus tesselatus (2N)	parthenogenesis	6/21	(29%)	Parker and Selander (1976)
C. tesselatus (3N)		0/21	(0%)	
Poeciliopsis 2 monacha-lucida	gynogenesis	4/23	(17%)	Vrijenhoek and Leslie (197)
Avena barbata	autogamous self-fertilization	5/16	(31%)	Marshall and Allard (1970)
Avena fatua		7/13	(54%)	

Table 1 : Electrophoretic variation in parthenogenetic and inbreeding organisms reported in the literature (cont.).

organism	mode of reproduction	variable loci / total loci	(%)	reference
Simocephalus serrulatus	parthenogenesis	5/16 to 9/16 (31% to 56%)		Smith and Fraser (1976)
Daphnia magna	parthenogenesis	4/13	(31%)	Hebert (1974)
D. pulex		3/8	(37%)	Young (unpubl. data)

overwinter in the lake and hatch after appropriate environmental stimulus. This reproductive strategy ideally covers all bases: individuals are not only capable of high fecundity and rapid colonization associated with parthenogenesis, but are also capable of dispersal of the eggs, and of reorganization of genetic material by sexual reproduction. Because they are acyclical parthenogens, Daphnia are interesting organisms for measurement and evaluation of the importance of genotypic and phenotypic variation.

Phenotypic and genotypic variation have previously been described for populations of Daphnia . Large amounts of phenotypic variation in Daphnia have been described among populations in British Columbia (Carl, 1940) and in North America (Brooks, 1957). Because of these regional differences in phenotype, accurate taxonomic characterizations of species has been difficult. Phenotypic variation in Daphnia may also be cyclomorphic, in that head and carapace morphology change through successive generations of parthenogenetic females. Cyclomorphosis resulting in changes in eye diameter and length of tail spine is thought to be an adaptation to predator avoidance (Jacobs, 1966; Zaret, 1972; Dodson, 1974) and is presumed to be induced by increasing temperature and correlated with food supply and turbulence of the environment (Brooks, 1946; review by Hutchinson, 1967).

Electrophoretic measures of genetic variation in cladocerans are comparable to those of sexually reproducing species. Harris (1966) and Lewontin and Hubby (1966) in studies

of humans and Drosophila concurred in finding approximately 30% variable loci (comparable to variation in Daphnia magna and Daphnia pulex which are variable at 31 to 38% of all loci). Simocephalus also was highly polymorphic with 33 to 60 % variable loci in several populations (Smith and Fraser, 1976).

Phenotypic variation is presumably influenced by the genotype of the individual and by the environment in which the organism lives, and in this study a model relating genotype, phenotype, and environment in Daphnia will be proposed. Falconer (1965) proposed an additive model of variances in which phenotypic variation is the sum of genetic and environmental variation. Further, one would expect some interaction of the genotype and the environment in describing mean phenotype in a population. This interaction of genotypic and phenotypic variation can be discussed in terms of two alternative strategies associated with individual and populational homeostasis (Thoday, 1953; Lewontin, 1957, and Levins, 1965). Populations may adapt to a variety of environments by individual flexibility in which each individual is capable of modifying the expression of the genotype in response to the environment. Phenotypic plasticity, the amount by which the expression of the characteristic of a genotype is changed by different environments (Bradshaw, 1965), can permit a single genotype to assume different phenotypes. This is particularly advantageous in acclimating to changes in the environment which are of shorter duration than the generation time of the organism. Populations may also adapt to a variety of environments by using the differential fitness of the individuals where multiple

genotypes are each adapted to a specific environment. Phenotypic plasticity in these populations can enable different genotypes to assume a single phenotype.

Levins (1965) provides a mathematical model of these strategies and suggests an inverse relationship of genotypic to phenotypic variation. Differences in the population structure of *Avena barbata*, the slender wild oat, and *A. fatua*, the common wild oat, have been explained by these two strategies (Jain and Marshall, 1967); and as predicted by Levins, *A. barbata* is genetically less variable and phenotypically more variable than *A. fatua*.

To describe the adaptive strategies in *Daphnia* one needs to evaluate the degree of adaptation, ie. the fitness of the organism, by measuring genotypic and phenotypic variation of ecologically important characters relative to the stability of the environment. In this analysis of variation a model describing the relationship of genotype, phenotype, and environment in *Daphnia pulex* populations will be proposed and discussed relative to individual and populational homeostasis. Phenotypic variation (determined by means and variances of morphological and physiological parameters) are compared in field, field and lab, and lab populations from three lower mainland ponds at 122 39' W, 49 01'N (designated P2, P4, and P5). Phenotypic variation is also compared for lab populations from two ponds, P2 in the lower mainland, and Near Roundup (NR) at 122 30' W, 52 00' N in central British Columbia. The rationale for comparisons of P2, P4, and P5 are based on the

electrophoretic similarity of the populations and the physical, and geographic similarity of the ponds. Rationale for comparisons of P2 and NR are based on the electrophoretic dissimilarity of these populations and the existence of environmental differences among ponds. Variation is compared hierarchically 1) between the lower mainland and central B.C. Regions, 2) among populations P2, P4, P5, and N.R., 3) among clones in each population and 4) among individuals within each clone.

MATERIALS AND METHODS

Daphnia were sampled in 22 ponds, two in central British Columbia about 35 km west of Williams Lake near Riske Creek and 20 in the lower mainland near Vancouver (Fig. 1). Three species, Daphnia pulex , Daphnia rosea , and Daphnia laevis , were sampled in 12, 5, and 5 ponds respectively. Several tows from various locations in the pond were taken using a Wisconsin net 30 cm in mouth diameter with 220 nitex mesh towed from the shore or from a boat. There has been little attempt to quantify physical and chemical properties in these ponds although area, depth, vegetation, and stability of these ponds are reported in Table 2. This study deals primarily with several ponds in the lower mainland and one in central British Columbia and further description of these ponds is given in Table 3.

Daphnia pulex used in the lab experiments were chosen arbitrarily from field samples and reared separately in 40 ml plastic vials in a 1:1 dilution of pond water and dechlorinated water. Animals were maintained at 15 C and at 16-8 light-dark hours and were fed on every third day an aquarium (lab) culture of unicellular algae, primarily Chlorella , diluted 1:4 with dechlorinated water.

Phenotypic variation at the first reproductive instar was determined by morphological and physiological measurements. Length, width, and head diameter (indicators of body size and shape); and length of tail spine and eye diameter (presumed to

Figure 1:Location of ponds in the lower mainland near
        Vancouver and in the Interior of British Columbia near
        Williams Lake.

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BRITISH COLUMBIA

LEGEND
1 — Pine Pass   4 — Crows Nest Pass
2 — Yellowhead Pass   5 — Rogers Pass
3 — Kicking Horse Pass

Main Highways
Railroads
Pass

Riske Creek

LEGEND
Land over 1,000 Feet
Metropolitan Vancouver
Sand and Silt
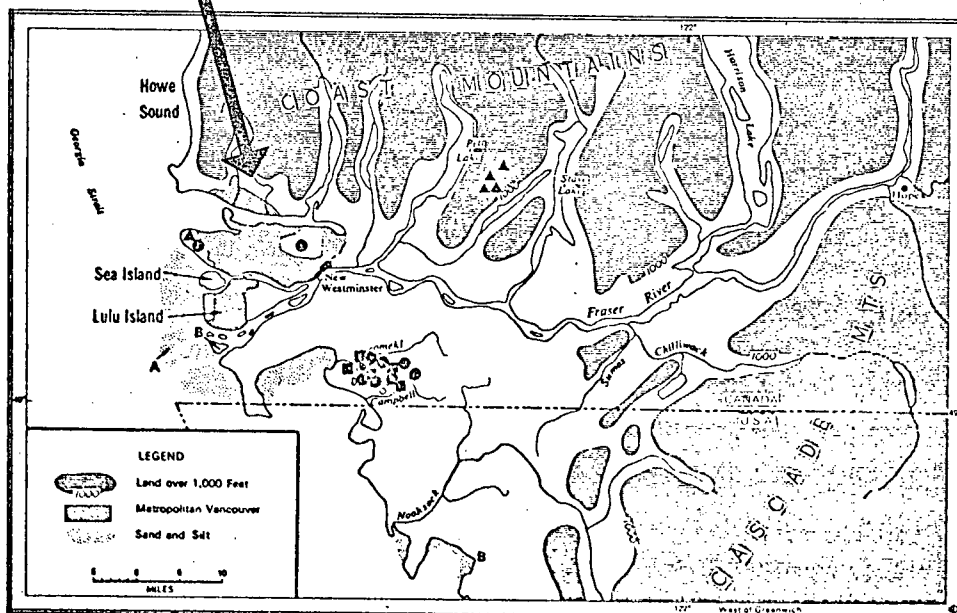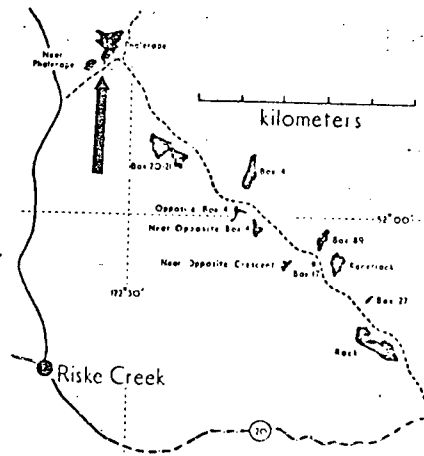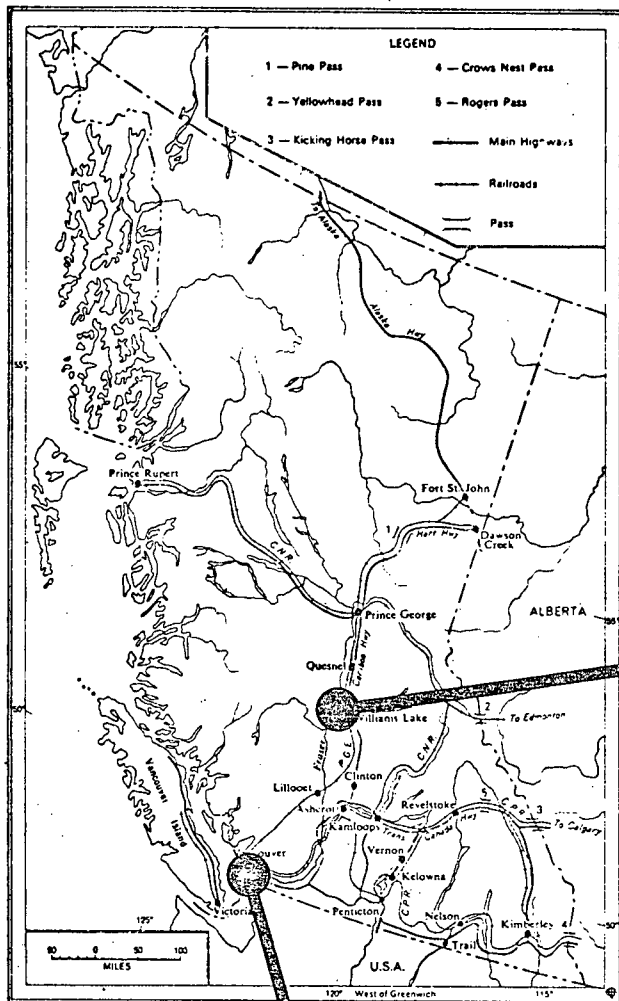
Table 2 : Summary of the available environmental data for the lakes in this study.

	Daphnia sp.	Fish	Elev. (m)	S.A. (ha)	max. depth (m)	pH	Stability
UBC Research Forest *	D. rosea						
Eunice		-	480	18.2	42	6.4	permanent
Placid		cutthroat trout	510	1.6	7	5.5-6.6	
Gwendoline		-	522	13.0	27 ·	6.6	
Katherine		-	505	20.7	29	6.6	
UBC Campus							
Nitobe Gardens		carp	30	<.05	@2		
Langley							
P1-A	D. laevis	-	10	<.01	<1	6.5-6.7	temporary
P2-A		-				6.5-6.7	
P7		-				6.4	
Riggs		-					
Newhouse		-					
P1	D. pulex	-					
P2		-				6.1	
P3		-				6.1	
P4		-				6.0-6.1	
P5		-				6.2-6.3	
P6		-				6.3-6.4	
P8		-.					
Mcleans		-		5	3	6.0	permanent
UBC Campus							
Library		-	30	<.01	<1		temporary
Burnaby							
Deer Lake		trout	100	36.0	6	6.8-7.0	permanent
Williams Lake **							
NR		-	945	5.06	3	8.1-8.6	
Box 22		-	945				

  * Northcote and Clarotto, 1975
** Topping, 1969

Table 3 : A summary of the physical chemical data for the Peterson ponds and NR.
Dates of collection are indicated in parentheses from 1976.

	Daphnia sp.	O_2 ppm		temp. (C)		µmho conductivity		pH	
P1A/P2A	laevis	(4/26)	13.2	(4/26)	14-17	(5/10)	50	(4/26)	6.1
P2	pulex	(5/19)	0.6	(5/19)	7-7.5	(5/10)	35	(4/26)	6.1
		(6/18)	0.8	(6/8)	10			(5/10)	6.1
		(6/23)	1.4	(6/23)	9				
P3	pulex	(4/26)	9.2	(4/26)	16	(5/10)	38	(4/26)	6.1
		(5/19)	8.1	(5/19)	17			(5/10)	6.1
			1.4						
P4	pulex	(4/26)	1.4	(4/26)	10-10.5	(5/10)	38	(4/26)	6.0
		(5/19)	1.2	(5/19)	7.5			(5/10)	6.1
		(6/8)	1.4	(6/8)	9				
		(6/23)	2.8	(6/23)	9				
P5	pulex	(4/26)	1.3	(4/26)	10.5	(5/10)	35	(4/26)	6.4
		(5/19)	1.2	(5/19)	8			(5/10)	6.4
				(6/8)	11				
P7	laevis	(4/26)	4.5	(4/26)	12-15	(5/10)	30	(5/10)	6.4
				(5/19)	11.5-15				
P8	pulex	(5/19)	4.7	(5/19)	7.5				
		(6/8)	3.6	(6/8)	9				
		(6/23)	2.2	(6/23)	9-9.5				
P1	pulex	(6/8)	0.9	(6/8)	11				
		(6/23)	1.2	(6/23)	9.5				
NR *	pulex	(5/12/66)	4.4	(5/12/66)	14.4	(5/12/66)	1,182	(5/12/66)	8.6
		(7/27/66)	1.2	(7/26/66)	16.4-18.9	(7/27/66)	1,485	(7/27/66)	8.1

* NR data from Toppings, 1969

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be ecologically important with regards to predator avoidance (Zaret, 1972; Dodson, 1974) were measured with an ocular micrometer at 50x magnification on a Wild dissecting microscope and are recorded as microns in the text (Fig. 2). The number of eggs at the primaparous instar was the primary measure of physiological variability, although, in one experiment comparing individuals from ponds P2 and NR, five physiological characters were measured: mortality, growthrates, number of broods/female, total eggs/female, total juveniles/female, and eggs/brood.

Genotypic variation described by 12 structural proteins was measured by horizontal starch gel electrophoresis using techniques similar to those described by Selander et al. (1971). Fifteen to twenty individuals from a single clone or from pooled field samples were homogenized by hand with a glass tissue grinder in an amount of buffer (0.01 M tris, 0.001 M EDTA, and 5 x 10-5 M NADP with pH adjusted to 6.8 with HCl) equivalent to the volume of the animals. The supernatant was absorbed into 9 x 6 mm pieces of number 1 Whatman filter paper and inserted into a slit in a 12% gel of Electrostarch (lot 302, Madison, Wisc.) and buffer. Three buffer types were used to assay for 22 loci (LiOH: esterase (ES-1, ES-2), and glutamate oxalate transaminase (GOT-1); Poulik: alkaline phosphatase (AKP-1, AKP-2, AKP-3, and AKP-4), acid phosphatase (AP-1 and AP-2), and leucine amino peptidase (LAP-1, LAP-2, LAP-3, and LAP-4); EDTA: malate dehydrogenase (MDH-1), octanol dehydrogenase (ODH-1), sorbitol dehydrogenase (SDH-1 and SDH-2), xanthine dehydrogenase (XDH-1 and XDH-2), phosphoglucose isomerase (PGI-1), aldehyde oxidase (AO-1) and indophenol oxidase (IO-1)). Buffers and stains are

Figure 2: Morphological measures of phenotypic variation.
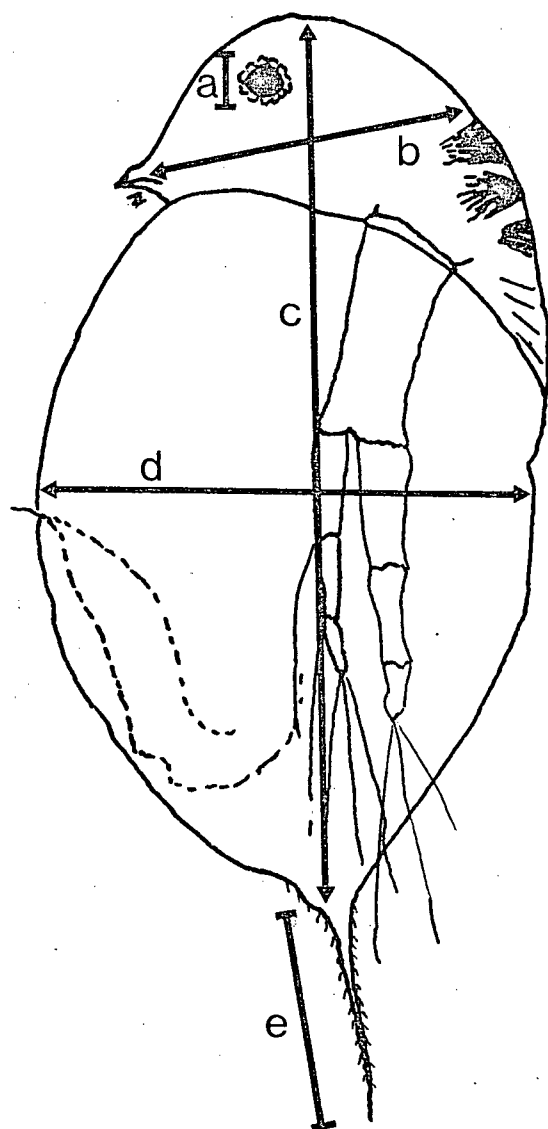
further described in the appendix.

To determine if there was any intraclonal variability individual animals were also assayed using a Tsuyuki apparatus. There was no detectable difference in electrophoretic mobility between siblings, and individuals within a clone were subsequently pooled and run on the previously described systems.

RESULTS AND DISCUSSION

Genotypic, phenotypic, and environmental variation are discussed in three sections with results and interpretation of the results incorporated into each section.

Electrophoresis

Three species of Daphnia were collected from 22 ponds and assayed electrophoretically for 12 enzymes. With the exception of Daphnia pulex from NR and Box 22 (two ponds near Riske Creek, 35 km from Williams Lake) all individuals of Daphnia pulex were monomorphic and identical in all populations (Table 4). NR individuals were variable for 38% of all loci assayed. Box 22 animals were also variable for the same loci, however too few animals were assayed for accurate measurment of % polymorphic loci. Activity at polymorphic loci PGI-1, AKP-2 and 3, and LAP 3 and 4 is shown in Figure 3, however, because of the complex banding patterns of these loci there has been no attempt made to measure gene frequencies or % heterozygosity/individual at variable loci. Daphnia pulex from three ponds in the lower mainland (P2, P4, and P8) were sampled semi-monthly for four months and all loci were monomorphic during this period.

Similarly Daphnia rosea were monomorphic in all populations although individuals from Placid Lake differed slightly from the other populations in the mobility of several alleles at the AKP,

Table 4 : Per cent monomorphic loci and number of individuals
assayed for each population and each species.

species	populations sampled	number of individuals	number of loci	monomorphic loci (%)
Daphnia rosea	UBC Research Forest:		18	100%
	Eunice	93		
	Placid	240		
	Gwendoline	124		
	Katherine	56		
	UBC campus			
	Nitobe Gardens	105		
Daphnia laevis	Langley:		12	100%
	P1-A	40		
	P2-A	82		
	P-7	18		
	Riggs	55		
	Newhouse	21		
Daphnia pulex	Langley:		18	100%
	P1	132		
	P2	250		
	P3	50		
	P4	342		
	P5	110		
	P6	41		
	P8	170		
	Mcleans	96		
	UBC campus:			
	Library	26		
	Burnaby:			
	Deer Lake	90		
	Williams Lake:			
	Near Roundup	153	18	62%
	Box 22	52		

Figure 3: Electrophoretic polymorphism at the AKP, ES, and LAP loci.
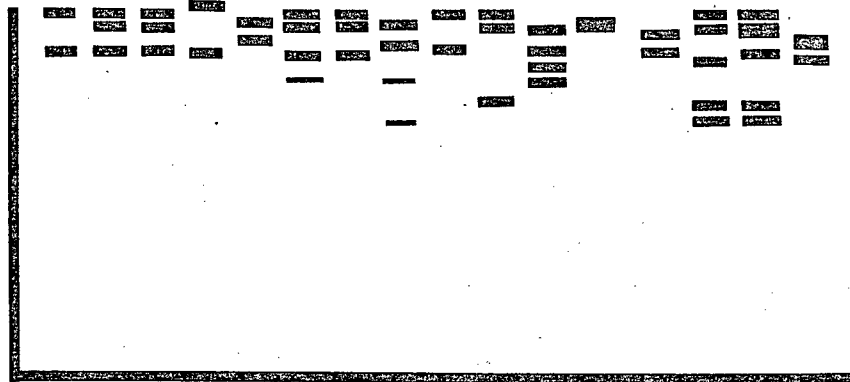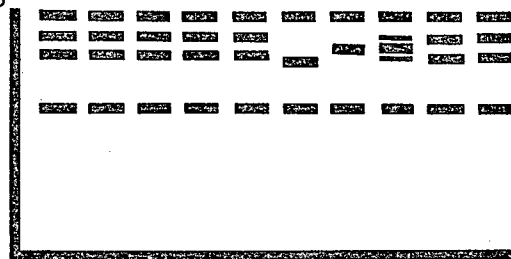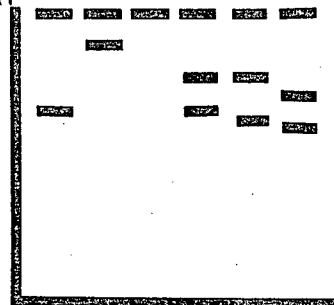
AP, and LAP loci (Tables 4 and 5). A third species, Daphnia laevis were also monomorphic at all loci and identical in the five populations assayed (Tables 4 and 5).

The three species differed from one another at several loci described in Tables 4 and 5). It is difficult to determine whether small differences in electrophoretic mobility are due to species differences or are a function of the technique. These differences, however, are consistent in all gels and are assumed to be biochemical species differences. Further detailed studies using isoelectric focusing, multidimensional gels, or amino acid composition and sequencing are needed to determine the magnitude of these differences.

Two species of another cladoceran, Simocephalus were also assayed in four populations. The two species, tentatively identified as S. serrulatus and S. vetulus, could be recognized by differences in mobility and banding patterns at several loci. These results are interesting not only because Simocephalus are parthenogenetically reproducing, but because 1) there are consistent biochemical differences between species, and 2) an occassional hybrid of the biochemical types suggests an intermediate or hybrid of the two species (Krepp, unpublished data).

No obvious explanation exists for electrophoretic homogeneity of each species in 20 ponds in the lower mainland. Electrophoretic differences were detected among species and polymorphisms observed in NR and Box 22 so it is unlikely that the observed monomorphism of lower mainland populations is a

Table 5 : Numerical designations for alleles measured from the origin
          (mm) and allele frequencies (%) for three species of Daphnia.

locus	D. rosea (all pops.)*	D. rosea (Placid)	D. laevis (all pops.)	D. pulex (all pops.)**	D. pulex (Near Roundup)
PGI-1	30 (100)	30 (100)	26 (100)	30 (100)	30 (80)
					31 (20)
GOT-1	43 (100)	43 (100)	40 (100)	43 (100)	43 (100)
XDH-1	32 (100)	32 (100)	34 (100)	32 (100)	32 (100)
2	30 (100)	30 (100)	28 (100)	30 (100)	30 (100)
IDH-1	----	----	----	15 (100)	15 (100)
SDH-1	32 (100)	32 (100)	33 (100)	32 (100)	32 (100)
2	23 (100)	23 (100)	----	25 (100)	25 (100)
AO-1	34 (100)	34 (100)	35 (100)	34 (100)	34 (100)
ODH-1	36 (100)	36 (100)	36 (100)	36 (100)	36 (100)
MDH-1	30 (100)	30 (100)	----	30 (100)	30 (100)
AKP-1	71 (100)	73 (100)	----	----	
2	67 (100)	67 (100)	67 (100)	68 (100)	polymorphic
3	57 (100)	58 (100)	57 (100)	50 (100)	polymorphic
4	53 (100)	54 (100)	----	----	
AP-1	71 (100)	74 (100)	68 (100)	68 (100)	----
2	----	67 (100)	----	63 (100)	----
ES-1	80 (100)	80 (100)	80 (100)	80 (100)	polymorphic
2	76 (100)	76 (100)	76 (100)	74 (100)	polymorphic
LAP-1	70 (100)	70 (100)	70 (100)	70 (100)	70 (100)
2	----	----	----	----	62 (100)
3	----	----	----	----	57 (36)
					54 (64)
4	53 (100)	55 (100)	55 (100)	55 (100)	37 (79)
					50 (21)

* excluding Placid
** excluding Near Roundup

function of the electrophoretic technique. Alternative explantations for the maintenance of variation in NR or lack of variation in all other ponds may relate variability to the temporal and spatial stability of the environment, to directional or stabilizing selection, and/or to population parameters such as the frequency of sexual reproduction, rate of reproduction, rate of recruitment from other populations, and rate of mutation. These explanations of variability are further considered in the final discussion.

The electrophoretic differences between the lower mainland populations and NR may suggest inherent differences in the amount of phenotypic variation in these populations and their ability to adapt to the environment. For this reason phenotypic variation among individuals has been compared for three electrophoretically identical populations, P2, P4, and P5, and between an electrophoretically monomorphic (P2) and electrophoretically polymorphic (NR) population.

Comparison Of P2, P4, And P5: Field Data

Phenotypic variation was measured in Daphnia collected from three ponds, P2, P4, and P5, in the lower mainland, by scoring body length and egg number, both ecologically important traits. Means, variances, and 95% confidence limits for the three populations are given in Table 6. Even in these three physically similar ponds, a one-way analysis of variance (ANOVA) comparing body length and egg number indicates significant differences

Table 6 : Estimates of the mean, variance and 95% confidence limits for body length and egg number for P2, P4, and P5 field populations.

population	N	body length mean±confidence limits variance	log body length mean±confidence limits variance	egg number mean±confidence limits variance
P2	140	1.99×10^3 136.97	$3.29 \pm .012$ 6.18×10^-3	8.1 ± 1.43 74.54
P4	120	2.57×10^3 219.88	$3.40 \pm .014$ 6.30×10^-3	13.0 ± 2.24 157.26
P5	109	2.30×10^3 134.59	$3.36 \pm .012$ 4.45×10^-3	15.3 ± 2.14 129.93

among populations for both characters. This suggests either that genetically similar organisms are phenotypically flexible, or that electrophoresis does not measure the genetic basis of phenotypic variability. A third explanation is that the large amount of phenotypic variation within ponds may be non-genetic and influenced by environmental heterogeneity among populations or by age differences among individuals in P2, P4, and P5. Histograms describing the distribution of body length (Fig. 4 to 6) in field animals indicate extreme variation among individuals in these three populations.

If variation in the variances and means of length and egg number is due to environmental differences or age variation among individuals and if the electrophoretic variation is a good indication of overall genetic variability, then one would expect 1) a decrease in variance in lab populations measured at a single physiological age, and 2) a convergence to a common mean for body length and for egg number in lab reared populations from P2, P4, and P5.

Figure 4: Distribution of body lengths in field and lab populations of _Daphnia pulex_ from P2.
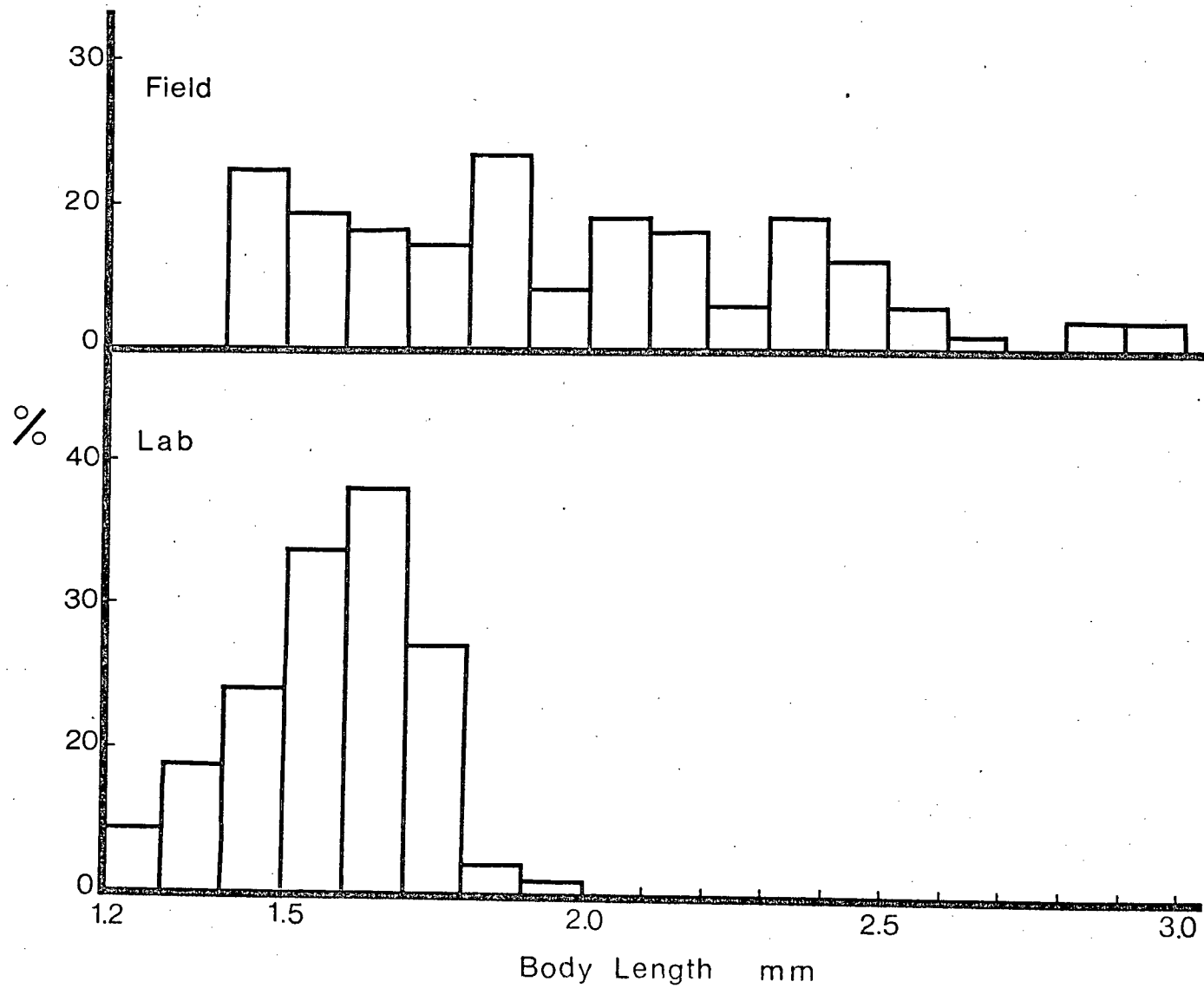
Body Length   mm

Figure 5 :Distribution of body lengths in field and lab
populations of _Daphnia pulex_ from P4.
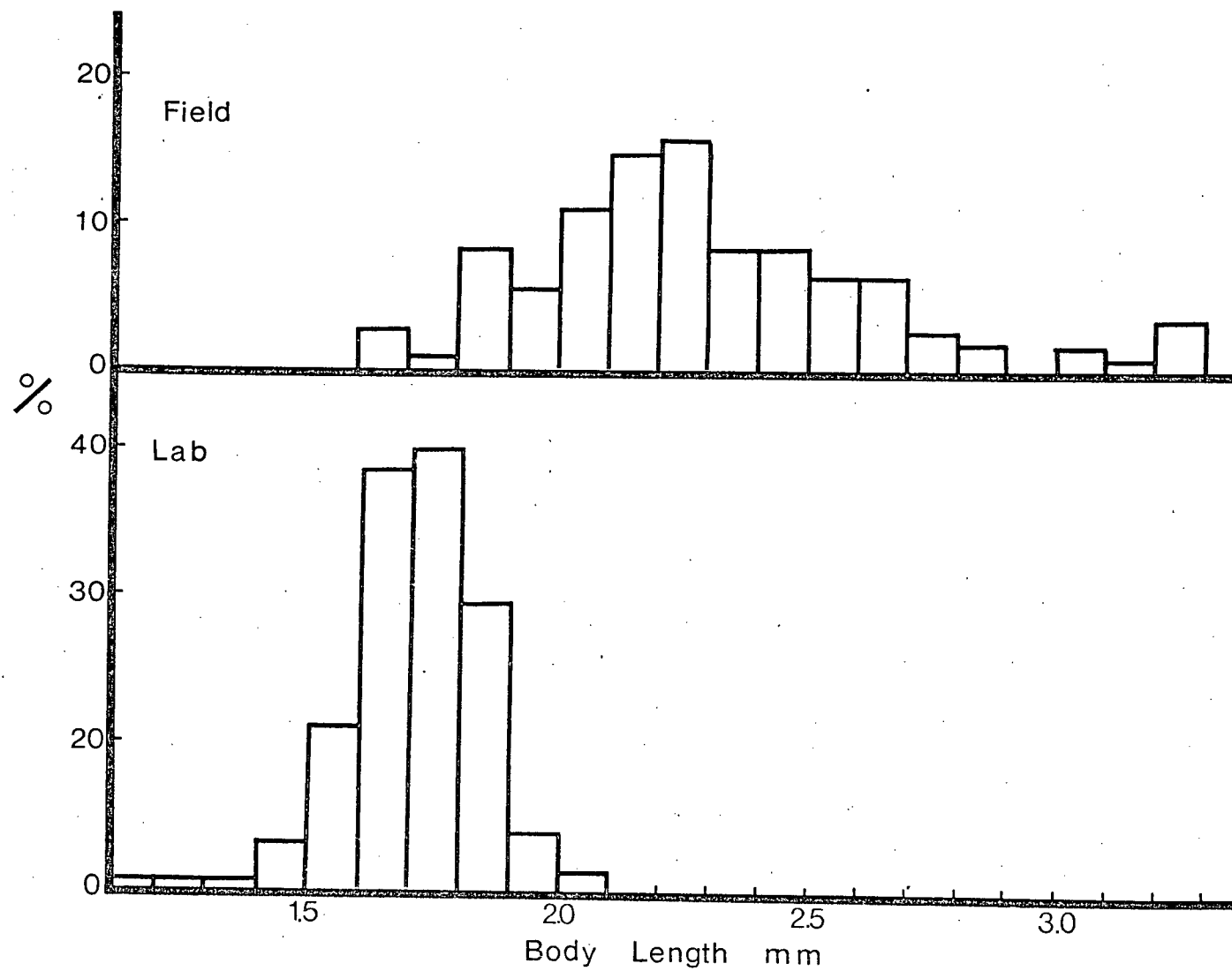
Body Length mm

Figure 6: Distribution of body lengths in field and lab
   populations of <u>Daphnia pulex</u> from P5.
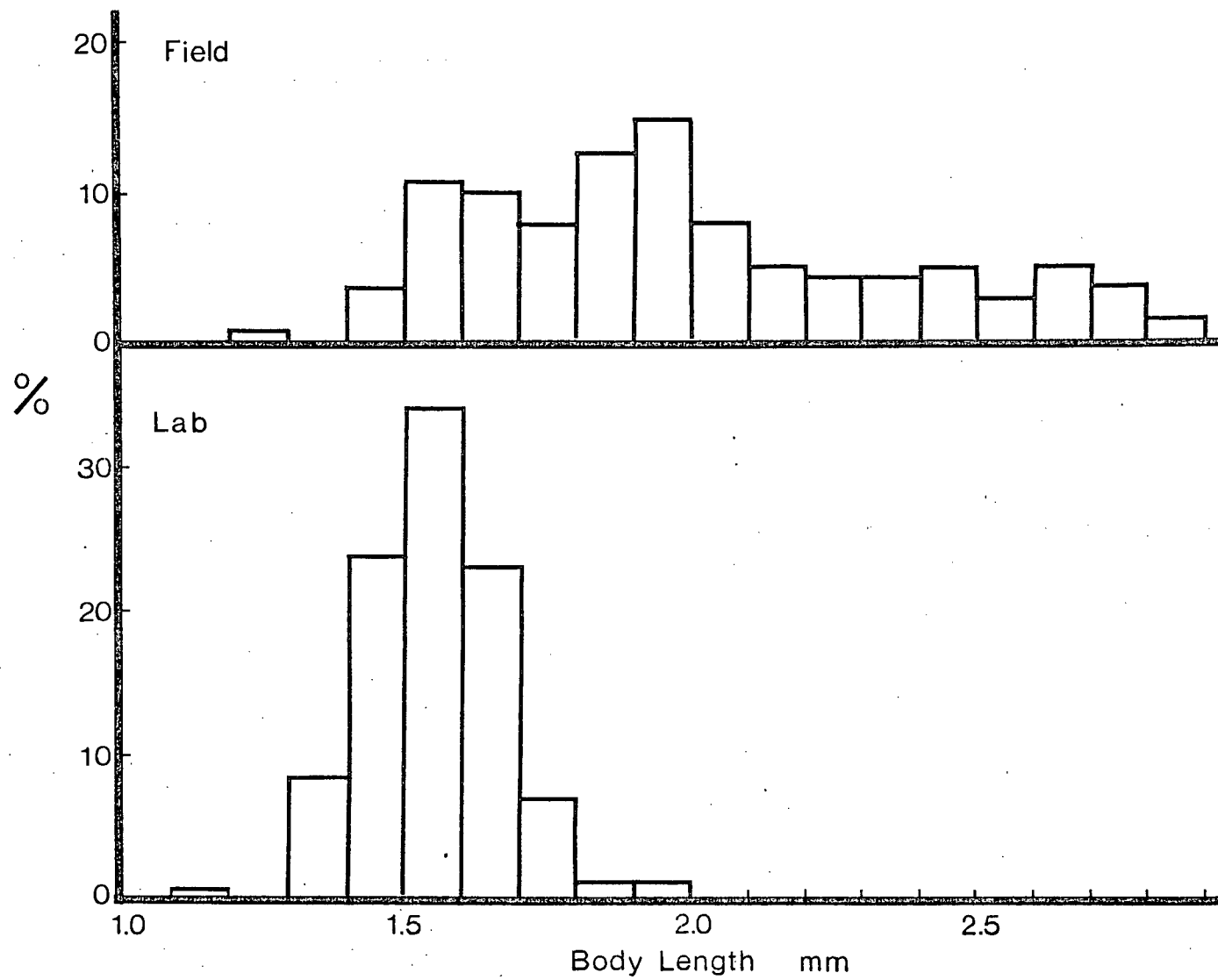
Comparison Of P2, P4, And P5: Lab And Field Data

To determine the impact of age differences among individuals and environmental heterogeneity on the amount of phenotypic variability, animals were collected from field populations P2, P4, and P5 and reared in separate vials under controlled lab conditions. Individuals from the first generation produced in the lab from each of these field animals were further separated into individual vials and six characters measured at the primaparous instar: body length, body width, eye diameter, head diameter, length of tail spine, and number of eggs.

Histograms describing the distribution of lengths (Fig. 4 to 6) in lab animals are compared to the distribution of lengths in field animals. It is evident even prior to statistical analysis that animals reared under controlled lab conditions show considerably less variation in this parameter. Variances of the field and lab populations were compared with an F test and differed significantly for body length and for egg number (Table 7). As expected field populations show significantly greater variation than lab populations reared from individuals from the same ponds. The ratio of lab variance to field variance (Table 8) indicates that of the total variation observed in the field the lab populations contain between 5 and 13% for body length and between 1 and 6% for egg number. However, to avoid biasing the variance because of differences in mean body length , the log of body length (Lewontin, 1966) was compared. A comparison

Table 7 : Comparison of lab and field variances of body length and egg number in P2, P4, and P5. All values are significant at $P < .01$.

population	body length V_{field}/V_{lab}	log body length V_{field}/V_{lab}	egg number V_{field}/V_{lab}
P2	$F_{155}^{139} = 10.13$	$F_{155}^{139} = 5.62$	$F_{155}^{139} = 15.89$
P4	$F_{75}^{119} = 17.43$	$F_{75}^{119} = 7.00$	$F_{75}^{119} = 68.20$
P5	$F_{163}^{108} = 7.91$	$F_{163}^{108} = 3.97$	$F_{163}^{108} = 31.39$

Table 8 : Comparison of lab and field variances of body length and
number (% variation) in P2, P4, and P5.

population	body length V_{lab}/V_{field}	log body length V_{lab}/V_{field}	egg number V_{lab}/V_{field}
P2	9.9%	17.8%	6.3%
P4	5.7%	14.3%	1.5%
P5	12.6%	25.2%	3.2%

of variances of log values also indicate significantly less relative variation (14 - 25%) in lab populations than field populations (Table 8).

These data suggest that a large amount of observed variation in a natural population is attributable to non-genetic factors: age differences among individuals and environmental heterogeneity.

This is similarly true in comparing mean values of length and egg number in lab and field populations (Figures 7 and 8). There were significant differences in mean body length and mean egg number in the lab and field populations from each pond. Means, variances and 95% confidence intervals are given in Table 9 for the lab populations. Lab reared individuals were on the average smaller with fewer eggs than field individuals. This again may be due to age differences among individuals particularly as older individuals tend to have larger clutches than primaparous adults. The reduction in body length and egg number may also be due to environmental differences particularly if the lab environment is poorer than the field environment.

Figure 7: Means and 95% confidence limits for body length in field (•) and lab (▽) populations from P2, P4, and P5.

Body Length mm

40
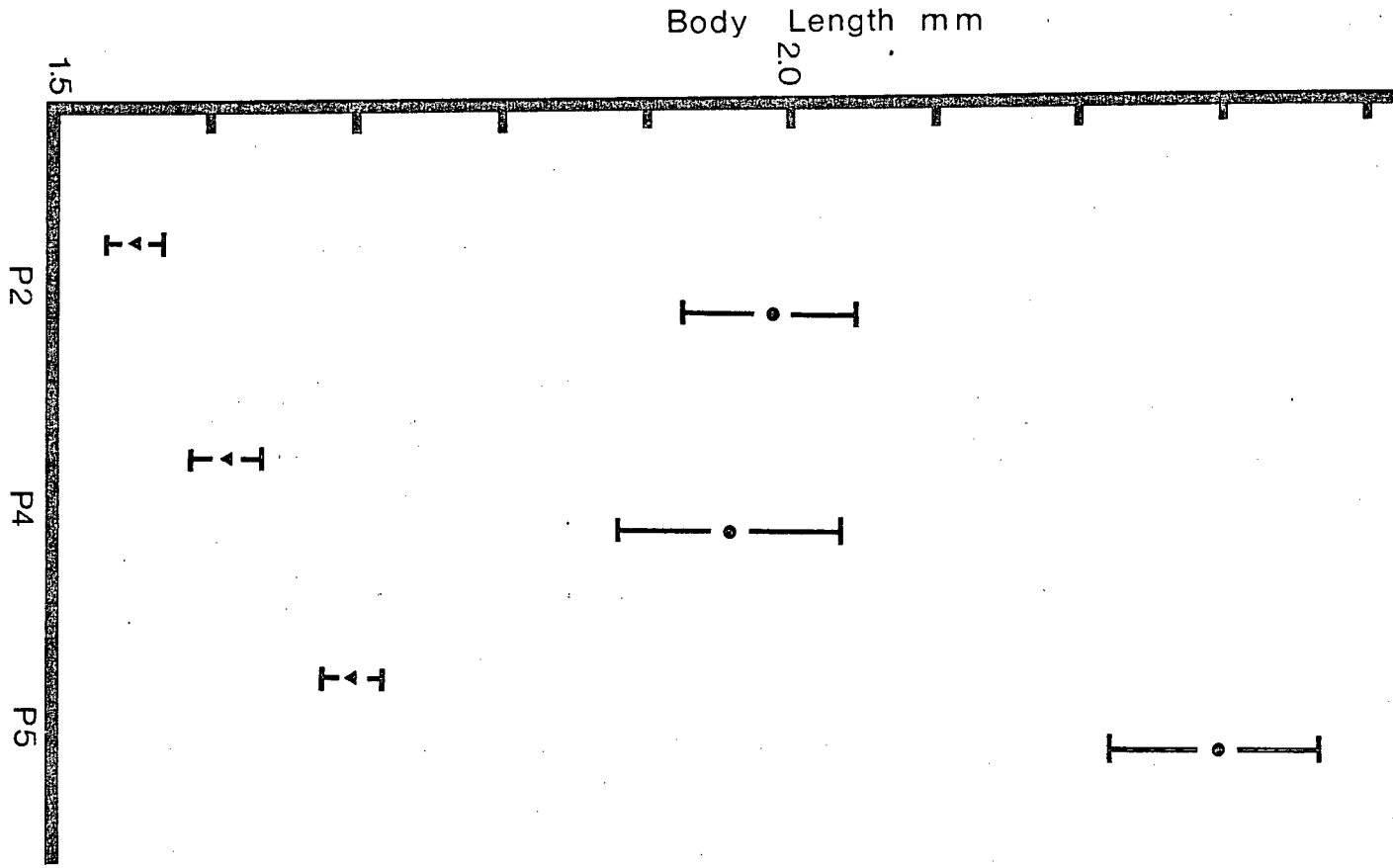
Figure 8: Means and 95% confidence limits for egg number in field (•) and lab (▽) populations from P2, P4, and P5.

Egg Number

0   5   10   15   20

P2

P4

P5

41
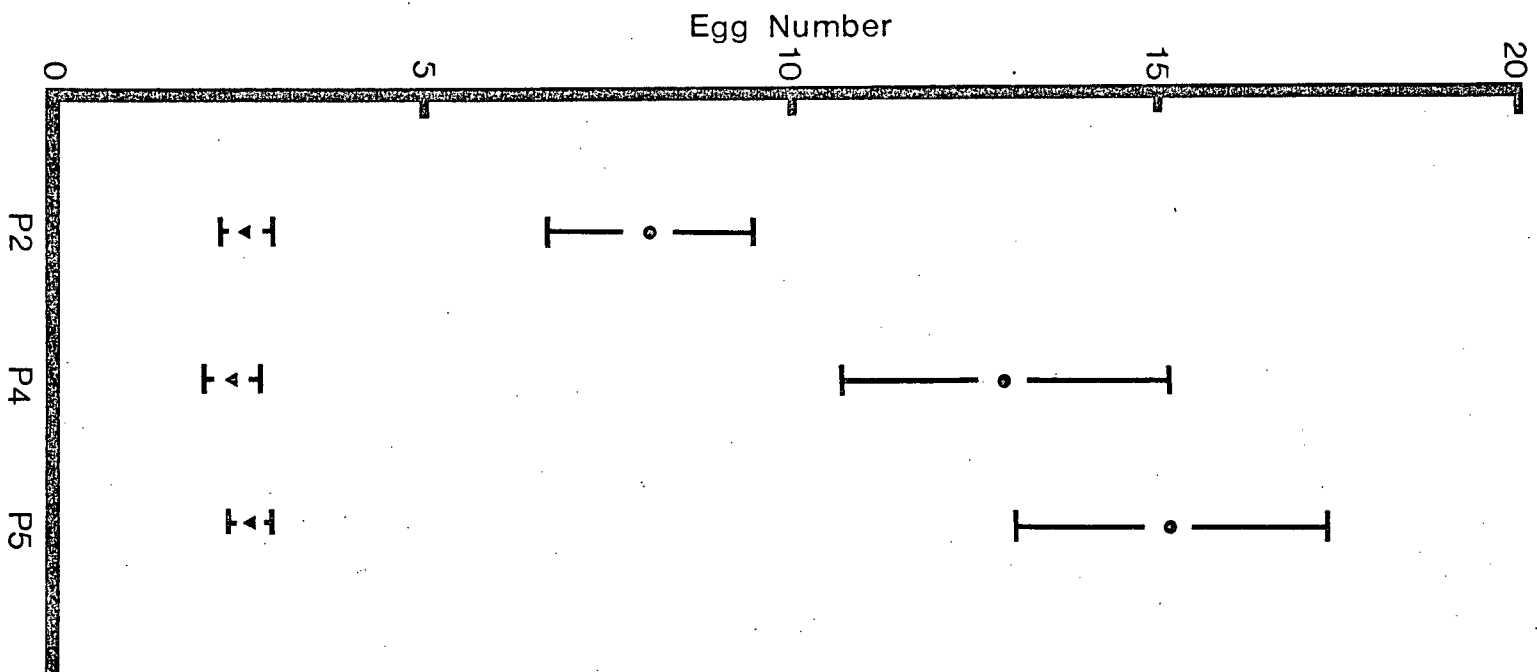
Table 9 : Estimates of the mean, variance, and 95% confidence intervals for P2, P4, and P5 lab populations. Sample sizes are indicated in parentheses.

	P2 (155)	P4 (76)	P5 (164)
body length	$1.56 \times 10^3 \pm 18$ 13.51	$1.62 \times 10^3 \pm 25$ 12.61	$1.71 \times 10^3 \pm 20$ 17.01
log length	$3.19 \pm .006$ 1.10×10^{-3}	$3.21 \pm .007$ $.90 \times 10^{-3}$	$3.23 \pm .006$ 1.12×10^{-3}
egg number	$2.67 \pm .340$ 4.69	$2.47 \pm .342$ 2.31	$2.75 \pm .312$ 4.14
log egg number	$.106 \pm .109$ .479	$.223 \pm .119$ .279	$.185 \pm .101$ .425
variable 1	$-.045 \pm .011$ .005	$-.004 \pm .016$ .005	$.046 \pm .013$ .007
variable 2	$-.021 \pm .005$ .001	$-.027 \pm .008$ .001	$.008 \pm .009$ .004
variable 3	$.025 \pm .006$ .002	$-.007 \pm .008$ .001	$-.021 \pm .007$ .002

Comparison Of P2, P4, And P5: Lab Data

Since age differences and environmental effects are responsible for 70 to 90% of the observed phenotypic variation in field animals, one might expect a convergence of mean lengths and egg number among the three populations if P2, P4, and P5 are genetically similar as suggested by the electrophoresis.

This is true for mean egg number in which there were no significant differences among populations. Egg number converges to a common mean of 2.7 eggs/female (Table 9). Mean body lengths among populations, P2, P4, and P5, however, differ significantly. This lack of convergence of body length may be explained by a residual maternal effect on body length to environmental change or to genetic differences among populations undetected by electrophoresis. The differences in egg number and body length suggest intrinsic differences in each characters' ability to respond to changes in the environment. It seems possible that body length may be insensitive to immediate environmental change whereas egg number may be very sensitive to the immediate environment and closely associated with the physiology of the parent.

If this is the case one might expect a convergence of mean body lengths only after a number of broods. This has not been demonstrated in P2 or in NR in four generations in the lab. In comparisons of mean length in each population in two separate experiments neither P2 nor NR showed any change in mean length

between experiments: likewise there was no convergence to a common mean. It may be argued that this was not long enough for the populations to respond to the change in the environment or that there are such large differences in the two populations that it is unreasonable to expect any convergence. More likely, however, the wide range of phenotypes reflects a lack of rigorous selection in the lab environment. There is nothing which suggests that a single genotype codes for a single phenotype or a constant fitness (Kojima, 1971) in any given environment, particularly if the expression of the genotype is fairly plastic.

Differences in mean length among populations may alternatively be due to genetic differences undetected by electrophoresis. This possibility will be further considered in the next section based on comparisons of inter- and intraclonal variation.

Comparisons Of P2, P4, And P5: Inter And Intraclonal Variation

Speculation on the source and maintenance of phenotypic variation in these organisms has relied on explanations of environmental and electrophoretic variation in comparison of lab and field populations. Since neither of these explanations is sufficient to account for all phenotypic variability among populations it is necessary to look at phenotypic variation at a finer level of resolution, within clones, where the genotypes of siblings is known.

Inter- and intraclonal variation is measured in P2, P4, and P5 and the analysis is described in five sections: 1) principle components analysis (PCA), 2) comparison of means for clones within each population, 3) comparison of means among populations, 4) calculation of components of variation from a one way ANOVA to determine % variation and absolute variation within and among clones in each population, and 5) calculation of the components of variation from a nested ANOVA to determine % variation and absolute variation within and among clones and among populations.

Ten to twenty siblings from each of 18 to 21 clones from P2, P4, and P5 were reared in the lab in individual vials and measured at the first reproductive instar for the six characters previously described. The morphological data was pooled by PCA and body length, egg number, and the three PCA variables were used to compare the three populations.

Principle components analysis pooled the morphological measures into three variables accounting for 95% of the total variation. Variable 1 is a measure of body length, body width, and head diameter, and is therefore an indicator of body size and shape. Variables 2 and 3 are composed of variation due to length of tail spine and eye diameter which are presumably ecologically important with respect to predator avoidance (Brooks and Dodson, 1965; Zaret, 1972; Dodson, 1974). The PCA variables and body length were used as measures of morphological variation rather than the individual morphological characters in

all further analyses.

Each character was compared among clones within each population using a one-way ANOVA and there were significant differences among clones within each population for all characters (Figure 9). This result is completely unpredicted by the electrophoretic data. That there are real phenotypic differences among electrophoretically identical clones suggests 1) that there may be genetic differences among clones or 2) that maternal effects among clones are sufficient to produce significant differences among clones.

Each character was also compared among the three populations and there were significant differences in body length and the three PCA variables among populations. There was no significant difference in mean egg number among P2, P4, and P5. As suggested in the previous section these differences may be due to the rate of response to changes in the environment or to genetic differences. Information on the comparison of electrophoretically identical clones seems to support the idea that there may be genetic differences undetected by electrophoresis which are responsible for the differences in phenotype.

Components of variation (Becker, 1967; Sokal and Rohlf, 1969) from a Model II ANOVA were computed for each character and provide estimates of the % variation attributable to variation within and among clones. Less variation is expected among in individuals within clones (which are genetically identical) than in individuals among clones, which although they are

Figure 9: Means and 95% confidence limits for the three principal component variables of P2, P4, and P5.
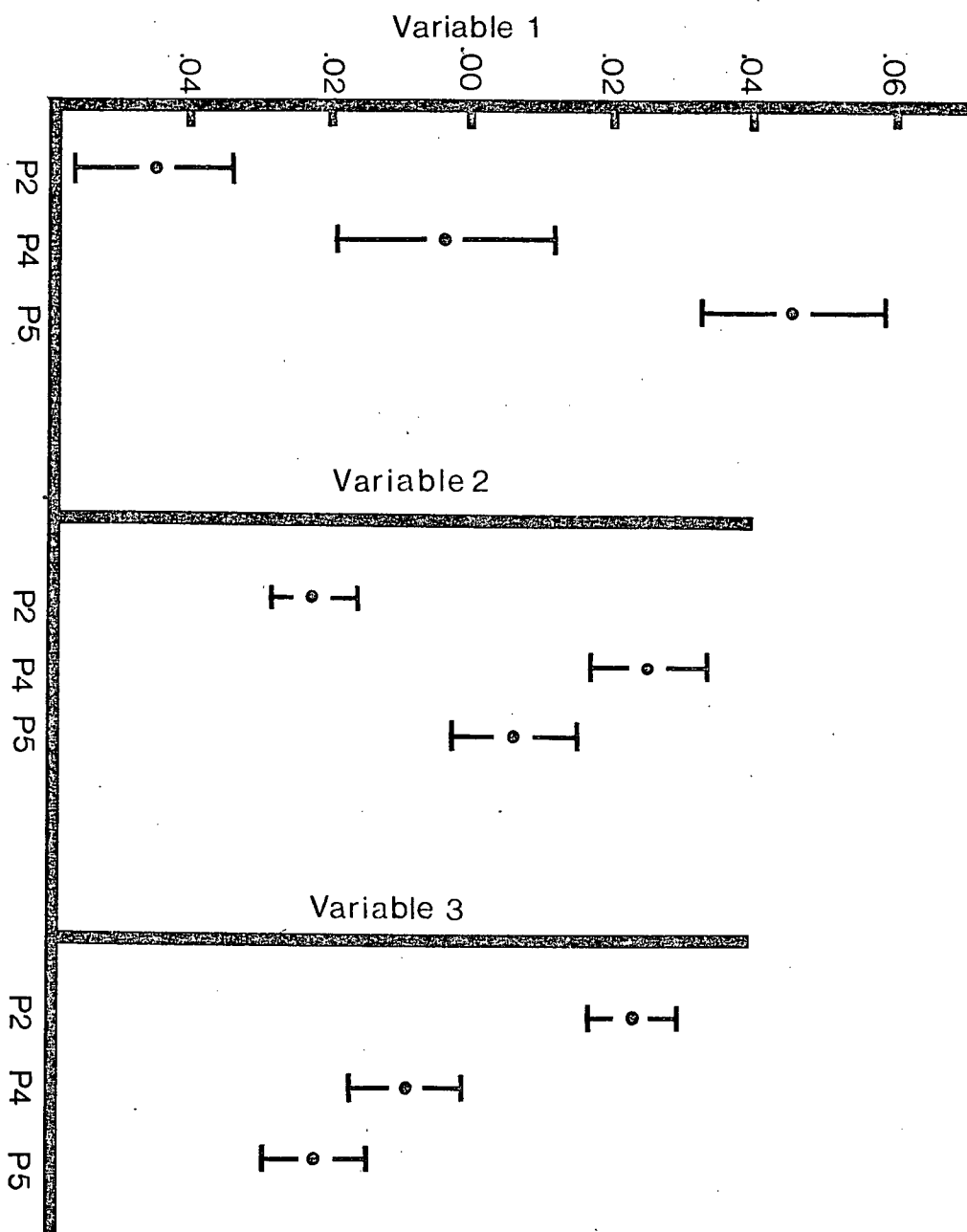
electrophoretically identical, are not necessarily genetically identical.

This expectation is not supported, however, in comparisons of variation within and among clones. With the exception of body length and body size (V1) in P4 the greater % variation for each character occurs within clones rather than among clones (Table 10). This large variation within clones may be a function of the statistical technique in that the within clone variation is the residual term of the ANOVA and includes any unexplained variation (experimental error) as well as any 'true' within clone variation. The within clone variation however, may actually represent a large degree of variation among genetically identical individuals due to non-genetic factors such as maternal effects or microhabitat differences among vials affecting development.

A large amount of phenotypic variation even among genetically identical sibs independent of statistical biases, may reduce the importance of the mean differences among electrophoretically identical clones described in the previous section. Based on the similarity within and among clones one would expect similar amounts of inter- and intraclonal phenotypic variation. Comparisons of the phenotypic variation within and among clones by an F test suggest there is no significant difference in inter- and intraclonal variation for several characters, supporting the electrophoretic data which infers that individuals within and among clones are electrophoretically identical.

Table 10 : Comparison of within and among clone variation in P2,
P4, and P5. Variances and % variation are given.

	P2		P4		P5	
body length*						
within	8.59	(62.4%)	5.40	(41.6%)	11.75	(68.0%)
among	5.17	(37.6%)	7.58	(58.4%)	5.54	(32.0%)
egg number						
within	3.06	(64.2%)	1.68	(72.0%)	2.92	(69.5%)
among	1.71	(35.8%)	.65	(28.0%)	1.28	(30.5%)
variable 1						
within	3.60×10^{-3}	(67.0%)	2.03×10^{-3}	(39.8%)	4.80×10^{-3}	(69.9%)
among	1.77×10^{-3}	(33.0%)	3.07×10^{-3}	(60.2%)	2.07×10^{-3}	(30.1%)
variable 2						
within	$.814 \times 10^{-3}$	(69.3%)	$.954 \times 10^{-3}$	(80.6%)	3.18×10^{-3}	(85.3%)
among	$.36 \times 10^{-3}$	(30.7%)	$.23 \times 10^{-3}$	(19.4%)	$.55 \times 10^{-3}$	(14.7%)
variable 3						
within	1.50×10^{-3}	(86.7%)	$.902 \times 10^{-3}$	(72.8%)	1.56×10^{-3}	(71.9%)
among	$.23 \times 10^{-3}$	(13.3%)	$.35 \times 10^{-3}$	(26.2%)	$.61 \times 10^{-3}$	(28.1%)

* Body length is measured in microns and variances associated with
body length within and among clones are x 10^3.

To evaluate the amount of additional variation found among populations, components of variation were also determined from a nested ANOVA partitioning variances for each character within and among clones and among populations. For all characters the greatest % variation again occurs within clones and with the exception of egg number, % variation among populations exceeds the variation among clones (Table 11). The amount of variation within and among clones is qualitatively similar to the amount of variation from the one-way ANOVA. Again, the variation within clones may be a function of the ANOVA, in which the residual term includes both non-genetic and unexplained variation, or the variation within clones may be genetic. As suggested by the electrophoresis these populations may be very similar, in which case one would expect comparable amounts of morphological variation among clones and among populations. Hence, even though populations differed in mean values for all characters (except egg number) they appear to be very similar in the amount of variation within and among clones and populations.

Table 11: A comparison of variation within and among clones and among populations P2, P4, and P5. Variances and % variation are listed for each character.

	variation within clones		variation among clones		variation among populations	
body length *	9.08	(41.0%)	5.66	(25.5%)	7.42	(33.5%)
egg number	2.75	(68.5%)	1.26	(31.5%)	<0	(0.0%)
variable 1	3.81×10^{-3}	(45.1%)	2.18×10^{-3}	(25.8%)	2.45×10^{-3}	(29.1%)
variable 2	1.86×10^{-3}	(67.5%)	$.374 \times 10^{-3}$	(13.5%)	$.523 \times 10^{-3}$	(19.0%)
variable 3	1.42×10^{-3}	(57.9%)	$.390 \times 10^{-3}$	(15.9%)	$.645 \times 10^{-3}$	(26.2%)

* variances associated with body length within and among clones and among populations are $\times 10^{3}$.

Comparison Of P2, P4, And P5: Summary

P2, P4, and P5 are small, physically similar ponds located within 10 m of one another in Langley, B.C. All individuals from these three ponds are electrophoretically monomorphic at the 16 loci examined. Results based on comparisons of field , lab and field , and lab populations are summarized in Table 12.

The differences in mean values for all phenotypic characters (except egg number in populations) 1) among field populations, 2) among lab populations, and 3) among clones within each population all suggest there are real differences inherent within and among populations which are not consistent with the electrophoretic data. Mean differences in phenotype may be largely attributed to environmental differences among field populations, although, this is not a practical explanation in lab reared individuals. Explanations for these differences have been tentatively suggested as due to 1) slow rate of change of morphological characters in response to the lab environment, 2) a large degree of phenotypic plasticity, or 3) genetic differences undetected by electrophoresis. Comparisons of variances indicate significantly less variation in lab populations than field populations, presumably due to environmental effects and age differences among field individuals. A large variance persists, however, even among genetically identical sibs within clones in lab reared populations. This suggests that these individuals are capable of a wide range of phenotypic expression from a single genotype .

Table 12: Summary of the results from comparisons of P2, P4, and P5.

P2	P4	P5
	FIELD DATA sig. differences in length and egg number among pop.	
FIELD/LAB DATA reduced variances and means for length and egg number in lab population	FIELD/LAB DATA reduced variances and means for body length and egg number in lab pop.	FIELD/LAB DATA reduced variances and means for length and egg # in lab pop.
LAB DATA electrophoretically homogeneous	LAB DATA electrophoretically homogeneous	LAB DATA electrophoretically homogeneous
	mean body length and PCA variables sig. dif. among populations	
	no sig. dif. in egg number among populations	
sig. dif. among clones for all characters	sig. dif. among clones for all characters	sig. dif. among clones for all characters
greatest % variation within clones for all characters	greatest % variation within clones for egg number,V2 and V3 greatest % variation among clones for length and V1	greatest % variation within clones for all characters
	greatest % variation within clones except egg number (nested ANOVA) variance among clones less than among pop. % variation within clones greater than among pop. which is greater than among clones	

The previous comparisons of P2, P4, and P5 are justified by the relative similarity of their electrophoretic data and the similarity of the physical environment among ponds. The genetic data, particularly the electrophoresis and the comparisons of variances, and the apparent phenotypic variation suggest these populations are individually buffered and respond to changes in the environment not by utilizing genetic heterogeneity but rather by exhibiting phenotypic plasticity. This conclusion will be discussed relative to the NR data and relative to the stability of the P2, P4, and P5 environments in the final discussion.

Comparison Of P2 And NR

Comparisons of P2 and NR are similarly justified by electrophoretic differences between populations and physical and geographic differences between ponds to determine if there is more or less phenotypic variation in an electrophoretically variant population than an elelctrophoretically invariant population. That is, do electrophoretically variant populations rely on large amounts of phenotypic variation or on genotypic variation to adapt to environmental change?

Twenty sibs from each of five clones from P2 and NR were reared in separate vials under controlled lab conditions. Six characters were measured at the first reproductive instar: body length, body width, head diamter, eye diamter, length of tail

spine, and number of eggs. Morphological characters were pooled by principal components analysis. Body length and egg number were also measured once a week in these animals until 1/2 of both populations had died. These latter measurements provide estimates of six additional parameters: 1) growthrate, 2) total number of eggs/female, 3) total number of juveniles/female, 4) total number of broods/female, 5) eggs/brood, and 6) % mortality ((eggs-juveniles)/eggs). Since total eggs and total juveniles is influenced by the number of broods produced by any female it seems that these characeters, although important in evaluating the fitness of the individual and of the clone, may overestimate variation. For this reason it seems practical to consider ratios of eggs/brood and % mortality as better indicators of actual variation. Body length was measured each week for each animal and plotted against log time. Growthrates were then determined from the slope of the line.

Results will be presented and discussed for comparisons within each population and for comparisons between populations.

Comparisons Of P2 And NR: Intrapopulation Results For P2

Results and interpretation of the P2 data in this experiment are similar to those described previously in comparisons of P2, P4, and P5. 1) _Daphnia_ from P2 were electrophoretically monomorphic and identical to animals in the previous experiment. 2) In the primaparous instar data, a comparison of means among clones in P2 indicated significant differences in length, egg number, and the three PCA variables although the mean values in this experiment tended to be larger than in the previous experiment (Table 13) perhaps due to food quality. In comparing interclonal variation of growthrates, number of broods/female, total eggs, and total juveniles, number of eggs/brood, and % mortality in P2 all characters differed significantly among clones except growthrate and % mortality. From analysis of the components of variation the % variation was significantly greater for egg number, V2 and V3, number of broods, total eggs, total juveniles, and % mortality within clones than % variation among clones (Tables 14 and 15). The interpretation of the results described previously is also applicable to these data.

Table 13 : Estimates of means, variances, and 95% confidence intervals for P2 and NR.

	P2		NR	
	means	variances	means	variances
body length	$1.63 \times 10^{3} \pm 32$	21.66×10^{3}	$2.19 \times 10^{3} \pm 39$	35.33×10^{3}
log length	$3.21 \pm .008$	1.52×10^{-3}	$3.34 \pm .008$	1.45×10^{-3}
egg number	$5.46 \pm .63$	8.40	$7.68 \pm .64$	9.36
log egg number	$.673 \pm .054$	.062	$.849 \pm .038$	.033
variable 1	$-.135 \pm .015$	.005	$.127 \pm .018$	.007
variable 2	$.021 \pm .015$	.005	$.020 \pm .015$	.005
variable 3	$-.003 \pm .005$	.001	$.003 \pm .008$	.001
growthrates	990.9 ± 19.45	31067.4	1361 ± 85.52	164672.7
log growthrates	$2.9 \pm .02$	.01	$3.1 \pm .02$	.01
number broods	$7.64 \pm .52$	5.81	$10.74 \pm .61$	8.53
log broods	$.86 \pm .035$	.027	$1.01 \pm .034$	.026
total eggs	57.99 ± 5.56	661.7	201 ± 16.04	5827.4
log eggs	$1.70 \pm .056$	.067	$2.26 \pm .047$	.051
total juveniles	53.03 ± 5.24	586.3	182.9 ± 13.39	4394.6
log total juv.	$1.66 \pm .062$	.083	$2.22 \pm .045$	.046
eggs/brood	$7.71 \pm .649$	9.01	$18.24 \pm .795$	14.32
log eggs/brood	$.848 \pm .044$	.041	$1.25 \pm .021$	.009
% mortality	$.098 \pm .019$	.008	$.081 \pm .009$	.002
log % mortality	$-1.44 \pm .221$	1.05	$-1.26 \pm .136$	.416

Table 14: Comparisons of within and among clone variation in P2 and NR. Components of variation and % variation are given for each character and sample size indicated in parentheses.

	P2		NR	
	within	among	within	among
body length	13.45×10^3	10.35×10^3	29.59×10^3	5.83×10^3
	56.5%	45.5%	80.7%	19.3%
egg number	6.31	2.60	9.10	0.32
	70.8%	29.2%	96.6%	3.4%
variable 1	2.94	2.75	6.27×10^{-3}	1.22×10^{-3}
	56.6%	43.4%	83.7%	16.3%
variable 2	4.01×10^{-3}	0.77×10^{-3}	3.66×10^{-3}	1.97×10^{-3}
	95.6%	4.6	65.0%	35.0%
variable 3	0.46×10^{-3}	0.10×10^{-3}	1.23×10^{-3}	0.17×10^{-3}
	81.5%	18.5%	87.6%	12.4%
growthrates	144780	24655	31982	−1152
	85.4%	4.6%	100.0%	0.0%
# broods	7.90	.78	5.14	.85
	91.0%	9.0%	85.9%	14.1%
total # eggs	5243.3	724.1	528.8	241.6
	87.9%	12.1%	68.6%	31.4%
total # juveniles	3985.4	507.3	464.2	153.8
	88.7%	11.3%	75.1%	24.9%
eggs/brood	12.89	1.77	5.47	4.45
	87.9%	12.1%	55.1%	44.9%
%mortality	.0018	.0000	.0077	.0006
	96.3%	3.7%	93.2%	6.8%

Table 15: Ratio of variances within and among clones in P2 and NR from untransformed and logarithmically transformed data.

	P2 F(81/81) V_{within}/V_{among}	NR F(86/86) V_{within}/V_{among}
body length	1.3	4.17*
log body length	1.2	4.00*
egg number	2.43*	28.4*
V1	1.26	5.25*
V2	5.00*	1.80*
V3	5.00*	6.00*
growth rate	-27*	5.87*
log growth rate	-32*	4.30*
# broods	6.05*	10.13*
log # broods	8.13*	8.50*
total eggs	2.18*	7.24*
log total eggs	4.35*	10.70*
total juveniles	3.01*	7.86*
log total juveniles	4.20*	13.90*
eggs/brood	1.23*	7.28*
log eggs/brood	1.61*	11.00*
% mortality	12.83*	division by 0
log % mortality	94.00*	219.4*

* $P < .05$

Comparisons Of P2 And NR: Intrapopulation Results For NR

Unlike P2, NR was electrophoretically polymorphic for 22 to 28% of all loci assayed. However, as in P2, there were significant differences among clones for all phenotypic characters except egg number and % mortality. Although there were significant differences in egg nummber among P2 clones, there were no differences in egg number among P2, P4, and P5, and the homogeneity of egg number is unique to this set of characters. Estimates of mean, variance, and confidence limits for the NR population are given for each character in Table 13. Interclonal differences may be related to electrophoretic differences among clones although this was shown not to be the case in P2, ie. clones in P2 differed phenotypically from one another even though they were electrophoretically identical. The variation in P2 was tentatively interpreted as genetic differences among clones or as phenotypic plasticity associated with the single genotype. NR individuals may also be highly variable, however, it seems more reasonable because of the electrophoretic heterogeneity to associate phenotypic variation with genotypic diversity.

These possibilities have been further explored in comparisons of inter- and intraclonal variability from components of variation. As in P2, the greatest % variation of the total variance in NR was within clones rather than among clones (Table 14) and, based on comparisons of absolute (not %) inter- and intraclonal variation by an F test, within clone

variation was significantly greater (P .05) than among clone variation for all characters (Table 15).

One might expect similar amounts of phenotypic variation within clones in P2 and NR, however, as suggested by the interclonal electrophoretic differences in NR there may be greater variation among clones in NR than in P2. Interpopulational comparisons of variances within and among clones between P2 and NR are described in the next section.

Interpopulation Comparisons Of P2 And NR: Means

Comparisons of means between P2 and NR indicate significant differences between populations for all characters. Only means of V2 and of % mortality were greater in P2 and NR. For all other characters NR was significantly larger than P2. Since there were significant differences among P2, P4, and P5 (populations which are electrophoretically and environmentally similar) these differences between NR and P2 are not unique or unexpected. The trend in differences is, however, more dramatic and unidirectional in comparisons of NR and P2. It seems possible that these differences may be genetic since the populations are electrophoretically dissimilar and since the phenotypic differences were maintained in the populations reared in the lab over several broods. It seems unlikely that these differences were environmentally induced. Alternatively these differences may have been due to a differential rate of change in characters in which animals are incapable of responding to

environmental changes in only a few generations.

Interpopulation Comparisons Of P2 And NR: Variances

Comparisons of variances include both comparisons of % variation and comparisons of absolute variation and it is important to make this distinction. Intrapopulational comparisons of variances within and among clones are based on comparisons of % variation or on comparisons of the absolute variances. All interpopulational comparisons are based on comparisons of the absolute variances of transformed and untransformed data. No statistical comparison of % variation has been made within and among clones between populations. Variances were compared with an F test (Table 16) and out of the comparisons of 11 untransformed characters only the variance associated with % mortality was greater in P2 than NR. Variances associated with untransformed body length, V3, growthrate, number of eggs/brood, and total eggs and total juveniles were greater in NR than P2. The remaining characters showed no significant differences in variances between populations. These differences in P2 and NR, however, do not mean that NR individuals were more variable in the essential zoological sense than P2 individuals: since NR individuals were significantly larger than P2 individuals it would be expected that variances would also be greater without any differences in functional variability (Simpson, Roe, and Lewontin, 1960). Lewontin, however, argues that by taking log transforms of the data, the

Table 16: Comparison of variances for NR and P2 (F test).
        Degrees of freedom: NR=86, P2=81

body length	* $F(86/81)=1.63$	log body length	$F(81/86)=1.05$
egg number	$F(86/81)=1.11$	log egg number	+ $F(81/86)=1.86$
variable 1	$F(86/81)=1.53$		
variable 2	$F(86/81)=1.14$		
variable 3	* $F(86/89)=2.54$		
growth rate	* $F(86/81)=5.30$	log growth rate	* $F(86/81)=1.62$
# of generations	$F(86/81)=1.47$	log # generations	$F(81/86)=1.02$
total # of eggs	* $F(86/81)=8.81$	log total eggs	$F(81/86)=1.33$
# of juveniles	* $F(86/81)=7.50$	log # juveniles	+ $F(81/86)=1.82$
# of eggs/genration	* $F(86/81)=1.59$	log eggs/gen.	+ $F(81/86)=4.31$
(eggs - juveniles)/eggs+	$F(81/86)=4.31$	log (eggs-juv)/eggs+	$F(81/86)=2.51$

* P < .05 NR being greater
+ P < .05 P2 being greater

variances, regardless of the mean, are put on the same scale and can be compared statistically. To estimate relative variability independent of mean differences between populations log transforms of the P2 and NR data were compared with an F test in the two populations (Lewontin, 1966). In sharp contrast to comparisons of the original data, P2 was relatively more variable than NR for egg number at the first reproductive instar, total number of juveniles/female, eggs/brood, and % mortality. In the eight transformed values of the total variances of log values of body length, total number of broods, and total eggs did not vary significantly between the two populations. Only in comparing relative growthrates was NR significantly more variable than P2 (Table 16).

Having compared the total variances between P2 and NR these variances were partitioned into components of variation and inter- and intraclonal variances of transformed data compared between populations with an F test. In comparisons of within clone variances growthrates and body length were more variable in NR than in P2 although the differences were not statistically significant at P<.05. In all other characters P2 was more variable than NR within clones. Intraclonal variation in P2 differs significantly from NR in total juveniles, eggs/brood and % mortality. There was no significant difference in number of broods/female or total eggs/female (Table 17).

In comparisons of interclonal variation there was a signficantly greater variance in growthrate in NR than P2. For all other characters except number of broods/female P2 was sign

Table 17: F tests comparing relative variances from transformed data within and among clones between populations P2 and NR. Degrees of freedom for P2 = 81 and for NR = 86.

	within clones	among clones
log body length	$F(86/81) = 1.32$	$F(81/86) = 2.53$ *
log growth rates	$F(86/81) = 1.36$	$F(86/81) = -9.0$ **
log # of broods	$F(81/86) = 1.02$	$F(81/86) = 1.07$
log total eggs	$F(81/86) = 1.21$	$F(81/86) = 2.98$ *
log total juvenile	$F(81/86) = 1.65$	$F(81/86) = 4.00$ *
log eggs/brood	$F(81/86) = 3.13$	$F(81/86) = 21.25$ *
log % mortality	$F(81/86) = 2.48$	$F(81/86) = -5.68$ *

* P2 significantly more variable than NR at $P < .05$
** NR significantly more variable than P2 at $P < .05$

ificantly more variable than NR among clones (Table 17).

Based on comparison of total variances and of intra- and interclonal variances, P2 was generally more variable than NR even though there was no electrophoretic variation in the P2 population. These results seem to corroborate the earlier suggestion that P2 is individually homeostatic with a single very flexible genotype and a great deal of phenotypic plasticity. Conversely NR with greater genetic variation and less phenotypic variation than P2 may adapt to the environment by populational homeostasis although no experiments have been done that would demonstrate differential fitnesses of the genotypes in different environments. NR was also capable of some phenotypic flexibility as evidenced by the large % variation within clones and it is difficult to explain adaptation in NR individuals by individual or populational buffering exclusively.

The untransformed variances in P2 and NR are further partitioned within and among clones and between populations in Table 18 in order to look at overall sources of variation irrespective of the particular population. The components of variation from a nested ANOVA indicate that the greatest % variation was either within clones or between populations in contrast with the comparisons of P2, P4, and P5 where the greatest variation for all characters was within clones. This result implies greater differences between P2 and NR for body length, V1, total eggs, total juveniles, and number of eggs/brood than among P2, P4, and P5 and,although comparisons of % variation do not indicate statistically the magnitude of these

Table 18: Comparison of variation within and among clones and between populations (P2 and NR) for primaparous instar. Variances and % variation (in parentheses) are listed.

	variation within clones		variation among clones		variation between pop.	
body length	21.77	(11.53%)	8.65	(4.58%)	158.41	(83.89%)
egg number	7.751	(68.95%)	1.4215	(12.64%)	2.07	(18.41%)
variable 1	.0047	(11.59%)	.00171	(4.27%)	.0338	(84.14%)
variable 2	.0038	(66.70%)	.00139	(24.22%)	.00052	(9.08%)
variable 3	.0009	(86.05%)	.00139	(13.95%)	-.00002	(0.00% (neg.))
growth rate	94976	(55.07%)	12888.6	(7.47%)	64599	(37.46%)
number of eggs	3161.6	(23.05%)	462.21	(3.37%)	10093	(73.58%)
# of generations	6.6852	(55.64%)	.7975	(6.64%)	4.5317	(37.72%)
# of juveniles	2430.6	(21.90%)	339.07	(3.05%)	8329.7	(75.05%)
# eggs/generation	9.6169	(14.30%)	2.9251	(4.35%)	54.70	(81.35%)
(eggs-juveniles)/eggs	.0044	(92.89%)	.00031	(6.46%)	.00003	(0.65%)

differences, they do suggest that there are real differences between P2 and NR and real similarities among P2, P4, and P5.

Comparison Of P2 And NR: Summary

The electrophoretic and environmental differences between populations form the rationale for the comparison of P2 and NR. The two populations differed electrophoretically from one another; P2 was monomorphic and NR polymorphic. Results are summarized in Table 19.

Mean values differed significantly among clones for most characters in P2 and NR independent of the presence or absence of electrophoretic variation. There were significant interclonal differences for all mean characters except growthrate and % mortality in P2 and egg number and % mortality in NR. As in comparisons of P2, P4, and P5 there were significant differences between populations for mean values of all characters. Differences among clones in each population were presumably due to maternal effects and related to the physiology of the female or to genetic differences among clones. The latter explanation is particularly convincing in NR since there were electrophoretic differences among clones. Mean population differences may be due to 1) differences in the physical and geographic environment, associated with 2) the ability of morphological characters to respond to changes in the environment over a short time period coupled with a lack of strong directional selection in the lab or 3) potential genetic

Table 19: A summary of the genetic data from P2 and NR.

P2	NR
electrophoretically homogeneous monomorphic	electrophoretically polymorphic heterogeneous
sig. difference in mean values of all characters among clones	sig. difference in mean values of all characters among clones

sig. differences in mean values for all characters between P2 and NR

greater % variation within clones than among clones	greater % variation within clones than among clones

P
P2 greater or similar variance for all characters except growth rate

greater % variation within clones in NR than in P2

greatest % variation is either within clones or between pop.

differences, electrophoretic or otherwise, among populations.

Components of variation based on a 1-way ANOVA in each population indicate greater % variation within clones than among clones in both P2 and NR. A comparison within NR of absolute inter- and intraclonal variances indicate all characters are significantly more variable within clones than among clones (P<.05). Within P2 there was significantly greater intraclonal variation than interclonal variation for all characters except body length and body size (V1) and for egg number/brood. A comparison of the total variance, interclonal variance, and intraclonal variance between populations from transformed data indicates P2 was relatively more variable for more characters than NR. A comparison of the % variation and absolute variation within each population however suggests the populations are partitioning the total variance differently within and among clones. There are similar amounts of variation within and among clones in P2 whereas the greater variation in NR is consistently within clones.

Comparisons of components of variation in P2, P4, and P5, and between P2 and NR indicate that a much greater % of the total variatnce is accounted for between populations of P2 and NR than among P2, P4, and P5. This supports the hypothesis, based on the electrophoretic and environmental data, that P2, P4, and P5 are more similar than P2 and NR and suggests the two populations may rely on different adaptive strategies balancing genotypic and phenotypic variation.

Comparison Of P2 And NR: Temperature Experiment

Although all experiments previously described here have been carried out in a single environment, all references to plasticity have referred to the ability of the organism to survive and reproduce in a range of environments. Previous experiments describe P2 as individually buffered with little genetic variation and large amounts of phenotypic variation compared to NR. NR on the other hand shows similar or less phenotypic variation and is electrophoretically polymorphic. How do these populations respond to different environments?

To further evaluate the plasticity of P2 and NR eight to ten siblings from each of ten clones from P2 and NR hatched at 15 C were reared at 10, 15, and 20 C in separate vials. Morphological characters and fecundity at first reproduction were measured and recorded and the morphological characters pooled by PCA (Table 20).

Since Daphnia are poikilothermic, any change in temperature in the external environment would have an effect on the rate of enzyme reactions. It has been repeatedly demonstrated that temperature is an important environmental parameter influencing feeding, growth, and egg production rates in these organisms and one might expect variation in the ability to respond to temperature changes related to the flexibility of the organism.

Analyses of variance were used to compare differences among clones within each population at each temperature treatment.

Table 20: Means and variances for morphological and reproductive characters from P2 and NR reared at three temperatures.

		10 C			15 C			20 C	
	N	mean	variance	N	mean	variance	N	mean	variance
P2									
body length *	33	1.857	11.00	50	1.519	10.47	35	1.543	9.78
log length	33	3.268	.04105	50	3.180	.00084	35	3.187	.00078
egg number	33	7.212	5.9849	50	3.080	1.7077	35	4.086	3.080
log egg number	33	.8233	.041047	50	.4611	.03456	35	.5753	.0360
variable 1	33	.00021	.00261	50	-1.454	.00139	35	-.1456	.00141
variable 2	33	.09574	.00346	50	-.00025	.00179	35	-.01576	.00158
variable 3	33	-.01522	.000635	50	.01107	.00051	35	.00197	.00060
NR									
body length *	35	2.192	25.11	47	2.241	22.03	45	2.180	27.16
log body length	35	3.340	.000939	47	3.349	.00087	45	3.305	.00095
egg number	35	7.429	9.19302	47	6.787	4.736	45	5.511	3.028
log egg number	35	.8313	.03884	47	.8074	.02304	45	.7106	.0346
variable 1	35	.08553	.00388	47	.1115	.00278	45	.09170	.00319
variable 2	35	.00720	.00241	47	-.0171	.00186	45	-.04540	.00162
variable 3	35	-.01558	.00105	47	.01047	.00178	45	-.001487	.00294

* Means and variances associated with body length within and among clones and among populations are x 10^3.

Unlike the previous experiments, there was no significant mean difference among clones in P2 and NR. There was no significant interclonal variation in P2 animals in body length, the three PCA variables, and in egg number at 10 and 15 C. However P2 clones did differ from one another in number of eggs. There was no significant difference among NR clones except in clones reared at 10 C which were significantly different at variables 2 and 3. The contrast of these results with those mentioned previously may be accounted for by the smaller sample sizes in this experiment.

Analyses of variance were also used to compare differences within each population at the three temperatures. P2 replicates at the three temperatures differed significantly from one another for all morphological and reproductive characters. Egg number and variables 2 and 3 were significantly different in the NR replicates. However, unlike P2 there were no differences in mean body length or mean body size (V1) in NR across temperatures (Table 20).

P2 and NR showed extremely different responses to environmental differences. These differences are consistent with other P2 and NR data and will be further discussed in the final discussion.

Differences between P2 and NR at each temperature were also determined by analyses of variance. Means and variances for P2 and NR were also compared to one another at each temperature and the data are summarized in Table 21 and Figures 10 and 11. NR individuals are significantly larger than P2 individuals at all

temperatures. However, a comparison of variances of the log length indicates neither population is significantly more variable.

Table 21: Comparison of means and variances in P2 and NR at three temperatures.

	10 C		15 C		20 C	
	MEANS	VARIANCES	MEANS	VARIANCES	MEANS	VARIANCES
body length	*	$F(34/32) = 2.28$ *	*	$F(46/49) = 2.11$	*	$F(44/34) = 2.78$*
log body length	ns	$F(34/32) = 1.49$	ns	$F(46/49) = 1.03$	ns	$F(44/34) = 1.22$
egg number	ns	$F(34/32) = 1.54$	*	$F(46/49) = 2.77$ *	ns	$F(34/44) = 1.02$
log egg number	-	$F(32/34) = 1.06$	-	$F(49/46) = 1.50$	-	$F(34/44) = 1.04$
variable 1	ns	$F(34/32) = 1.49$	*	$F(46/49) = 2.00$	*	$F(44/34) = 2.26$*
variable 2	*	$F(32/34) = 1.44$	ns	$F(46/49) = 1.03$	*	$F(44/34) = 1.03$
variable 3	ns	$F(34/32) = 1.65$	ns	$F(46/49) = 3.46$ *	ns	$F(44/34) = 4.91$*

* $P < .05$
ns not significant

	TEMP		
	10	15	20
D.F. of P2	32	49	34
D.F. of NR	34	46	44

77

Figure 10: Means and 95% confidence limits for body length in P2 (º) and NR (■) <u>Daphnia</u> reared at three temperatures.

79
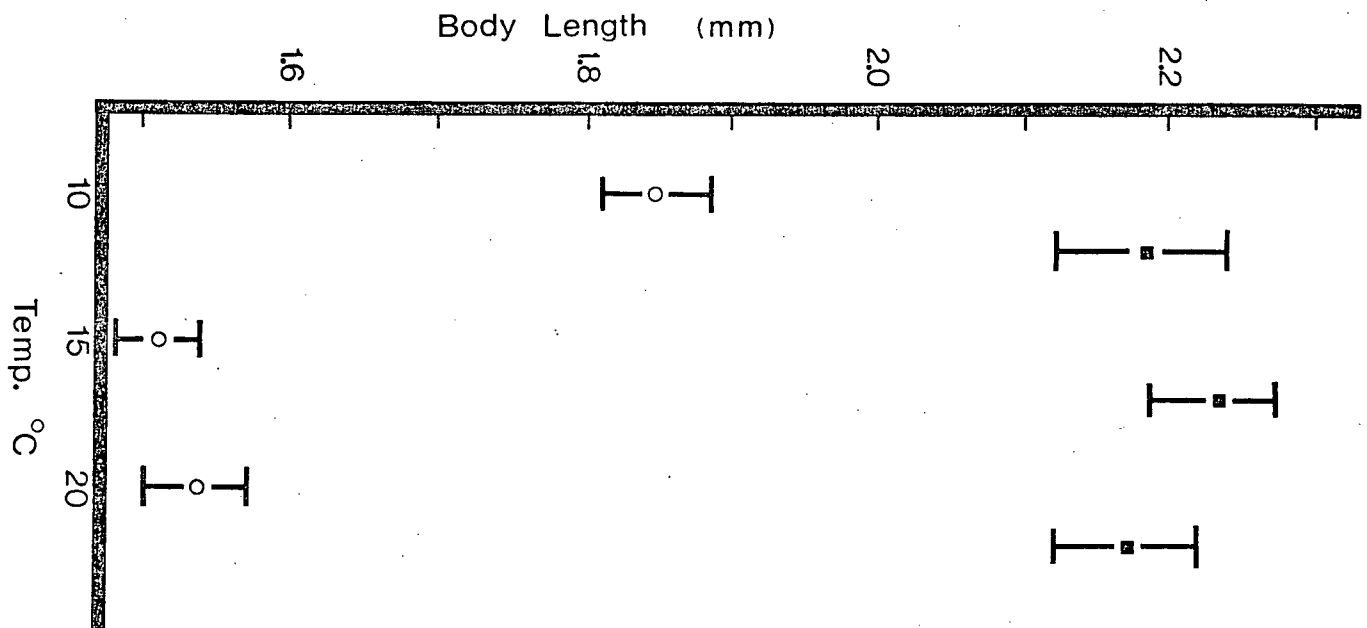
Figure 11: Means and 95% confidence limits for egg number in P2 (°) and NR (■) <u>Daphnia</u> reared at three temperatures.
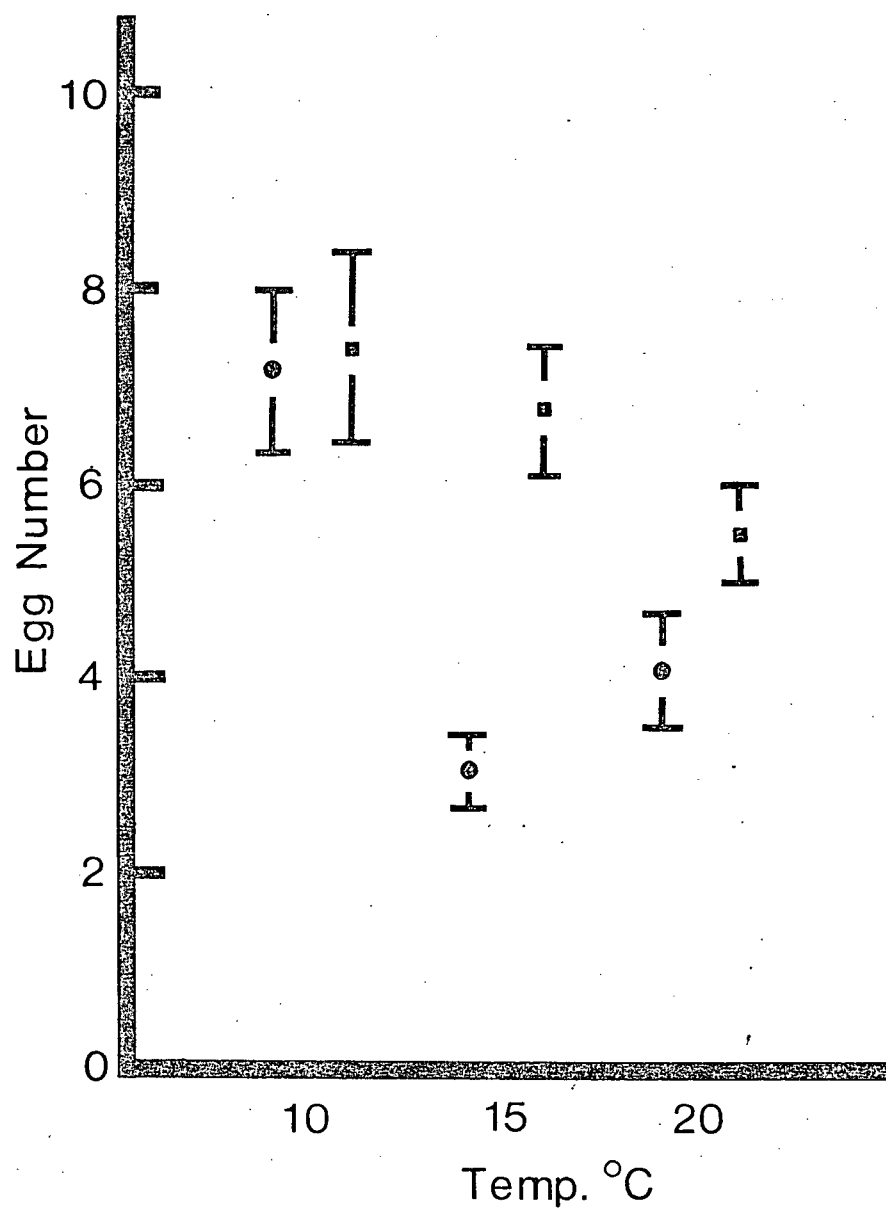
FINAL DISCUSSION

Because of their reproductive biology, _Daphnia_ which reproduce by acyclical parthenogenesis, are useful organisms in which to quantify genotypic and phenotypic variation and in which to evaluate the influence of genetic and environmental variation on phenotype. It is important to interpret this information relative to adaptation and fitness of parthenogenetic animals generally and of _Daphnia_ specifically.

Because of the lack of recombination and independent assortment in ameiotic parthenogens, one might expect _Daphnia_ to be less variable genotypically than sexually reproducing organisms. Using electrophoresis to measure enzyme variation, I observed a total lack of variation in three _Daphnia_ species in 20 ponds. This extreme homogeneity may be explained by the founder principle: that each new population was started by a small number of females (one?) which were capable of rapidly colonizing the environment to the exclusion of all other genotypes. Only a profound founder effect, however, would explain the complete and consistent lack of variation in all ponds and the electrophoretic similarity of animals from different ponds within each species. This explanation, thus, seems unlikely.

If it is assumed that enzymes are selectively important or closely linked to selectively important characters, the electrophoretic variation in Box 22 and NR as well as in the

lower mainland populations may be associated with the temporal and spatial stability shown by the environment. The lower mainland ponds are geographically similar and exposed to similar external environmental conditions ,although they do differ slightly in size, depth, vegetation, and temporal stability (during the course of this study one pond (P5) dried up considerably in advance of the others) from one another. Thus, it seems unlikely that electrophoretic homogeneity within a species can be attributed to any precise spatial homogeneity of physical characters within the ponds.

In NR, which is physically and geographically different from the lower mainland ponds, the electrophoretic differences (polymorphic vs. monomorphic) may be due to both temporal and spatial heterogeneity. However, without further data concerning the temporal variation in physical characteristics of this pond this must remain speculation.

Other effects of environmental stability on _Daphnia_ will be considered later in this discussion.

Population parameters, specifically frequency of sexual reproduction, rate of recruitment from other populations, and the rate of mutation may also explain differences in the amount of variation in NR and lower mainland ponds although again there is little information on these parameters. There was no evidence of change in genotype due to mutation or immigration in P2, P4, or P8 which were periodically sampled for four months. There was no sexual reproduction in these ponds during this time although it is unlikely that recombination of gametes from genetically

identical individuals with few heterozygotes would result in genetic changes in the offspring.

The variation observed electrophoretically is genetic. However, whether this genetic variation is ecologically important is unknown (Lewontin, 1974). The significant phenotypic differences among electrophoretically identical clones suggest differences, possibly genetic, which are undetected by electrophoresis. Ideally, to evaluate the importance of electrophoretic variability it is necessary to link the function of the enzyme to the environment and to demonstrate selection acting at the enzyme level. Since this is impractical in most studies, it is possible alternatively to correlate identifiable enzyme types with the environment, regardless of the specific function of the enzyme, as in _Avena_ or with data on phenotypic variation as done in this study. Clearly, detailed studies of individual responsiveness to different environments, population dynamics, and environmental fluctuations need to be coupled to determine the mechanisms for maintaining these different genetic structures in different populations.

In populations of _Daphnia_ there are differences in _means_ of morphological and physiological traits among populations regardless of the electrophoretic or geographical similarity of the populations. Likewise there are differences in means of some characters among clones within populations, again independent of electrophoretic and environmental similarity. There is no obvious explanation for these mean differences in such similar

populations or clones. These differences suggest that electrophoretic similarity does not necessarily provide a good indication of phenotypic similarity. Mean phenotypes may be influenced by the environment or by genetic differences undetected by electrophoresis; however, with no information on how selection operates on these phenotypes, mean differences among clones and between populations provide little information on the adaptive strategies of these organisms.

The existence of mean differences among clones and among populations for some characters and not for other suggests that different characters respond differently both in degree and in rate of change. Bradshaw (1965) argues that plasticity, the amount by which the individual genotype can be modified by its environment, is specific for each character and specific in relation to particular environmental influences. It is difficult however to assess whether characters in _Daphnia_ are varying independently of one another. Morphological characters were relatively related based on correlation coefficients among characters (Table 22). There was no evidence, however, that the characters associated with body size and those associated with predator avoidance were more or less plastic or varying independently of one another. Similarly there was no obvious difference in the amount of plasticity in morphological and physiological characters, although egg number showed greater conformity among populations (P2, P4, and P5) and among clones (NR) than other characters. This may imply that egg number is an extremely plastic character capable of responding in a very short time to changes in the environment. Egg number is most

Table 22: Comparisons of morphological characters in P2 and NR.

correlation matrix   N=169

body length	1.000				
body width	.986	1.000			
tail spine	.873	.876	1.000		
eye diameter	.343	.333	.193	1.000	
head width	.976	.973	.873	.287	1.000

Comparisons of morphological characters in P2,P4,andP5.

correlation matrix   N=391

body length	1.000				
body width	.908	1.000			
tail spine	.061	.057	1.000		
eye diameter	.320	.239	-.009	1.000	
head width	.914	.858	.084	.287	1.000

likely correlated with body length and with the general physiology of the parent. In this respect it does seem to differ at least qualitatively from the other characters examined in its response to environmental change.

Comparisons of electrophoretic variability, variances among populations, and components of variation within and among clones suggest differences between P2 and NR and a negative correlation of genetic and phenotypic variability in populations of Daphnia pulex . P2 Daphnia with less electrophoretic variability and more relative phenotypic variation than NR animals partitioned the total variance equally within and among clones and among populations. NR Daphnia were electrophoretically polymorphic and showed less absolute phenotypic variation between populations, within clones, and among clones than P2 with the greatest % variation in NR within clones.

Similar data have been described in Avena barbata and A. fatua (Marshall and Allard, 1970) and in Drosophila (Carson, 1965) and interpreted relative to the contribution of individual homeostasis and genetic polymorphism to adaptability of the population. Lewontin (1957) points out that populations may adapt to change in the environment either 1) by populational homeostasis, where the genotypic composition of the population may be flexible, 2) by individual homeostasis where each individual is fit in a number of environments by being phenotypically plastic, or 3) by some combination of indivuudal flexibility and genetic diversity that maximizes fitness. These differences in P2 and NR may be similarly interpreted as

different adaptive strategies though they differ in degree rather than kind. Whether these differences are quantitative or qualitative and whether they are pathological or strategic are undetermined and it seems more practical to look at P2 and NR as populations which need to deal with different amounts of seasonal change and, within any individual lifetime, similar amounts of environmental change. This will be done by 1) further estimating environmental stability of P2 and NR from field data, 2) measuring fitness in populations of Daphnia pulex reared in the lab at three different temperatures, and 3) by interpreting genetic and phenotypic variability relative to selection and stability in a model environment.

Environmental Stability Of P2 And NR

A further interpretation of environmental stability of P2 and NR is necessary before continuing the discussion of the genetic and phenotypic data in Daphnia . However, the interpretation is primarily speculative, based only on field observations and the data described in Tables 2 and 3.

The P2, P4, and P5 environment may or may not be stable over short periods of time. The three ponds are all small and fairly shallow and thus may be sensitive to any external environmental change. However, the ponds are well shaded and there is a large reserve of ground water which may sufficiently buffer these ponds against any severe short term changes in volume. Long term seasonal changes in the lower maniland are not

particularl y dramatic and populations may be able to survive by phenotypic plasticity alone. Populations of <u>Daphnia</u> in the Peterson ponds, however, are temporary, dying out in the fall either due to the actual disappearance of the ponds as they dry up or by some other environmental stimulus, presumably decreased temperature or amounts of food, or an increased population density as a consequence of the smaller volume of the pond. <u>Daphnia</u> in these ponds seem to respond to these long term changes associated with the disappearance of these ponds by forming ephippial eggs rather than by genetic changes in the population or by phenotypic flexibility. It seems logical that if there is no possibility of continued survival in a pond, then an individual increases its fitness by producing ephippial eggs which will increase probability of progeny in the next season.

There are probably very few short term changes occurring in NR because of the large size and depth of this pond. Comparisons of temperature profiles from two similar lakes in Riske Creek indicate much less variation in daily minimum/maximum temperatures at depths greater than 30 cm than at shallow depths (surface) (Toppings, 1969; Jansonn, 1971). If the <u>Daphnia</u> <u>pulex</u> are located at depths greater than 30 cm then they probably do not experience much environmental variability over a short period of time. It is not known whether <u>Daphnia</u> survive throughout the winter in NR though it seems unlikely since the pond freezes over. However, since the pond itself is permanent, changes in the genetic structure of the population may be adaptive in surviving long term environmental changes.

These differences in genotypic and phenotypic variation may suggest differences in the ability of these organisms to adapt to different environments. Adaptability in these populations has been described relative to the environmental stability and to their response to temperature. P2 and NR show extremely different responses to different temperatures, however, both responses may be explained by phenotypic plasticity. Phenotypic flexibility associated with a single genotype in P2 may be responsible for the observed differences in means among genetically identical replicates. In electrophoretically polymorphic individuals in NR, however, phenotypic flexibility by extreme developmental canalization may have been responsible for convergence of means at different temperatures.

It is difficult to determine if the divergence in P2 and the convergence in NR actually confer an adaptive advantage to either population. In comparisons of mean adaptive values (W) determined from mean number of survivors and mean number of eggs at all temperatures NR was greater than P2 for both characters. P2 however has the greater variance in fitness than NR (Table 23).

Greater mean fitness generally associated with a low variance in fitness has similarly been observed in _Drosophila pseudoobscura_ (Lewontin, 1957) from a single population where homozygotes showed less average fitness and greater variance in fitness than heterozygotes. This does not indicate which population of _Daphnia_ or which genotype of _Drosophila_ is more fit; however, it does indicate two types of

Table 23: Means and variances for fitness based on number of
survivors and number of eggs from P2 and NR in the
temperature experiment.

NUMBER OF SURVIVORS

	10C	15C	20C	$\overline{W}$	σ_w^2
NR	35	47	44	42.0	78.0
P2	33	51	35	39.7	194.7

TOTAL EGGS

	10C	15C	20C	$\overline{W}$	σ_w^2
NR	240	301	242	261	2402
P2	232	149	153	178	4382

Qualitative comparison of mean fitness and variance in fitness in P2 and NR

	MEAN FITNESS	VARIATION IN FITNESS
NR	HIGHER	LOWER
P2	LOWER	HIGHER

fitness, one associated with larger means and smaller variances,the other with smaller means and larger variances.

Since there is little environmental data available a hypothetical model relating phenotypic and genotypic variation to selection and to the temporal stability of the environment is described. The apparent trade-off of individual homeostasis and genetic polymorphism described previously in P2 and NR may be closely associated with selective pressures on the organism. If the NR environment is such that a single phenotype is advantageous, all individuals regardless of their electrophoretic genotype will tend to converge on that optimal phenotype, either by phenotypic flexibility associated with developmental canalization or by selection for genotypes coding for that phenotype. This convergence would account for the for the reduced variance among clones and in the population. In this model any intraclonal variation observed in this study is attributed to experimental error. If in P2 the environment is less restrictive and there is little selection for a single phenotype, then this would account for greater absolute variation in the population and the distribution of variances within and among clones.

The severity of selection in these populations may be influenced by the environmental stability. If changes in the environment are of the same or shorter duration than the generation time of the organism adaptation can only take place by individual homeostasis. The organism cannot respond to short term environmental changes by genetic changes unless they are

associated with the development of the organism. If, however, changes in the environment take longer than the generation time adaptation may take place by genetic changes in the population..

If the measures of genotypic and phenotypic variation in P2 and NR are a real indication of the adaptive strategies then one might assume P2 is well adapted to short term environmental fluctuations with little ability to adapt to severe long term changes. NR may also be sufficiently individually buffered to adapt to short term changes. Further, because of its genetic diversity the NR population is also buffered over long term environmental differences.

Populational homeostasis is dependent on genetic variability in the population and is maintained in part by sexual reproduction. Since parthenogenetic organisms cannot necessarily rely on recombination and random assortment to maintain genetic diversity phenotypic variability and individual homeostasis seem to be a more reliable means of adapting to the environment. This has been proposed for populations of the snail, Rumina decollata and for populations of wild oats, Avena barbata which are electrophoretically homogenous and phenotypically variable. However, all other studies measuring genetic variability in parthenogenetic populations report large amounts of variation apparently unaffected by the lack of sexual reproduction and maintained by selection or some other mechanism. These differences in the amount of variability in parthenogenetic organisms seem to suggest that variation may not be as rigorously linked to the mode of reproduction as to

selection and environmental stability.

Daphnia are apparently capable of large amounts of phenotypic flexibility and both genetic and phenotypic variability seem to be more closely associated with environmental parameters than reproductive strategy. Hebert (1974) does, however, presents data from temporary and permanent populations that suggest genetic variation is closer to Hardy-Weinberg equilibrium in temporary ponds, which presumably undergo more frequent sexual reproduction. An alternative adaptation to both phenotypic flexibility and genetic polymorphism in Daphnia is their ability to produce overwintering ephippial eggs. Regardless of the genetic consequences of sexual reproduction in these individuals the formation of ephippial eggs provides a means of surviving difficult times in these ponds.

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APPENDIX

Buffers:

LiOH

    Stock A (electrode buffer)   pH = 8.1
                    2.4 g LiOH
                    23.8 g boric acid
                    make up to 2 l with distilled water

    Stock B   pH = 8.4
                    12.4 g TRIS buffer
                    3.2 g citric acid
                    make up to 2 l with distilled water

    Gel buffer   25 ml Stock A + 215 ml Stock B

250 volts for 3 hours

Poulik

    Electrode buffer   pH 8.12
                    37.10 g boric acid
                    4.8 g NaOH
                    make up to 2 l with distilled water

    Gel buffer   pH = 8.62
                    18.42 g TRIS buffer
                    2.10 g citric acid monohydrate
                    make up to 2 l with distilled water

    250 volts for 3 hours

EDTA

    Gel and Electrode buffers   pH = 9.00
                    42.2 g TRIS buffer
                    1.2 g EDTA
                    2.0 g boric acid
                    make up to 4 l with distilled water
add 20 mg NAD to gel buffer when making gel

350 volts for 4 hours

<u>Stains</u>:

Used with LiOH:

    <u>ES</u>

    incubate in TRIS malate:
                    Tris malate   100 ml (see LAP stain)
                    fast blue RR   20 mg
                    Na napthyl acetate   10 ml
                        (100mg in 5 ml water + 5 ml acetone)

    <u>GOT</u>

                    TRIS (0.1 M)   pH = 8.5   100 ml
                    aspartic acid   4.40 mg
                     ketoglutaric acid   240 mg
                    fast blue BB   80 mg
                    pyridoxal 5' phosphate   2 mg

Used with Poulik:

    <u>AKP</u>

                    TRIS HCl (0.1 M)   pH = 8.5   100 ml
                    PVP   500 mg
                    fast blue BB   100 mg
                      napthyl acid phosphatase   100 mg
                    $MgCl_2$ 60 mg
                    $MnCl_2$ 60 mg
                    NaCl   2 g

    <u>AP</u>

    incubate for 30 min. in 0.5 M boric acid, then to:
                    0.125 M sodium acetate   pH=5.0   100 ml
                    PVP   500 mg
                    Na-napthyl acid phosphatase   100 mg
                    fast blue BB   100 mg

    <u>LAP</u>

    incubate for 30 min. in 0.5 M boric acid, then to:
                    TRIS malate   100 ml
                        12.1 g TRIS
                        11.6 g maleic acid
                        1.0 N NaOH   1 ml
                        make up to 1 l with distilled water
                    fast black K   20 mg
                    Na L-leucine   20 mg

Used with EDTA:

MDH

    TRIS HCl 0.1 M pH=8.5
    malic acid   50 mg
    NAD   20 mg
    KCl   10 mg
    PMS   2 mg

ODH

    TRIS HCl 0.1 M pH=8.5   100 ml
NAD   25 mg
 NBT   20 mg
 Octanol 5 ml
 PMS   2 mg

SDH

    TRIS HCL 0.1 M pH=8.5   100 ml
    NAD   25 mg
    NBT   20 mg
    D-sorbitol   500 ug
    PMS   2 ug

XDH

    TRIS HCl 0.1 M pH 7-7.4   100 ml
    hypoxanthine   100 mg
    NAD   25 mg
    PMS   5 mg
    NBT   20 mg
    KCL   100 mg

PGI

    TRIS HCl 0.05 M pH 8.5   100 ml
    MgCl$_2$ 100 mg
    fructose-6-phosphate   25 mg
    NADP   15 mg
    MTT   25 mg
    G-6-PDH   25 units
    PMS   10 mg

AO

    TRIS HCl 0.05 M   pH 8.5   100 ml
    benzaldehyde   1 ml
    NBT   20 mg
    NAD   25 mg
    EDTA   10 mg
    PMS   10 mg