Genetic Variation and Evolutionary Divergence Within and Among Populations, Species, and Genera of the Cambarinae

by

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To Jo Anne and my family

Wisdom without knowledge is a blessing.
Knowledge without wisdom is a key with
no lock to open.

S.T.N.

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Abstract

Seven crayfish species from three genera of the subfamily Cambarinae were electrophoretically examined for genetic variation at a total of twenty-six loci. Polymorphism was detected primarily at three loci: Ao-2, Lap, and Pgi. The average heterozygosities over all loci for each species were found to be very low when compared to most other invertebrate species that have been examined electrophoretically.

With the exception of Cambarus bartoni, the interpopulation genetic identities are high within any given species. The average interspecific identities are somewhat lower and the average intergeneric identities are lower still. Populations, species and genera conform to the expected taxonomic progression. The two samples of C. bartoni show high genetic similarity at only 50 percent of the loci compared. Locus by locus identity comparisons among species yield U-shaped distributions of genetic identities.

Construction of a phylogenetic dendrogram using species mean genetic distances values shows that species grouping is in agreement with morphological taxonomy with the exception of the high similarity between Orconectes propinquus and Procambarus pictus. This high similarity suggests the possibility of a regulatory change between the two species.

It appears that the low heterozygosities, high interpopulation genetic identities, and taxonomic mispositioning can all be explained on the basis of low mutation rates.

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INTRODUCTION

The Measurement of Genetic Variation

Techniques of electrophoresis were developed by Tiselius (1937; cited by Brewer, 1970) in order to separate fractions of serum proteins migrating through solution under the influence of an electric current. The next 25 years saw developments in electrophoretic technique which included the use of starch gels as a support medium for proteins (Smithies, 1955), the development and use of a large number of histochemical staining methods (Hunter and Markert, 1957) for enzymatic proteins, and the demonstration that protein variation was inherited largely in a simple Mendelian manner. It was not until after 1960 that electrophoretic techniques were adequately developed to allow large multi-locus studies of proteins in populations of organisms (Hubby, 1963; Hubby and Throckmorton, 1965; Hubby and Lewontin, 1966; Lewontin and Hubby, 1966; Harris, 1966). These techniques provided geneticists with a direct method of analyzing population structure and genetic variability within a species (see review by Gottlieb, 1971).

Gel electrophoresis is now the most common method of analysis for the study of genetic variation. Following the initial reports, a great many studies encompassing scores of species from almost all animal phyla have been published. The large number of species examined has prompted the publication of papers which compare the amounts of variation among species. Most notable of these are reviews by J.R. Powell (1975) and

R.K. Selander (1976). A summary of Powell's review is listed in Table 1 showing only those populations for which heterozygosity values were given.

The averages in Table 1 are drawn from five separate phyla and over 150 individual species studies. A number of generalizations may be concluded from all genetic variation studies carried out thus far. First, the majority of natural populations contain a good deal of genetic variability. There are a few exceptions, notably the gastropod mollusc, Rumina decollata (Selander and Kaufman, 1973), the lizard, Anolis augusticeps (Webster, Selander, and Yong, 1972), and the elephant seal, Mirounga angustirostris (Bonnell and Selander, 1974), in which no genetic variation was detected at any of the loci examined. These studies have also demonstrated that parthenogenic species can contain as much genetic variability as sexually reproducing species (Suomalainen and Saura, 1973).

In the majority of cases, invertebrates have been found to have more genetic variability than vertebrates, the mean heterozygosities being 0.146 ± 0.009 and 0.050 ± 0.004 respectively (from Table III in Powell, 1975). This difference may possibly be due to differences in evolutionary strategies between vertebrates and invertebrates or differences in ecological niches.

No evidence exists which suggests that there is a difference in genetic variability between tropical species and temperate species. The mean heterozygosities for invertebrates from the tropical zone and the temperate zone are 0.109 ± 0.009 and 0.132 ± 0.012 respectively. Analogous values for vertebrates from the tropical zone and the temperate zone are 0.047 ± 0.010 and 0.049 ± 0.005 respectively (from Table IV in Powell, 1975).

Table 1. Summary of genetic variation studies listing the mean heterozygosities of various phyla examined. Means are calculated from only those studies for which heterozygosity values are given (from J.R. Powell, 1975).

Phylum	Average Heterozygosity	Average Number of Loci per Study	Number of Studies
Mollusca	0.148 ± 0.084	17.3 ± 8.4	6
Arthropoda	0.154 ± 0.058	21.8 ± 7.8	56
Bryozoa	0.082 ± 0.016	11	2
Echinodermata	0.078 ± 0.08	22.7 ± 6.7	3
Vertebrata	0.054 ± 0.035	22.3 ± 7.3	89

The amount of genetic variation occurring in any given enzyme appears to be related to its metabolic function. Enzymes which control metabolic pathways have, in general, more variation than those that do not, for example, enzymes in the glycolytic pathway (see Selander, 1976). The reasons for the differences in levels of variation from one species to the next are not clear. However, some authors have attempted to demonstrate that differences do exist in the levels of genetic variation among species inhabiting constant environments and those that inhabit variable environments do exist (Selander, 1976; Soulé, 1976; and Valentine, 1976). Others have argued that the differences are attributable to differences in effective population size and mutation rate (Ohta, 1974).

Genetic Variability and Environmental Heterogeneity.

Genetic variation has been found in virtually every species examined; the problems arise when one attempts to account for this variation. One may deduce that if a population shows a relatively high degree of genetic variability and exists in an environment that is also highly variable, the genetic variation may be accounted for by environmental heterogeneity. By the same reasoning one would expect a species inhabiting a constant environment to have very little polymorphism among its enzymes. The answer is not quite so clear-cut.

Powell (1971) and McDonald and Ayala (1974) electrophoretically examined genetic responses to environmental heterogeneity in Drosophila willistoni and D. pseudoobscura respectively. They tested the hypothesis that different genetic variants are favoured in different niches. They found a positive correlation between genetic and environmental heterogeneity. In contrast Minawa and Birley (1975) found that D. melanogaster

from populations maintained in variable environments were not, on average, the most genetically heterogeneous.

A number of studies have also been conducted which compare genetic variability with environmental parameters in natural populations (Levinton, 1973; Somero and Soulé, 1974; Selander, Hunt, and Yang, 1969; Bryant, 1974). In each case the authors concluded that there exists a positive correlation between and among species, demonstrating that the degree of habitat variability is directly related to the degree of genetic polymorphism.

Other studies can be found that are not in agreement with the proposed correlation. Schopf and Gooch (1971), Gooch and Schopf (1972), and Ayala, Hedgecock, Zumwalt, and Valentine (1974) have studied a variety of deep sea invertebrates collected from as deep as 2,000 metres. If there are ecosystems which are stable and constant, the sea depths should be one of these. The levels of genetic variation found in these surveys were comparable to those of organisms which inhabited highly variable environments. Nevo (1976) also cites a relatively large amount of genetic variation in a species of subterranean spadefoot toads, Pelobates syriacus that inhabit an environment he describes as constant.

Inasmuch as there are no actual indices of environmental heterogeneity, it is difficult at best to make any type of environment-genetic variability correlation. Selander and Kaufman (1973) have argued that genetic variability should not be correlated directly to environment, but rather to the individual species' adaptive strategy in response to its environment. Furthermore, in order to properly test the niche-variation

hypothesis (when employing gel electrophoresis) one must be absolutely certain that the enzymes and proteins under scrutiny come in contact with the environmental parameters being studied (Somero and Soulé, 1974).

Electrophoretic Variation and Selectively Neutral Mutations.

A protein which exhibits differing electrophoretic mobility in a population is assumed to differ by at least one amino acid between any two variants. A difference in mobility between the two proteins therefore implies that at least one nucleotide base substitution has taken place in the DNA codon. However, due to the redundancy of the genetic code, a codon change could take place which does not change the amino acid. About one-fourth or 134 of the 549 possible DNA base substitutions are of this type. These mutational changes are called synonymous since they do not affect the protein (King and Jukes, 1969).

Another type of neutral mutation can occur in proteins. These neutral mutations may be detected by electrophoresis, but may not be detected by the organism and are hence called neutral. Such mutations can be described by imagining that a single base change in the DNA codon produced a change in amino acids from one which was positively charged or neutral to one which was negatively charged and structurally similar (glutamic acid and glutamine, for example). If this change occurs far from the active site of the enzyme, it may make no difference at all to the overall functioning of the organism. Such mutations may be responsible for maintaining certain enzyme polymorphisms in natural populations (see G.B. Johnson, 1973; and Ayala, Tracey, Barr, McDonald and Perez-Salas, 1974). This is one of the major reasons the concept of selectively neutral

mutations was put forth. This concept, or non-Darwinian evolution as it is called, assumes first that neutral mutations can occur in structural genes and second that since neutral alleles are selectively neither advantageous nor disadvantageous, they are free to drift in a gene pool either toward fixation or extinction. King and Jukes (1969) describe this phenomenon as random walk; under the neutral model protein polymorphism is not selectively maintained. The observed variation is transient rather than stable.

Electrophoretic Variation and the Study of Systematics and Speciation.

With the accumulation of electrophoretic data from a large number of species came the development of various mathematical methods for analyzing these data (see for example Crow and Denniston, 1974). Among these methods were formulae, developed by M. Nei, which assigned mathematical values of genetic similarity and genetic distance for comparisons between two or more populations using allele frequency data from genetic variation studies (Nei, 1971 and 1972). These formulae and their interpretations are described in MATERIALS and METHODS.

Genetic variation data coupled with the calculations of genetic similarity and genetic distance have been demonstrated to be powerful tools in the study of systematics and speciation. J.C. Avise (1974) points out that recent multi-locus electrophoretic studies show high levels of genetic similarity between conspecific populations and that similarities between different species are, in general, much lower. He also discusses the theoretical advantages and disadvantages of electrophoretic data in the study of systematics. Advantages such as objectivity,

the ability to collect large amounts of genetic information, precision, equal weighting of information, and the comparison of relative similarities between species groups are discussed as well as disadvantages such as restriction to extant species, chance identity in band mobilities, scoring difficulties, more than one mutational step having taken place, and non-detected protein differences.

Despite the disadvantages, electrophoresis has been demonstrated to be a very valuable tool in the study of speciation (Avise, 1976). Many populations and species of Drosophila have been electrophoretically examined and the resulting data used to characterize the populations according to geographic populations, subspecies, semispecies, and sibling species (Ayala, Tracey, Hedgecock, and Richmond, 1975). The conclusions are generally in accord with phylogenies based on non-electrophoretic criteria.

Genetic Variation in Crustaceans

Among the many electrophoretic studies of genetic variation that have been carried out, very few have been conducted on crustaceans which, as a group, comprise a relatively large portion of the animal kingdom. With the exception of a study done on the cladoceran Daphnia magna (Hebert, 1974 a and b), all of the crustaceans examined thus far are decapods. Detailed multi-locus studies have been carried out on galatheid crabs (Gooch and Schopf, 1972), fiddler crabs (Selander et al., 1971), and the American and European lobster of the species Homarus (Tracey et al., 1975; Hedgecock et al., 1976 and 1977). In all of these studies, the observed average heterozygosities were found to be low when compared to most other invertebrate species.

To date, no electrophoretic variation studies have been carried out on species of crayfish from the subfamily Cambarinae of the family Astacidae, although species diversity among crayfish was described as far back as 1880 in a textbook published by T.H. Huxley (1973). This study examines seven species from three separate genera of the subfamily Cambarinae: Orconectes, Cambarus and Procambarus. Three other genera are also found among the Cambarinae: Paracambarus, Faxonella, and Troglocambarus which are respectively composed of 2, 2 and 1 species. Only the first three genera mentioned above show any degree of species diversity. Procambarus is composed of approximately 102 species, Cambarus, 48 species, and Orconectes, 51 species (Crocker and Barr, 1968). We therefore decided to focus attention on the species-rich genera to establish baselines for future phylogenetic studies and because these species are more readily obtained.

This study examines genetic variation within a population of a given species, between populations of the same species, between populations of different species of the same genus, and between different genera. Measures of genetic similarity and genetic distance are used to characterize differences between the populations and to cluster them phylogenetically. Comparison of these results with those of other crustacean studies will permit extension of the low crustacean heterozygosity observation and speculation as to its cause. Lastly, a comparison of these results to the general results of all electrophoretic variation studies will determine whether or not they are consistent with any trends among invertebrates as a whole.

MATERIALS and METHODS

Collections

All samples of crayfish were collected during the months of April through September in 1976 and 1977 for the purpose of examining the amount of genetic variability and divergence within and between taxa. Collecting localities for Orconectes propinquus, O. immunis, O. virilis, Cambarus robustus, C. bartoni, Procambarus clarkii, and P. pictus are as follows:

Orconectes propinquus

1. Hart Creek, stream connecting Hart Lake and Lake Opinicon, 56 km northeast of Kingston, Ontario on Highway 15, near Queen's University field station (September 1976).
2. Chippawa Creek I, on the north shore of the creek where the Welland River (Chippawa Creek) begins at the Niagara River in Niagara Falls, Ontario (September 1976).
3. Twelve-Mile Creek I, where the creek crosses Decew Road, 4 km west of St. Catharines, Ontario, off Regional Road 69 (April-May 1976).
4. St. John's Pond I, in the St. John's Conservation Area 8 km south of St. Catharines, Ontario in the Effingham Valley (June-July 1976).
5. Oliphant, on the shore of Lake Huron 80 km south of Tobermory, Ontario and about 15 km west of Wiarton, Ontario (August 1976).
6. Tobermory, off Light House Point in Tobermory, Ontario where Highway 6 terminates at the end of the Bruce Peninsula (May 1977).

Orconectes virilis

7. St. John's Pond II, in the same area and at the same time as that indicated for (4) above.

Orconectes immunis

8. St. John's Pond III, in the same area and at the same time as that indicated for (4) and (7) above.
9. Stinking Barn I, a marshy area about 6 km south of Welland, Ontario. This sample was taken from a drainage pond on the east side of a farm road (August 1976).
10. Stinking Barn II, in the same area as (9) above except that this sample was taken from the pond on the west side of the same farm road (August 1976).

Cambarus robustus

11. Chippawa Creek II, in the same area as that indicated for (2) above (September 1976).
12. Twelve-Mile Creek II, in the same area as that indicated for (3) above. This sample was taken at a point 300 m upstream from that of (3) (April 1976).

Cambarus bartoni

13. Opinicon, a small wooded stream about 15 km southwest of Queen's University field station at Lake Opinicon (September 1976).
14. Georgia, near Jackson Lake in Jackson County, Georgia, U. S. A. (April 1976).

Procambarus clarkii

15. Texas, collected off Interstate 10, 50 to 75 km east of Houston in culverts and ephemeral ponds (May 1977).

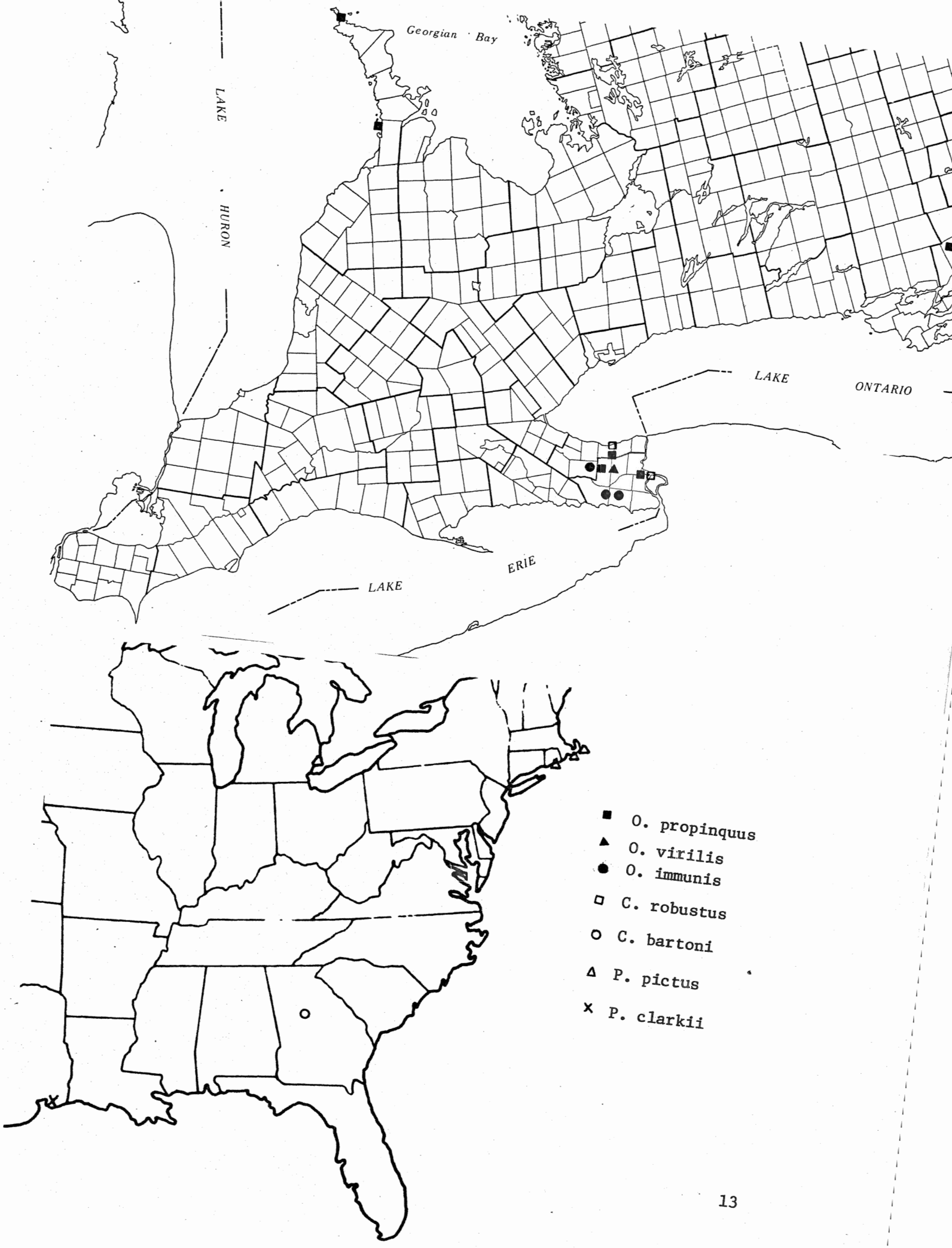
Procambarus pictus

16. Cape Cod I, collected at Fisherman's Landing, Sheep's Pond, Brewster, Massachusetts (July 1976).
17. Cape Cod II, collected at a pond near Orleans, Massachusetts, just off Route 6 (July 1977).

Table 2. Population sample summary

	Sample Name and Number	Species	Sample Size Males	Females	Total
1.	Hart Creek (HC)	O. propinquus	22	8	30
2.	Chippawa Creek I (CCR-I)	O. propinquus	24	24	48
3.	Twelve-Mile Creek (TMC-I)	O. propinquus	28	2	30
4.	St. John's I (SJ-I)	O. propinquus	36	24	60
5.	Oliphant (OLP)	O. propinquus	15	10	25
6.	Tobermory (TOB)	O. propinquus	20	20	40
7.	St. John's II (SJ-II)	O. virilis	2	58	60
8.	St. John's III (SJ-III)	O. immunis	33	27	60
9.	Stinking Barn I (SB-I)	O. immunis	36	44	80
10.	Stinking Barn II (SB-II)	O. immunis	14	16	30
11.	Chippawa Creek II (CCR-II)	C. robustus	35	5	40
12.	Twelve-Mile Creek II (TMC-II)	C. robustus	12	18	30
13.	Opinicon (OPIN)	C. bartoni	12	4	16
14.	Georgia (GG)	C. bartoni	17	16	33
15.	Texas (TEX)	P. clarkii	15	15	30
16.	Cape Cod I (CC-I)	P. pictus	10	6	16
17.	Cape Cod II (CC-II)	P. pictus	14	11	25
18.	Rhode Island (RI)	P. pictus	12	5	17

Figure 1. Relative locations of sampling sites of all species collected


Georgian Bay
LAKE HURON
LAKE ONTARIO
LAKE ERIE
O. propinquus
O. virilis
O. immunis
C. robustus
C. bartoni
P. pictus
P. clarkii

18. Rhode Island, collected at Echo Lake, Chepachet, Rhode Island (July 1976).

In this study a collecting site constitutes a population. Therefore when the word "population" appears with reference to this study it refers to a species taken from one of the listed sample sites.

Electrophoretic Techniques

Genetic variation in natural populations of crayfish was examined by employing starch gel electrophoresis. This method allows the user to study an individual at many different genetic loci simultaneously by choosing a variety of the histochemical assays available. Also, many individuals may be run simultaneously on the same starch gel, hence allowing one to compare many individuals simultaneously over a number of loci. The zymograms or banding patterns obtained following selective staining after electrophoresis are in accordance with simple codominant models of Mendelian inheritance. A single band at a locus signifies the presence of a homozygote and a double or triple band pattern indicates that a heterozygote is present, the triple banding being an enzyme molecule that is at least a dimer.

Tissue Preparation

All collected individuals were either kept alive or frozen at -78°C until dissection. Freezing was found to have no effect upon any enzyme assays used in this study. Four tissues were removed from each animal larger than 16 mm carapace length (carapace lengths are included in Appendix A): liver, muscle, gill, and eye. Crayfish of 16 mm carapace

length and shorter were too small to obtain any significant amounts of each tissue in a preparation and were therfore finely minced with scissors and homogenized whole. Each tissue or whole animal was homogenized in an equal volume of deionized water using an ice-chilled 10 ml homogenizing tube. After a thorough homogenization of each tissue or whole specimen (using a Black and Decker variable speed drill for about 20 seconds), the crude homogenate was immediately transferred to a 3 ml plastic cryogenic vial. The vial was then capped and immersed in liquid nitrogen to flash-freeze the sample. All samples were stored at -78°C until electrophoresis. During gel loading the vials were put on ice while the frozen sample was chipped out. At no time were the samples allowed to completely thaw. After obtaining the sample, the vials were returned to the freezer.

Whatman No. 4 filter paper wicks 10 mm x 4 mm were saturated with the crude extract and applied to the gel. As many as 24 of these wicks may be placed in each gel.

Gel Preparation

The gel molds used were made of plexiglas with the dimensions 15.2 x 11.9 x 1 cm. Each mold has a trough section on two opposing sides so that the gel, when placed in the electrode buffer trays, makes direct contact with the electrode buffer (Figure 2).

The type of starch used for all electrophoresis in this study was a 1:1 (w/w) mixture of Sigma starch (S-4501, Sigma Chemical Company, St. Louis, Missouri) and Electrostarch, lot 303 (Electrostarch Company, Madison, Wisconsin). It was found that such a mixture made the gels easy to handle without loss of resolution.

The gels were prepared by measuring out 557 ml of the appropriate gel buffer and heating all but 150 ml of it to a boil. The remaining 150 ml was used to suspend 68 g of starch (12.2:100 w: final v) in a 1000 ml side-arm flask. After bringing to a boil, the boiling buffer was quickly poured into the side-arm flask with the suspended starch and swirled vigorously until a homogeneous solution of starch resulted. The flask was then stoppered and subjected to vacuum so that the solution boiled and was degassed. When the solution boiled evenly with large bubbles, the vacuum line was removed and the solution was poured into the gel mold. After the gel had cooled, it was covered with Saran Wrap and allowed to sit overnight at room temperature before being used.

Filter paper wicks with absorbed sample were inserted in pockets in the gel made by a metal template (Figure 2). The contact portions of the gel were then placed in the electrode trays containing platinum electrodes and connected to a constant current power supply (Figure 3). The electrode trays were filled with the appropriate buffer and the gel run in a cold room at 4°C. When electrophoresis was complete, the contact portions of each gel were cut off and discarded and the wicks were removed to facilitate slicing.

Gel and Electrode Buffers

Three buffer systems were used.

A. Gel buffer: 75 mM Tris and 5 mM citric acid, pH 8.65; electrode buffer: 300 mM boric acid and 60 mM NaOH, pH 8.1 (Poulik, 1957).

Figure 2. Electrophoretic apparatus.

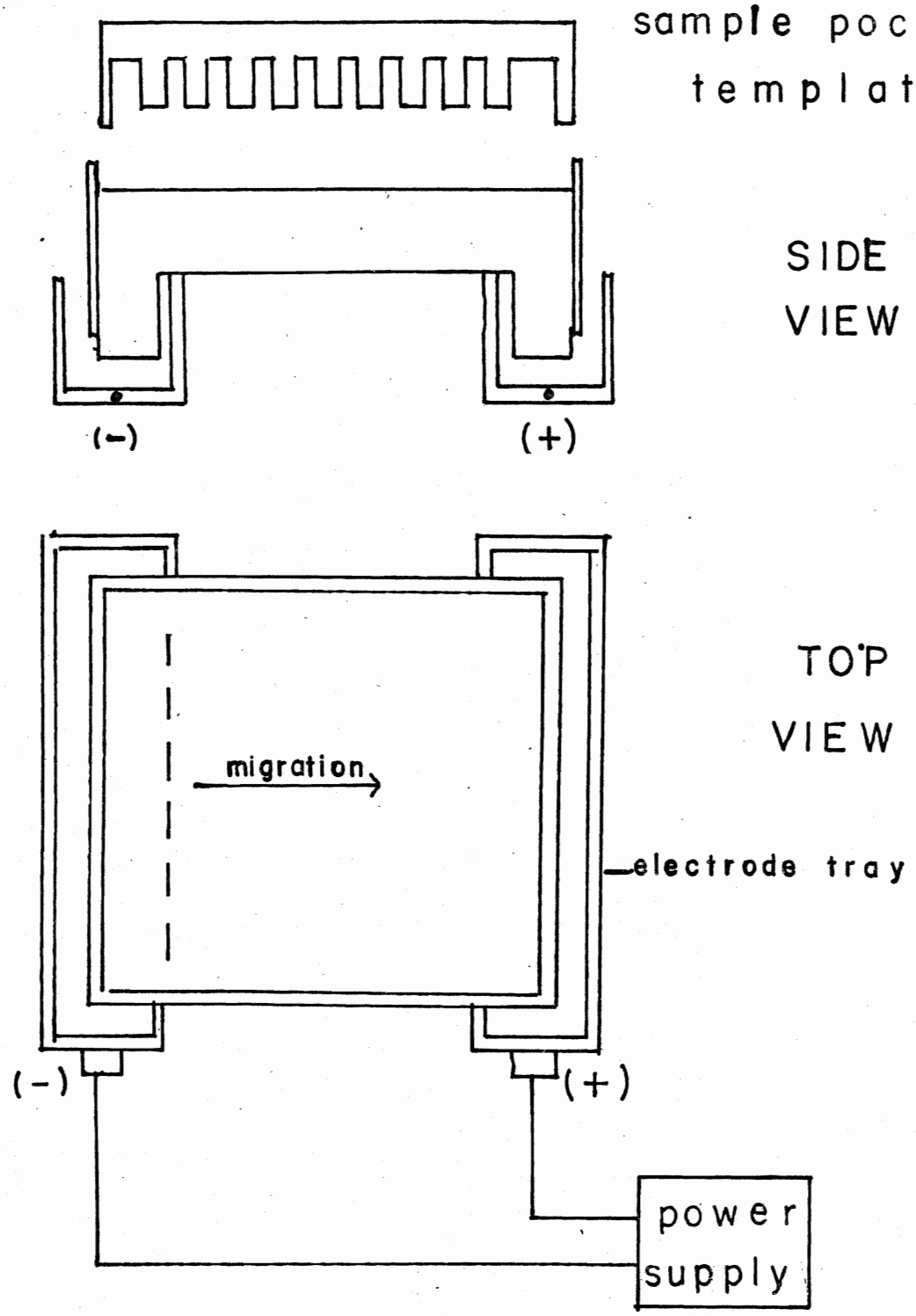
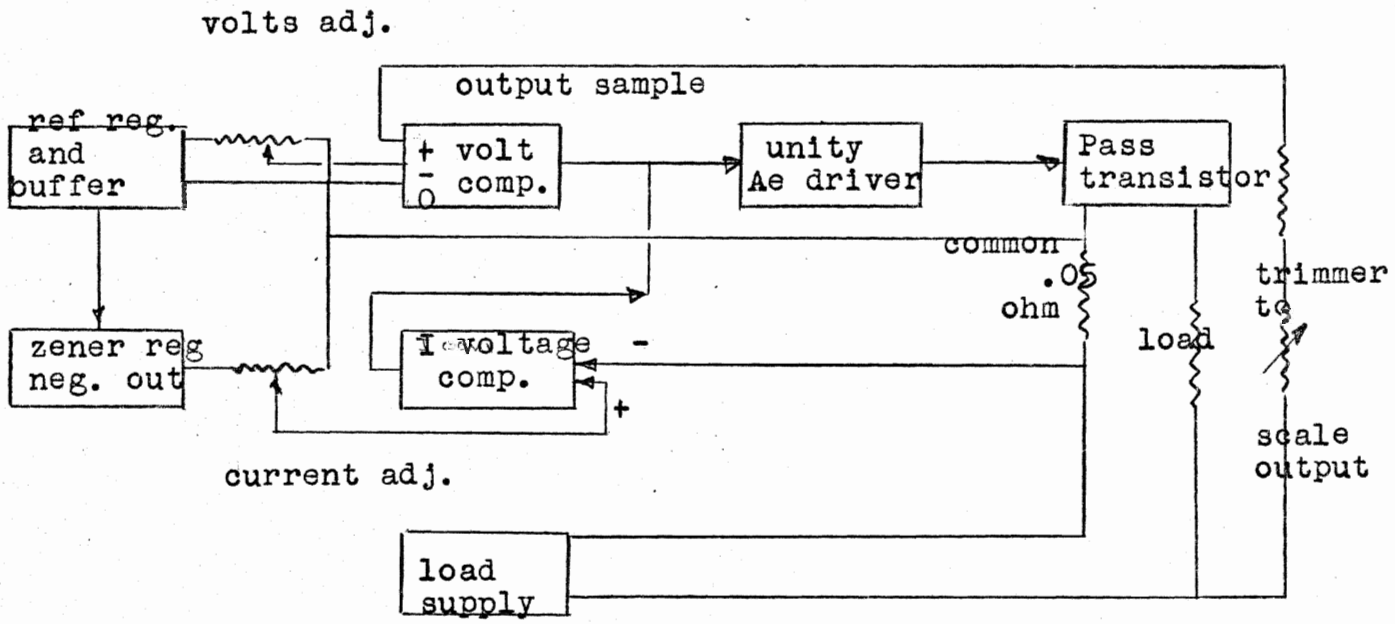

sample pocket template
SIDE VIEW
(-)
(+)
TOP VIEW
migration
electrode tray
(-)
(+)
power supply

Figure 3. Block diagram of constant current/voltage regulator. Designed and constructed by John Rustenberg, Brock University Technical Services.

OCT. 75

CONSTANT CURRENT/VOLTAGE REGULATOR

BLOCK DIAGRAM



The .05 ohm resistor in common load circuit develops a voltage output proportional to the load current.

B. Gel and electrode buffer: 87 mM Tris, 8.7 mM boric acid, and 1 mM EDTA, pH 9.1 (F.J. Ayala, J.R. Powell, M.L. Tracey, C.A. Mourão, S. Perez-Salas, 1971).

C. Gel buffer: 5 mM histidine, pH 7.0;
electrode buffer: 510 mM sodium citrate adjusted to pH 7.0 with 0.41 M citric acid (Brewer, 1970).

The pH of all buffers, with the exception of the electrode buffer of (C), is adjusted with either 1N HCl or 4 N NaOH.

Power Applied for Electrophoresis.

All three buffer systems were started and maintained at 80 mA. For buffer system A, the run was terminated when the visible boric acid front had migrated 10 cm from the origin.

The runs for both B and C were terminated after no less than 4 h nor more than 4.5 h.

Fixing, Wrapping, and Reading Gels.

After the bands of each assay had reached optimum density, any gels to be kept were rinsed twice with distilled water and soaked overnight in a fixing solution of 60 parts 95% ethanol to 40 parts water. The following day the gel slices were wrapped in Saran wrap and labelled. With the exception of esterases, leucine amino peptidases, and protein, all gels were scored without being fixed because of the rapidity of staining. The individual genotypes were characterized by comparing the band mobilities of the samples with those of the controls (described below).

Enzymes Assayed

All enzymes and nonenzymatic proteins used in this study are listed in Table 3 with their genetic symbols for the genes which are assumed to encode their respective primary structure. Also listed are their tissue sources, buffer system used for each assay, and the total number of loci scored for each assay over all species examined.

Assays for each of the enzymes and proteins listed in Table 3 are as follows:

Acid phosphatase: soak gel slice 30 minutes in 0.5 M boric acid; rinse with distilled water. Then to 100 ml of ACPH stain buffer (0.2 M glacial acetic acid, 0.13 M NaOH, pH 5.0) add 150 mg fast blue BB salt, 150 mg α-naphthyl acid phosphate. Allow to stain at room temperature.

Amylase: any gel run on buffer system A with hepatopancreas tissue was allowed to sit overnight at room temperature. The bands will show up as clear spots in the starch.

Aldehyde oxidase: dissolve in 100 ml 0.05 M Tris-HCl buffer, pH 8.6, 20 mg MTT, 25 mg nicotinamide adenine dinucleotide (NAD), 10 mg EDTA, 1.0 ml benzaldehyde, 5 mg phenazine methosulphate (PMS). Allow to stain at room temperature.

Esterase: Soak gel slice 30 minutes in 0.5 M boric acid. Rinse with distilled water. Dissolve in 100 ml phosphate buffer pH 6.5 (0.03 M Na_2HPO_4, 0.07 M KH_2PO_4), 60 mg Fast Garnet GBC salt, 1.5 ml 1% β-naphthyl acetate made by dissolving 1 g β-naphthyl acetate in 50 ml of acetone and 50 ml distilled water.

Table 3. Enzymes and proteins assayed, their symbols, tissue sources, and buffer systems.

Enzyme	Symbol	Buffer System[1]	Best Tissue[2]	Number of loci Scored over all species
Acid phosphatase	Acph	B	HP	1
Amylase	Amy	A	HP	2
Aldehyde oxidase	Ao	A	Hp, M	4
Esterase	Est	A	HP, M, G, E	4
Leucine amino peptidase	Lap	A	HP, M, E	1
Malate dehydrogenase	Mdh	C	M	2
Octanol dehydrogenase	Odh	B	HP	1
Phosphoglucose isomerase	Pgi	A	HP, M, G, E	1
Phosphoglucomutase	Pgm	A	M, HP	2
Protein	Pt	C	M, G	5
Tetrazolium oxidase	To	B	HP	2
Xanthine dehydrogenase	Xdh	B	HP	1

[1] All starch used is a 1:1 (w/w) mixture of Sigma and Electrostarch.

[2] Symbols for tissues are HP = hepatopancreas, M = abdominal muscle, G = gill, E = eye; best tissues are those having highest activity and/or best resolution.

Leucine amino peptidase: Soak gel slice 30 min in 0.5 M boric acid. Rinse with distilled water. Add 70 mg L-leucyl-β-naphthylamide and 30 mg Black K salt to 50 ml LAP solution A (0.2 M NaOH, 0.2 M maleic anhydride), 10 ml LAP solution B (0.35 M NaOH), 40 ml distilled water.

Malate dehydrogenase: dissolve in 100 ml 0.05 M Tris-HCl buffer, pH 8.6, 150 mg L-malic acid, 20 mg MTT, 25 mg NAD, and 5 mg PMS.

Octanal dehydrogenase: dissolve in 100 ml 0.05 M Tris-HCl buffer, pH 8.6, 20 mg MTT, 25 mg NAD, 5 mg PMS, 1.0 ml octanol-ethanol solution (20 ml octanol in 80 ml ethanol). Allow octanol-ethanol solution to mix with buffer for two hours before using.

Phosphoglucose isomerase: dissolve in 75 ml 0.2 M Tris-HCl, pH 8,0, 20 ml distilled water, 5 ml 0.1 M $MgCl_2$, 25 mg NADP, 30 mg MTT, 50 mg D-fructose-6-phosphate, 20 units glucose-6-phosphate dehydrogenase and 10 mg PMS

Phosphoglucomutase: dissolve in 100 ml 0.1 M Tris-HCl, pH 7.1, 20 mg MTT, 10 mg NADP, 200 mg $MgCl_2$, 600 mg glucose-1-phosphate, 80 units glucose-6-phosphate dehydrogenase, 5 mg PMS.

Protein: combine 50 ml 0.25% coomassie blue (2.5 g/1 distilled water), 50 ml methanol, and 10 ml glacial acetic acid. Destain with gel fixing solution.

Tetrazolium oxidase: appears as white bands on the blue background produced by MTT in assays using buffer systems A and B. It can be scored most clearly on gel slices assayed for octanol dehydrogenase.

Xanthine dehydrogenase: To 100 ml of 0.05 M Tris-HCl, pH 8.0 add 200 mg hypoxanthine. Heat buffer to boiling until hypoxanthine goes into solution. Cool to room temperature and add 20 mg MTT, 25 mg NAD, 15 mg KCl, and 5 mg PMS.

All assays were allowed to stain at room tempature. Those assays utilizing MTT were allowed to stain in the dark.

Genetic Hypotheses.

All zymograms are in accord with simple patterns expected from codominant expression of allozymes. On any gel stained for any given enzyme or protein different zones of activity are evident. Within these zones, the position or the number of these bands may vary, but they will always occupy the same region of the gel relative to a standard. The position of these zones is consistent and is observed to be maintained throughout all genera studied. The enzymes or proteins of a given zone are generally considered to be the products of a simple genetic locus. When more than one locus is detected by a particular assay (multiple isozymes) they are designated by adding a hyphenated numeral to the gene symbol. The numeral one is assigned to the zone closest to the origin (least anodally migrating zone).

The standards used as the basis of comparison for all recorded runs were individuals #571 to 576 from the Twelve-Mile Creek population of Orconectes propinquus. Since there were 2-3 ml of homogenate for each individual there was more than enough to serve as controls for all runs.

The following are the migration distances from the origin of the 100 allele of each locus:

Acid phosphatase, 60 mm; Amylase-1, 3 mm; Amylase-2, 7 mm; Aldehyde oxidase-1, 12 mm; Aldehyde oxidase-2, 20 mm; Aldehyde oxidase-3, 30 mm; Aldehyde oxidase-4, 34 mm; Esterase-1, 45 mm; Esterase-3, 62 mm; Esterase-4, 65 mm; Esterase-5, 70 mm; Leucine amino peptidase, 55 mm; malate

dehydrogenase-1, 18 mm; Malate dehydrogenase-2, 40 mm; Octanol dehydrogenase, 35 mm; Phosphoglucose isomerase, 35 mm; Phosphoglucomutase-1, 60 mm; Phosphoglucomutase-2, 68 mm; Protein-1, 18 mm; Protein-2, 20 mm; Protein-3, 35 mm; Protein-4, 40 mm, Protein-5, 45 mm, Tetrazolium oxidase-1, 24 mm; Tetrazolium oxidase-2, 65 mm; Xanthine dehydrogenase, 32 mm.

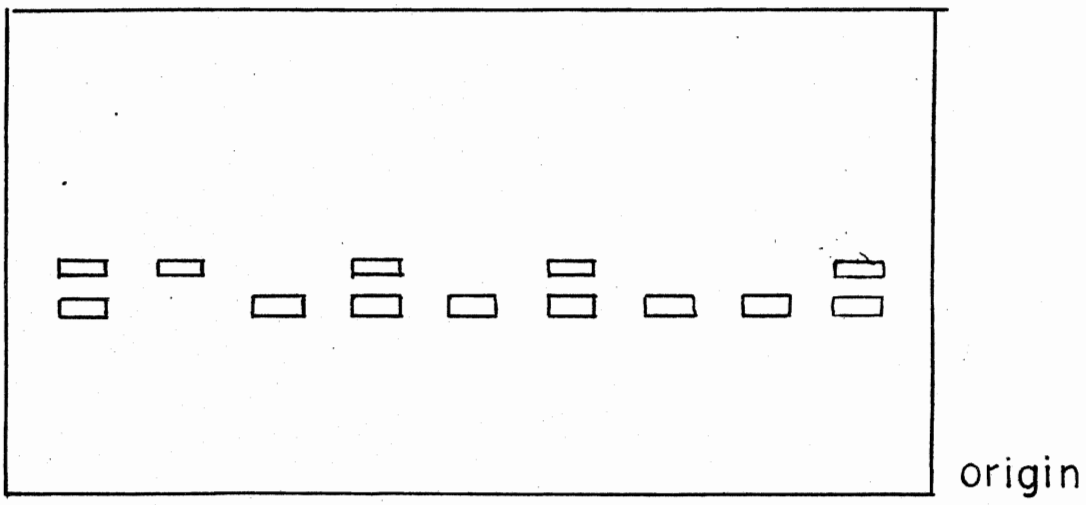
If all phenotypes in a zone are identical single bands in all individuals of a population or taxon, the enzyme or protein is assumed to be controlled by a single, monomorphic locus. If variation within a zone occurs with the presence of one- and two-banded phenotypes, the protein is assumed to be controlled by a single polymorphic locus and the active enzyme or protein is a monomer. The two bands signify an individual that is heterozygous for both allozyme alleles. If zonal variation exists in the form of one- and three-banded phenotypes, the protein is assumed to be encoded by a single polymorphic locus. In this case the active enzyme or protein is a dimer. The three-banded phenotypes signify heterozygous individuals for two different allozyme alleles. These protein products randomly associate to form two types of homodimers and one heterodimer. In crayfish, phosphoglucose isomerase is such an enzyme (Figure 4).

An allele is designated 100 if it migrates to the same position in the zone as that of the most common allele of the control population (*O. propinquus*, Twelve-Mile Creek). Other alleles are assigned numbers which are obtained by adding or subtracting the number of millimeters by which their positions differ from the 100 alleles. For example, an allele which migrates 2 mm farther than the 100 allele is designated 102. Allozyme genotypes are written with the gene symbol followed by a superscript giving the alleles present (*e.g.*, $Acph^{100}$).

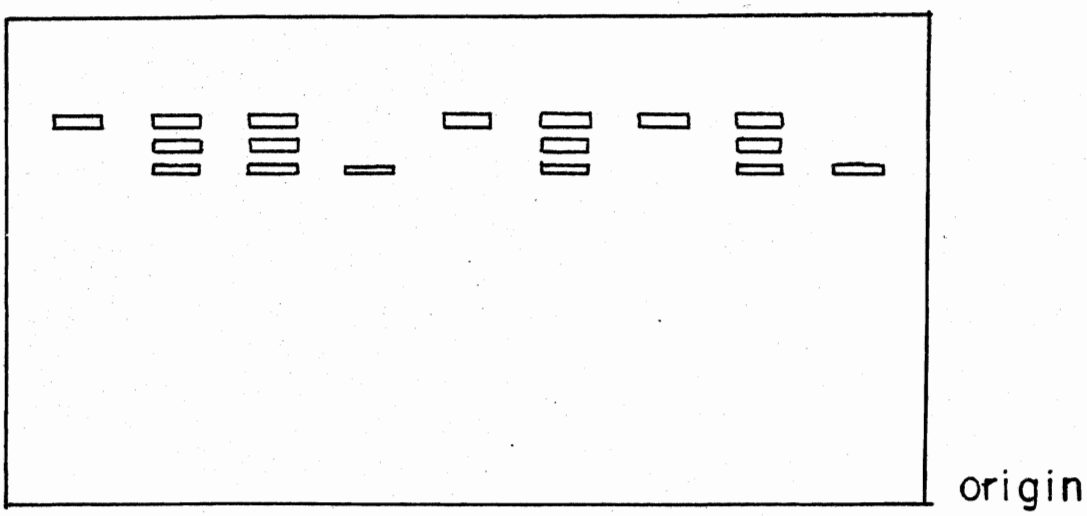
Figure 4. Polymorphic enzyme banding patterns

(a) monomeric enzyme

(b) dimeric enzyme



2a



2b

The Hardy-Weinberg equilibrium law was used to compare expected and observed genotypic distributions based on the genetic hypothesis for each polymorphic locus.

Computation of Genetic Identity and Genetic Distance.

The allele frequency data generated from the scoring of the zymograms can be utilized to produce measurements of genetic identity and genetic distance using the statistics developed by Nei (1971, 1972). The statistic of genetic identity or genetic similarity is based on Malecot's concept of the identity of genes within and between populations. It is the probability of obtaining two copies of the same allele from each of two populations and is calculated from the allele frequency data in the following way.

Let X and Y be two different populations (of the same or of different species) and j a given locus. The normalized probability that two alleles, one from each of the populations, are identical is given by:

$$I_j = \frac{\Sigma_i x_i y_i}{\sqrt{\Sigma_i x_i^2 \Sigma y_i^2}}$$

where x_i and y_i are the frequencies of the i-th allele in populations X and Y respectively. The mean "genetic similarity" over all loci scored simultaneously in both X and Y is given by

$$I = \frac{J_{xy}}{\sqrt{J_x J_y}}$$

where J_{xy}, J_x, and J_y are the arithmetic means over all loci of the terms $\Sigma x_i y_i$, $\Sigma {x_i}^2$, and $\Sigma {y_i}^2$ respectively. The value of I can range from 0, when allelic frequencies of two populations do not overlap, to 1 when the allelic frequencies are identical in both the compared populations.

The average "genetic distance" between two populations is given by:

$$D = -\ln I$$

The value of D can range from 0, when I = 1, to infinity. If mutations occur at random in the cistrons coding for the enzymes and proteins assayed, D can then be interpreted as the average number of electrophoretically detectable amino acid substitutions per locus which have occurred since populations X and Y diverged from one another (Nei, 1971, 1972).

RESULTS

Genetic Variability Within Populations

Genotypes and carapace lengths for all crayfish examined are presented in Appendix A.

Orconectes propinquus. Six natural populations of O. propinquus have been assayed for protein variation. Allele frequencies at each locus are presented in Table 4. Twenty-one loci were scored in the Hart Creek, Chippawa Creek I and Twelve-Mile Creek I samples, while sixteen, eighteen, and twenty loci were scored in the St. John's I, Oliphant, and Tobermory samples respectively. Sample sizes shown for each locus are equal to the number of genes sampled or twice the number of individuals.

A total of eight polymorphic loci appear over all six populations. A locus is considered to be polymorphic whenever two or more alleles appear in a sample. The polymorphic loci in these six populations are listed in Table 5 with the corresponding observed and expected heterozygosities. The expected heterozygosity is calculated using Levene's formula for small samples (Levene, 1949):

$$\text{Exp (H)} = \sum_{ij} \frac{4x_i x_j}{2n - 1}$$

where x_i and x_j are gene frequencies and 2n is the number of genes. Chippawa Creek I, Twelve-Mile Creek I, St. John's I, and Tobermory were all found to have four polymorphic loci, while Hart Creek and Oliphant had only

Table 4. Allele frequencies in all populations of Orconectes propinquus. A locus is arbitrarily classified as polymorphic if variants are observed in any population of any species.[1]

Locus	Allele[2,3]	Populations					
		HC	CCR-I	TMC-I	SJ-I	OLP	TOB
	(n)	(60)	(96)	(60)	(120)	(50)	(80)
Acph	98	--	--	0.17	--	--	--
	100	1.00	1.00	0.83	1.00	1.00	1.00
	101	--	--	--	--	--	--
	(n)	--	--	(60)	(120)	--	(80)
Amy-1	100			1.00	1.00		1.00
	(n)	--	--	(60)	--	--	--
Amy-2	100			1.00			
	(n)	--	(96)	--	--	(50)	--
Ao-1	100		1.00			1.00	
	(n)	(56)	(96)	(60)	(120)	(50)	(80)
Ao-2	94	--	--	--	--	--	0.12
	95	--	--	--	--	--	0.08
	96	--	--	--	--	--	0.11
	98	--	--	0.10	0.08	00	0.03
	100	0.45	1.00	0.87	0.92	1.00	0.66
	101	--	--	0.03	--	--	--
	102	0.55	--	--	--	--	--
	(n)	(60)	(96)	(60)	(120)	(50)	--
Ao-3	100	1.00	1.00	1.00	1.00	1.00	
	(n)	(60)	(96)	(60)	(120)	(50)	--
Ao-4	100	1.00	1.00	1.00	1.00	1.00	
	(n)	--	--	(60)	--	--	(80)
Est-3	100			1.00			1.00
	(n)	(60)	(96)	(60)	--	(50)	(80)
Est-4	100		1.00	1.00		1.00	1.00
	101	1.00					
	(n)	(60)	--	--	(120)	--	--
Est-5	100	1.00			1.00		
	(n)	(60)	(88)	(60)	(120)	(50)	(80)
Lap	95	--	0.05	0.02	0.10	--	--
	98	--	0.68	0.53	0.20	0.02	--
	100	0.73	0.27	0.45	0.70	0.60	0.34
	102	0.27	--	--	--	0.38	0.65
	104	--	--	--	--	--	0.01

Table 4, page 2.

Locus	Allele	Populations					
		HC	CCR-I	TMC-I	SJ-I	OLP	TOB
	(n)	(60)	(90)	--	--	--	(80)
Mdh-1	100	0.67	0.90				0.62
	102	0.33	0.10				0.38
	(n)	(60)	(96)	(60)	(120)	(50)	(80)
Mdh-2	100	1.00	1.00	1.00	1.00	1.00	1.00
	(n)	(60)	(96)	(60)	(120)	(50)	(80)
Odh	100	1.00	1.00	1.00	1.00	1.00	1.00
	(n)	(60)	(96)	(60)	(120)	(50)	(80)
Pgi	95	--	0.60	0.13	0.17	0.72	0.88
	100	1.00	0.40	0.87	0.83	0.28	0.12
	(n)	(60)	(96)	(60)	(120)	(50)	(80)
Pgm-1	98	--	0.05	--	--	--	--
	100	1.00	0.95	1.00	0.98	1.00	1.00
	102	--	--	--	0.02	--	--
	(n)	(60)	(96)	(60)	(120)	(50)	(80)
Pgm-2	100	1.00	1.00	1.00	1.00	1.00	1.00
	(n)	(60)	(96)	(60)	(120)	(50)	(80)
Pt-1	100	1.00	1.00	1.00	1.00	1.00	1.00
	(n)	(60)	(96)	--	(120)	--	(80)
Pt-2	100	1.00	1.00		1.00		1.00
	(n)	(60)	(96)	(60)	--	(50)	(80)
Pt-3	100	1.00	1.00	1.00		1.00	1.00
	(n)	(60)	(96)	(60)	--	(50)	(80)
Pt-4	100	1.00	1.00	1.00		1.00	1.00
	(n)	(60)	(96)	(60)	--	(50)	(80)
Pt-5	100	1.00	1.00	1.00		1.00	1.00
	(n)	(60)	(96)	(60)	--	--	(80)
To-1	100	1.00	1.00	1.00			1.00
	(n)	(60)	(96)	(60)	(120)	(50)	(80)
To-2	100	1.00	1.00	1.00	1.00	1.00	1.00
	(n)	(58)	(96)	(60)	(120)	(50)	(80)
Xdh	100	1.00	1.00	1.00	1.00	0.88	1.00
	102	--	--	--	--	0.12	--

Table 4, page 3.

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[1] Any population not assayed for a particular locus is represented with a dash in the sample size space.

[2] Allele 100 is the most common variant in O. propinquus (Twelve-Mile Creek I) and all others are identified by adding or subtracting the migration distance in millimeters relative to this standard.

[3] The number in parentheses represents the sample size at each locus.

Table 5. Observed and expected heterozygosities of all polymorphic loci in Orconectes propinquus.

Population	Locus	Heterozygosity		$\frac{H_E - H_O}{H_E}$ [2]
		Observed	Expected[1]	
Hart Creek	Ao-2	0.250	0.504	0.504
	Lap	0.533	0.397	-0.343
	Mdh-1	0.333	0.453	0.265
Chippawa Creek I	Lap	0.182	0.466	0.609
	Mdh-1	0.156	0.182	0.143
	Pgi	0.458	0.483	0.052
	Pgm-1	0.104	0.100	-0.040
Twelve-Mile Creek I	Acph	0.333	0.283	-0.177
	Ao-2	0.300	0.243	0.177
	Lap	0.400	0.520	0.231
	Pgi	0.267	0.237	-0.127
St. John's I	Ao-2	0.177	0.140	0.164
	Lap	0.317	0.463	0.315
	Pgi	0.267	0.280	0.046
	Pgm-1	0.033	0.033	0.000
Oliphant	Lap	0.800	0.504	-0.587
	Pgi	0.380	0.412	-0.165
	Xdh	0.240	0.216	-0.111
Tobermory	Ao-2	0.675	0.533	-0.266
	Lap	0.350	0.470	0.255
	Mdh-1	0.400	0.475	0.158
	Pgi	0.150	0.222	0.324

[1] Computed using Levene's formula for small samples (Levene, 1949).

[2] The mean $\left|\frac{H_E - H_O}{H_E}\right| = 0.229 \pm 0.167$

three. The percentages of polymorphic loci are as follows: Hart Creek, 14.3 percent; Chippawa Creek I, 19.0 percent; Twelve-Mile Creek I, 19.0 percent; St. John's I, 25.0 percent; Oliphant, 16.7 percent; Tobermory, 15.0 percent.

The proportion of loci observed to be heterozygous in the average individual at only the polymorphic loci and at all loci is given in Table 6. For the six samples Hart Creek, Chippawa Creek I, Twelve-Mile Creek I, St. John's I, Oliphant, and Tobermory the average individual is heterozygous at 5.3 ± 13.8 percent, 4.3 ± 10.7 percent, 5.7 ± 12.2 percent, 4.6 ± 9.8 percent, 8.4 ± 21.1 percent, and 7.9 ± 17.9 percent, respectively. This proportion is averaged over all loci in a particular sample and hence may be interpreted as the proportion of loci heterozygous in the average individual. Since approximately 80 percent of the loci in any given sample have no heterozygotes and the remaining polymorphic loci contain proportions of heterozygotes ranging from 0.033 to 0.800, the standard deviations will, of course, be large.

The average heterozygosity observed over all polymorphic loci in all six samples expressed as a percentage is 35.1 ± 8.1 percent. This means that approximately 35 percent of all polymorphic loci in the six samples of O. propinquus are heterozygous. The average heterozygosity observed over all loci in all six samples is 6.0 ± 1.7 percent. Neither value above is significantly different from the expected values.

Orconectes virilis and Orconectes immunis. Samples from one natural population of O. virilis and three natural populations of O. immunis were assayed for genetic variation. A total of eighteen loci were studied in

Table 6. Summary of genetic variation in samples from six natural populations of Orconectes propinquus.

	HC	CCR-I	TMC-I	SJ-I	OLP	TOB
No. of loci studied	21	21	21	16	18	20
No. of individuals	30	48	30	60	25	40
Proportion of polymorphic loci per population[1]	0.143	0.190	0.190	0.250	0.167	0.150
Average proportion of heterozygotes over polymorphic loci						
observed	0.372±0.119	0.225±0.137	0.300±0.074	0.184±0.114	0.507±0.229	0.394±0.187
expected[2]	0.445±0.051	0.308±0.169	0.321±0.116	0.229±0.161	0.377±0.120	0.425±0.120
Average proportion of heterozygotes over all loci studied						
observed	0.053±0.138	0.043±0.107	0.057±0.122	0.046±0.098	0.084±0.211	0.079±0.178
	0.064±0.159	0.059±0.142	0.061±0.137	0.057±0.128	0.063±0.149	0.085±0.178

[1] Loci with two or more alleles at any frequency are classified as polymorphic.

[2] Computed as the average over loci of the proportion of heterozygotes expected at each locus, using Levene's formula for small samples (Levene, 1949).

the O. virilis (St. John's I) sample and in the three O. immunis samples, St. John's III, Stinking Barn I, and Stinking Barn II, twelve, fourteen, and seventeen loci were studied respectively. Table 7 gives the allele frequencies for all four samples, Table 8, the observed and expected heterozygosities for each of the polymorphic loci, and Table 9 presents a summary of genetic variation.

The proportion of polymorphic loci in the O. virilis sample, is 11.1 per cent. The average proportion of heterzygotes observed for polymorphic loci is 25.8 ± 24.2 percent and that observed over all loci is 2.9 ± 11.4 per cent. The standard deviations are large since almost 90 per cent of the loci assayed have no heterozygotes and the polymorphic loci, Amy-1 and Lap, have heterozygote proportions of 0.017 and 0.500 respectively.

The proportion of polymorphic loci in the three samples of O. immunis are as follows: St. John's III, 8.3 per cent; Stinking Barn, 14.3 per cent; Stinking Barn II, 11.8 per cent. The average observed proportion of heterozygotes at the polymorphic loci averaged over all three samples is 33.8 ± 20.1 per cent. Over all loci the observed averaged proportion is 4.2 ± 3.4 per cent. It can be seen from summary Table 9 that the Stinking Barn I heterozygosities are considerably higher than both St. John's III and Stinking Barn II. This discrepancy accounts for the high standard deviations and is due to the Est-4 locus. Also, the observed values in Stinking Barn II show a much larger departure from the expected values than the other two samples; the Ao-2 heterozygote deficiency produces this disagreement.

Table 7. Allele frequencies in all populations of Orconectes virilis and Orconectes immunis. A locus is classified as polymorphic if variants are observed in any population of either species.

		Populations			
		O. virilis	O. immunis		
Locus	Allele[1]	SJ-II	SJ-III	SB-I	SB-II
	(n)	(120)	(120)	(160)	(60)
Acph	100	1.00	1.00	1.00	1.00
	(n)	(120)	(120)	(160)	(60)
Amy-1	100	0.99	1.00	1.00	1.00
	102	0.01	--	--	--
	(n)	(120)	--	--	--
Amy-2	100	1.00			
	(n)	(120)	--	--	--
Ao-1	100	1.00			
	(n)	(120)	(120)	(160)	(60)
Ao-2	98	1.00	0.02	0.01	0.02
	99	--	--	0.04	--
	100	--	0.02	0.18	0.23
	101	--	0.09	0.17	0.18
	102	--	0.87	0.60	0.57
	(n)	(120)	--	--	(60)
Ao-3	100	1.00			1.00
	(n)	(120)	(120)	(160)	(60)
Ao-4	100	1.00	1.00	1.00	1.00
	(n)	(120)	--	--	--
Est-1	100	1.00			
	(n)	--	--	(160)	--
Est-4	99			0.10	
	101			0.74	
	102			0.16	
	(n)	--	--	--	(60)
Est-5	(100)				1.00
	(n)	(120)	--	--	--
Lap	95	0.67			
	98	0.33			
	(n)	--	--	(160)	--
Mdh-1	102			1.00	

Table 7, page 2.

Locus	Allele	Populations			
		O. virilis	O. immunis		
		SJ-II	SJ-III	SB-I	SB-II
	(n)	(120)	(120)	(160)	(60)
Mdh-2	97	--	--	--	0.02
	100	1.00	1.00	1.00	0.98
	(n)	(120)	(120)	(160)	(60)
Odh	102	--	1.00	1.00	1.00
	108	1.00	--	--	--
	(n)	(120)	(120)	(160)	(60)
Pgi	100	1.00	1.00	1.00	1.00
	(n)	(120)	(120)	(160)	(60)
Pgm-1	100	1.00	--	--	--
	103	--	1.00	1.00	1.00
	(n)	(120)	(120)	(160)	(60)
Pgm-2	100	1.00	1.00	1.00	1.00
	(n)	(120)	(120)	(160)	(60)
Pt-1	100	1.00	1.00	1.00	1.00
	(n)	(120)	--	--	--
Pt-2	100	1.00			
	(n)	--	--	--	(60)
Pt-3	100				1.00
	(n)	--	--	--	(60)
Pt-4	100				1.00
	(n)	--	--	--	(60)
Pt-5	100				1.00
	(n)	(120)	(120)	(160)	(60)
To-2	100	1.00	1.00	1.00	1.00
	(n)	(120)	(120)	(160)	(60)
Xdh	101	--	1.00	1.00	1.00
	103	1.00	--	--	--

[1] Standards used for identifying alleles are the same as those for O. propinquus.

Table 8. Observed and expected heterozygosities of all polymorphic loci in Orconectes virilis and O. immunis.

Population	Locus	Heterozygosity		$\frac{H_E - H_O}{H_E}$ [2]
		Observed	Expected[1]	
St. John's II (O. virilis)	Amy-1	0.017	0.016	-0.062
	Lap	0.500	0.442	-0.116
St. John's III (O. immunis)	Ao-2	0.250	0.227	-0.101
Stinking Barn I (O. immunis)	Ao-2	0.662	0.586	-0.130
	Est-4	0.475	0.422	-0.126
Stinking Barn II (O. immunis)	Ao-2	0.357	0.596	0.401
	Mdh-2	0.033	0.033	0.000

[1] Computed using Leven's formula for small samples (Levene, 1949).

[2] The mean $\left|\frac{H_E - H_O}{H_E}\right| = 0.134 \pm 0.126$

Table 9. Summary of genetic variation in samples from four natural populations of *Orconectes virilis* and *O. immunis*.

	O. virilis	*O. immunis*		
	SJ-II	SJ-III	SB-I	SB-II
No. of loci studied	18	12	14	17
No. of individuals	60	60	80	30
Proportion of polymorphic loci per population	0.111	0.083	0.143	0.188
Average proportion of heterozygotes over polymorphic loci				
observed	0.258 ± 0.242	0.250	0.568 ± 0.094	0.195 ± 0.162
expected[1]	0.229 ± 0.213	0.227	0.502 ± 0.082	0.314 ± 0.282
Average proportion of heterozygotes over all loci studied				
observed	0.029 ± 0.114	0.021 ± 0.069	0.081 ± 0.202	0.023 ± 0.084
expected[1]	0.025 ± 0.101	0.019 ± 0.063	0.072 ± 0.179	0.037 ± 0.140

[1] Computed as the average over loci of the proportion of heterozygotes expected at each locus, using Levene's formula for small samples (Levene, 1949).

Cambarus robustus and Cambarus bartoni. Two populations each of Cambarus robustus and C. bartoni were sampled. The two C. robustus populations, Chippawa Creek II and Twelve-Mile Creek II were assayed at nineteen and eighteen loci respectively. The Opinicon sample of C. bartoni was assayed at eighteen loci and that from Georgia at fifteen loci.

Looking first at the two C. robustus samples, one can see from the allele frequency data in Table 10 that both samples are polymorphic at the Ao-2 and Lap loci. As can be seen in Table 11, both the observed and expected heterozygosities differ considerably at the Lap locus. Therefore although the proportion of polymorphic loci in each sample is about the same (Chippawa Creek II, 10.5 per cent; Twelve-Mile Creek II, 11.1 per cent, from Table 12), the average proportion of heterozygotes observed over the polymorphic loci is different (29.2 ± 24.2 per cent and 45.0 ± 15.0 per cent respectively). The average heterozygosity over all examined loci is 3.1 ± 11.9 per cent in Chippawa Creek II and 5.0 ± 15.0 per cent in Twelve-Mile Creek II with a mean of 4.0 ± 1.3 per cent for both samples. When the observed data are compared with the expected data, one sees that there is a trend toward heterozygote deficiency in both populations.

Looking next at the two samples of C. bartoni, the gene frequencies in Table 10 show that both samples are polymorphic at the Ao-2 and Lap loci, as are the samples of C. robustus, but the Opinicon sample is also polymorphic at the To-2 locus and the Georgia sample is polymorphic at the Pgi locus. The average proportion of heterozygotes observed at polymorphic loci is 50.0 ± 23.4 per cent for the Opinicon sample and 38.2 ± 25.1 percent for the Georgia sample with a mean for both of 44.1 ± 8.3 per cent (Table 12). The overall average observed hetero-

Table 10. Allele frequencies in all populations of Cambarus species. A locus is arbitrarily classified as polymorphic if variants are observed in any population of any species. Standards are the same as for Orconectes species.

		Populations			
		C. robustus		C. bartoni	
Locus	Allele	CCR-II	TMC-II	OPIN	GG
	(n)	(80)	(60)	(32)	(68)
Acph	101	1.00	1.00	1.00	1.00
	(n)	(80)	--	(32)	--
Amy-1	102	1.00		1.00	
	(n)	(76)	(60)	(32)	(68)
Ao-2	96	--	--	0.53	--
	97	0.16	0.02	--	--
	98	0.16	0.42	0.47	--
	100	9.67	0.56	--	--
	101	0.01	--	--	--
	102	--	--	--	0.66
	103	--	--	--	0.01
	104	--	--	--	0.32
	(n)	(80)	(60)	(32)	--
Ao-3	100	1.00	1.00	1.00	
	(n)	(80)	(60)	(32)	--
Ao-4	100	1.00	1.00	1.00	
	(n)	(80)	(60)	--	--
Est-3	105	1.00	1.00		
	(n)	(80)	(60)	(32)	--
Est-4	102	--	--	1.00	
	105	1.00	1.00	--	
	(n)	(80)	(60)	(32)	(68)
Lap	102	--	--	0.44	0.32
	103	0.10	0.55	--	--
	104	--	--	0.56	0.68
	105	0.90	0.45	--	--
	(n)	(80)	(60)	(32)	(68)
Mdh-2	100	1.00	1.00	1.00	1.00
	(n)	(80)	(60)	(32)	(68)
Odh	100	1.00	1.00	--	1.00
	104	--	--	1.00	--
	(n)	(80)	(60)	(32)	(68)
Pgi	100	1.00	1.00	1.00	0.99
	105	--	--	--	0.01

Table 10, page 2

Locus	Allele	Populations			
		C. robustus		C. bartoni	
		CCR-II	TMC-II	OPIN	GG
	(n)	(80)	(60)	(32)	(68)
Pgm-1	100	1.00	1.00	1.00	1.00
	(n)	(80)	(60)	(32)	(68)
Pgm-2	100	--	--	1.00	--
	102	1.00	1.00	--	1.00
	(n)	(80)	(60)	(32)	(68)
Pt-1	96	--	--	--	1.00
	97	1.00	1.00	--	--
	102	--	--	1.00	--
	(n)	(80)	(60)	(32)	(68)
Pt-2	95	--	--	--	1.00
	98	1.00	1.00	1.00	--
	(n)	(80)	(60)	(32)	(68)
Pt-3	86	--	--	--	1.00
	96	1.00	1.00	--	--
	98	--	--	1.00	--
	(n)	(80)	(60)	(32)	(68)
Pt-4	85	--	--	--	1.00
	96	1.00	1.00	--	--
	98	--	--	1.00	--
	(n)	--	--	--	(68)
Pt-5	85				1.00
	(n)	(80)	(60)	(32)	(68)
To-2	97	--	--	0.09	--
	100	--	--	0.91	1.00
	101	1.00	1.00	--	--
	(n)	(80)	(60)	(32)	(68)
Xdh	100	1.00	1.00	1.00	1.00

Table 11. Observed and expected heterozygosities of all polymorphic loci in *Cambarus robustus* and *C. bartoni*.

Population	Locus	Heterozygosity		$\frac{H_E - H_O}{H_E}$ [2]
		Observed	Expected[1]	
Chippawa Creek II (*C. robustus*)	Ao-2	0.533	0.505	-0.055
	Lap	0.050	0.182	0.725
Twelve-Mile Creek (*C. robustus*)	Ao-2	0.600	0.513	-0.170
	Lap	0.300	0.503	0.404
Opinicon (*C. bartoni*)	Ao-2	0.562	0.512	-0.098
	Lap	0.750	0.506	-0.482
	To-2	0.188	0.175	-0.074
Georgia (*C. bartoni*)	Ao-2	0.588	0.465	-0.265
	Lap	0.529	0.444	-0.191
	Pgi	0.029	0.029	0.000

[1] Computed using Levene's formula for small samples (Levene, 1949).

[2] The mean $\left|\frac{H_E - H_O}{H_E}\right| = 0.246 \pm 0.228$

Table 12. Summary of genetic variation in samples from four natural populations of Cambarus robustus and C. bartoni.

	C. robustus		C. bartoni	
	CCR-II	TMC-II	OPIN	GG
No. of loci studied	19	18	18	15
No. of individuals	40	30	16	34
Proportion of polymorphic loci per population	0.105	0.111	0.167	0.200
Average Proportion of heterozygotes over polymorphic loci				
observed	0.292 ± 0.242	0.450 ± 0.150	0.500 ± 0.234	0.382 ± 0.251
expected[1]	0.344 ± 0.162	0.508 ± 0.005	0.398 ± 0.157	0.313 ± 0.201
Average proportion of heterozygotes over all loci studied				
observed	0.031 ± 0.119	0.050 ± 0.150	0.083 ± 0.209	0.076 ± 0.190
expected[1]	0.036 ± 0.118	0.056 ± 0.160	0.066 ± 0.162	0.063 ± 0.154

[1] Computed as the average over loci of the proportion of heterozygotes expected at each locus, using Levene's formula for small samples (Levene, 1949).

zygosity for the Opinicon sample is 8.3 ± 20.9 percent and that for the Georgia sample is 7.6 ± 19.0 percent with a mean of 7.9 ± 0.5 percent.

The average heterozygosity for all four samples of both species at polymorphic loci and at all loci is not largely different from the species means: average heterozygosity, polymorphic loci, 40.6 ± 9.0 percent, all loci, 6.0 ± 2.4 percent. The amount of genetic variation in these four samples of Cambarus species is thus of the same order as that for Orconectes species.

Procambarus clarkii and Procambarus pictus. Only one sample of Procambarus clarkii could be obtained (Texas). A total of thirty individuals were assayed at fifteen loci, 13.3 percent of which were polymorphic. The allele frequency data in Table 13 show that the Ao-2 and Lap loci are the only two polymorphic loci.

The average proportion of heterozygotes observed over polymorphic loci and over all loci are 38.4 ± 11.6 percent and 5.1 ± 13.7 percent, respectively. These values do not differ greatly from the expected averages of 36.8 ± 12.5 percent and 4.9 ± 13.3 percent as seen in Table 15.

Three natural populations of P. pictus were sampled: Cape Cod I, assayed for eighteen loci; Cape Cod II for seventeen loci; Rhode Island, for eighteen loci. All three samples were found to be polymorphic at the Lap locus with the amount of observed heterozygosity differing in all three samples (Table 14). The Rhode Island sample was polymorphic at three of the eighteen loci assayed (16.7 percent) while the samples of Cape Cod I and Cape Cod II were polymorphic at two of eighteen loci (11.1 per cent) and two of seventeen loci (11.8 percent) respectively.

Table 13. Allele frequencies in all populations of Procambarus species. A locus is arbitrarily classified as polymorphic if variants are observed in any population of any species. Standards are the same as for Orconectes species.

		Populations			
		P. clarkii	P. pictus		
Locus	Allele	TEX	CC-I	CC-II	RI
	(n)	(60)	(32)	(50)	(34)
Acph	100	1.00	1.00	1.00	1.00
	(n)	(60)	(32)	(50)	(34)
Amy-1	100	1.00	1.00	1.00	1.00
	(n)	--	(32)	--	(34)
Amy-2	100		1.00		1.00
	(n)	(60)	(32)	(50)	(34)
Ao-2	96	0.02	--	--	--
	98	0.10	--	--	--
	99	0.02	--	--	--
	100	0.87	0.25	--	--
	101	--	--	1.00	--
	102	--	0.75	--	0.76
	104	--	--	--	0.24
	(n)	--	(32)	(50)	(34)
Ao-3	100		1.00	1.00	1.00
	(n)	--	(32)	(50)	(34)
Ao-4	100		1.00	1.00	1.00
	(n)	(60)	(32)	(50)	(16)
Lap	97	0.42	--	--	--
	98	0.58	0.16	--	0.19
	100	--	--	0.02	--
	102	--	0.84	0.22	0.75
	104	--	--	0.76	0.06
	(n)	(60)	(32)	(50)	(34)
Mdh-2	100	1.00	1.00	1.00	1.00
	(n)	(60)	(32)	(50)	(34)
Odh	100	1.00	1.00	1.00	1.00
	(n)	(60)	(32)	(50)	(34)
Pgi	100	--	1.00	1.00	1.00
	105	1.00	--	--	--
	(n)	(60)	(32)	(50)	(34)
Pgm-1	100	--	1.00	1.00	1.00
	104	1.00	--	--	--

Table 13, page 2.

Locus	Allele	Populations			
		P. clarkii	P. pictus		
		TEX	CC-I	CC-II	RI
	(n)	--	(32)	(50)	(34)
Pgm-2	102		1.00	1.00	1.00
	(n)	(60)	(32)	--	(34)
Pt-1	100	1.00	1.00		1.00
	(n)	--	--	(50)	--
Pt-2	100			1.00	
	(n)	(60)	(32)	(50)	(34)
Pt-3	100	1.00	1.00	1.00	1.00
	(n)	(60)	(32)	(50)	(34)
Pt-4	100	1.00	1.00	1.00	1.00
	(n)	(60)	(32)	(50)	(34)
Pt-5	100	1.00	1.00	1.00	1.00
	(n)	(60)	(32)	--	(34)
To-1	100	1.00	1.00		1.00
	(n)	(60)	(32)	(50)	(34)
To-2	97	--	--	--	0.03
	100	1.00	1.00	1.00	0.97
	(n)	(60)	--	(50)	--
Xdh	100	1.00		0.86	
	102	--		0.14	

Table 14. Observed and expected heterozygosities of all polymorphic loci in *Procambarus clarkii* and *P. pictus*.

Population	Locus	Heterozygosity		$\frac{H_E - H_O}{H_E}$ [2]
		Observed	Expected[1]	
Texas (*P. clarkii*)	Ao-2	0.267	0.243	-0.099
	Lap	0.500	0.493	-0.014
Cape Cod I (*P. pictus*)	Ao-2	0.375	0.388	0.034
	Lap	0.188	0.275	0.316
Cape Cod II (*P. pictus*)	Lap	0.400	0.380	-0.053
	Xdh	0.200	0.244	0.180
Rhode Island (*P. pictus*)	Ao-2	0.235	0.371	0.367
	Lap	0.250	0.425	0.412
	To-2	0.059	0.059	0.000

[1] Computed using Levene's formula for small samples (Levene, 1949).

[2] The mean $\left|\frac{H_E - H_O}{H_E}\right| = 0.164 \pm 0.162$

For polymorphic loci, the average heterozygosity observed over all three samples is 25.4 ± 6.4 percent and that for all loci is 3.2 ± 0.3 percent. As seen in Table 15 the individual overall observed heterozygosities barely differ from one another. If one averages the observed heterozygosities over all four samples of Procambarus species one finds the polymorphic average to be 28.7 ± 8.3 percent and the average over all loci is 3.7 ± 1.0 percent which is again the same low level of heterozygosity as for Orconectes and Cambarus species.

Genetic Divergence between Populations

Orconectes propinquus. The previous section shows how genetic variation is distributed over loci within a population of a given species. Examining each column in Table 4, one can see that all variation within a population occurs at eight loci: Acph, Ao-2, Est-4, Lap, Mdh-1, Pgi, Pgm-1, and Xdh. Scanning across the rows of that same table and comparing allele frequencies one can get a rough idea of the variation that occurs between populations. For example, the 100 allele of the Pgi locus is fixed at a frequency of 1.00 in the Hart Creek sample of O. propinquus while in the Chippawa Creek I sample the same allele has a frequency of 0.40.

From the allele frequency data and the differences that arise between them for each sample, a measure of genetic similarity or identity (I) and genetic distance (D) may be calculated using the formulae for I and D given in MATERIALS and METHODS. Table 16 gives the genetic I and D for the fifteen pairwise comparisons among the six samples of O. propinquus. The mean I and D values for all of these comparisons are 0.946 ± 0.040 and 0.056 ± 0.043 respectively.

Table 15. Summary of genetic variation in samples from four natural populations of *Procambarus clarkii* and *P. pictus*.

	P. clarkii	*P. pictus*		
	TEX	CC-I	CC-II	RI
No. of loci studied	15	18	17	18
No. of individuals	30	16	25	17
Proportion of polymorphic loci per population	0.133	0.111	0.118	0.167
Average proportion of heterozygotes over polymorphic loci				
observed	0.384 ± 0.116	0.282 ± 0.094	0.300 ± 0.100	0.181 ± 0.087
expected[1]	0.368 ± 0.125	0.332 ± 0.056	0.312 ± 0.068	0.285 ± 0.161
Average proportion of heterozygotes over all loci studied				
observed	0.051 ± 0.137	0.031 ± 0.094	0.035 ± 0.103	0.030 ± 0.076
expected[1]	0.049 ± 0.133	0.037 ± 0.106	0.037 ± 0.103	0.048 ± 0.125

[1] Computed as the average over loci of the proportion of heterozygotes expected at each locus, using Levene's formula for small samples (Levene, 1949).

Table 16. Genetic distance (below diagonal) and genetic identity (above) for six Orconectes propinquus populations.

	HC	CCR-I	TMC-I	SJ-I	OLP	TOB
HC	--	0.888	0.911	0.975	0.884	0.872
CCR-I	0.118	--	0.983	0.969	0.978	0.959
TMC-I	0.093	0.018	--	0.991	0.961	0.941
SJ-I	0.026	0.031	0.009	--	0.965	0.928
OLP	0.123	0.023	0.040	0.036	--	0.987
TOB	0.137	0.042	0.061	0.074	0.013	--

The largest value of I, and hence the smallest D value, occurs between the St. John's I and Twelve-Mile Creek I samples where I = 0.991 and D = 0.009. These samples are separated by about 5 km and are in the same drainage system. The slight deviation from total identity can be attributed to slight differences in allele frequencies at the following loci: Acph, Ao-2, Lap, Pgm-1, Pgi. The smallest value of I, and hence the largest D value occurs between the samples from Hart Creek and Tobermory where I = 0.872 and D = 0.137. The Hart Creek sample was taken from north of Kingston, Ontario, and that of Tobermory from the tip of the Bruce Peninsula, jutting out into Georgian Bay at Tobermory, Ontario. One sample is from the eastern side of Southern Ontario and the other from the western side.

The Hart Creek sample differs considerably more from the other samples than the others do from one another. This is due to the fact that at the Est-4 locus $I_j = 0$ when compared with all samples except St. John's I for which there was no Est-4 assayed. This is the only locus in all six samples for which $I_j = 0$. This also accounts for the reason that the St. John's I sample is much more similar to Hart Creek than the others. If the genetic I and D were calculated for all samples with the Est-4 locus excluded, the other samples would also appear more similar to Hart Creek. For example, leaving out the Est-4 locus, the I and D values between Hart Creek and Chippawa Creek I would be 0.938 and 0.064 respectively.

Orconectes species comparison

Table 17 shows the genetic identities and genetic distances of forty-five pairwise comparisons between all ten samples from Orconectes propinquus, O. virilis, and O. immunis. The mean I and D values for all

Table 17. Genetic distance (below diagonal) and genetic identity (above) for Orconectes species populations.

	HC	CCR-I	TMC-I	SJ-I	OLP	TOB	SJ-II	SJ-III	SB-I	SB-II
HC	--	0.888	0.911	0.975	0.884	0.872	0.750	0.704	0.705	0.803
CCR-I	0.118	--	0.983	0.969	0.978	0.959	0.744	0.606	0.551	0.737
TMC-I	0.093	0.018	--	0.991	0.961	0.941	0.777	0.671	0.648	0.776
SJ-I	0.026	0.031	0.009	--	0.965	0.928	0.770	0.674	0.697	0.745
OLP	0.123	0.023	0.040	0.036	--	0.987	0.702	0.596	0.577	0.730
TOB	0.137	0.042	0.061	0.074	0.013	--	0.669	0.584	0.556	0.695
SJ-II	0.288	0.296	0.253	0.261	0.353	0.401	--	0.675	0.684	0.709
SJ-III	0.351	0.501	0.399	0.395	0.518	0.538	0.394	--	0.996	0.994
SB-I	0.350	0.596	0.434	0.361	0.550	0.586	0.380	0.004	--	1.000
SB-II	0.219	0.305	0.253	0.294	0.315	0.364	0.343	0.006	0.000	--

three species are 0.686 ± 0.070 and 0.381 ± 0.106 respectively. For *O. immunis* the mean I value for the three samples is 0.997 ± 0.003 and that of D is 0.003 ± 0.003. Thus there is a high degree of similarity between the three samples, ranging from I = 1.00 (Stinking Barn I and Stinking Barn II) to I = 0.994 (St. John's III and Stinking Barn II). *O. immunis* and *O. propinquus* samples show values of $I_j = 0$, complete genetic divergence, at the *Odh* and *Pgm-1* loci.

The one sample obtained of *O. virilis* can be seen to have approximately the same degree of similarity to *O. propinquus* as does *O. immunis*. Values of I and D between *O. virilis* and *O. propinquus* range from 0.669 and 0.401 respectively to 0.777 and 0.253. Values of I and D between *O. virilis* and *O. immunis* are also of the same order, with I from 0.675 to 0.709 and D from 0.343 to 0.394.

Between *O. virilis* and *O. propinquus* I_j was found to equal zero at the *Odh* and *Xdh* loci. *Ao-2*^{98} is fixed in the *O. virilis* population, but polymorphic in four of six *O. propinquus* populations. Between the samples of *O. virilis* and *O. immunis*, $I_j = 0$ at the *Odh*, *Pgm-1*, and *Xdh* loci. Thus the differences between *O. virilis* and *O. propinquus* are not the same as those for *O. virilis* and *O. immunis*. Summarizing these differences, *O. propinquus* is fixed for the *Odh*^{100} and *Pgm-1*^{100} alleles and fixed for the *Xdh*^{100} allele in five of six samples (Oliphant has *Xdh*^{102} allele in low frequency). *O. virilis* is fixed for the *Ao-2*^{98}, *Odh*^{108}, *Pgm-1*^{100}, and *Xdh*^{103} alleles. *O. immunis* is fixed for the *Odh*^{102}, *Pgm*^{103}, and *Xdh*^{101} alleles. At no loci other than those listed above does the value of $I_j = 0$ between the three species.

Cambarus robustus and Cambarus bartoni. Table 18 shows the six pairwise comparisons of genetic identity and genetic distance between four samples representing the genus Cambarus in this study. The two C. robustus populations show a very high degree of similarity, but the two samples of C. bartoni do not. In fact, the two C. bartoni samples are very dissimilar. Although the two samples key out taxonomically to be the same species, the genetic data suggest that they probably are not: the genetic identities of the two C. bartoni samples are more similar to the C. robustus samples than they are to one another.

When on examines the allele frequency data of the two C. robustus samples, one sees that at no single locus does $I_j = 0$. The allele frequencies vary only at the Ao-2 and Lap loci. The two samples were collected from populations that are separated by approximately 25 km and are not in the same drainage systems.

Examining the allele frequency data for both C. bartoni samples, it can be seen that $I_j = 0$ at the following loci: Ao-2, Odh, Pgm-2, Pt-1, Pt-2, Pt-3, and Pt-4. Of the fifteen loci in common between the two samples, seven loci were completely dissimilar with a resulting I value of 0.495 and D value of 0.703.

Between C. robustus samples and that of the Opinicon sample of C. bartoni values of 0 for I_j were found at the following loci: Est-4, Lap, Odh, Pgm-2, Pt-1, To-2, Pt-2, and Pt-4. Between the samples of C. robustus and the Georgia sample of C. bartoni I_j was equal to zero at the following loci: Ao-2, Lap, Odh, Pt-1, Pt-2, Pt-3, Pt-4, and To-2. It must be kept in mind that the two C. bartoni samples came from populations that are separated by about 2400 km. However, this still

Table 18. Genetic distance (below diagonal) and genetic identity (above) for Cambarus species populations.

	CCR-II	TMC-II	OPIN	GG
CCR-II	--	0.985	0.531	0.529
TMC-II	0.015	--	0.515	0.536
OPIN	0.632	0.664	--	0.495
GG	0.637	0.624	0.703	--

does not account for the fact that the C. robustus samples are genetically more similar to both C. bartoni samples. The mean genetic identities for all four samples of Cambarus species is 0.528 ± 0.009 and that for genetic distance is 0.639 ± 0.017. There is obviously a very low degree of similarity over all samples of C. robustus and C. bartoni, considerably lower than that for all species of Orconectes studied (mean I = 0.686 ± 0.070, mean D - 0.381 ± 0.106).

Procamburus clarkii and Procambarus pictus. Table 19 contains the genetic identity and genetic distance values for the six pairwise comparisons of the four Procambarus species studied, one of P. clarkii and three of P. pictus. The mean I and D values for P. pictus are 0.912 ± 0.004 and 0.092 ± 0.004 respectively. Examining the allele frequencies for the three samples of P. pictus one can see that at only one locus does the value of I equal zero. The Cape Cod II sample, at the Ao-2 locus, shows complete dissimilarity from both the Cape Cod I and Rhode Island samples. However, the Cape Cod I and Rhode Island samples do have common alleles in different frequencies. The only other loci between the three samples found to be polymorphic are the Lap and To-2 loci. These data thus show that there is a relatively high degree of similarity between the three samples of P. pictus.

Examining next the one sample of P. clarkii from Texas, the allele frequency data shows that values of zero for I_j are found at the Pgi, Pgm-1, and To-2 loci when compared to the three P. pictus samples. Also, the Texas sample of P. clarkii shows that $I_j = 0$ when compared at the Ao-2 locus of the Cape Cod II and Rhode Island samples of P. pictus, but has a common allele with the Cape Cod I sample at the same locus. This

Table 19. Genetic distance (below diagonal) and genetic identity (above) for Procambarus species.

	TEX	CC-I	CC-II	RI
TEX	--	0.699	0.637	0.689
CC-I	0.358	--	0.910	0.996
CC-II	0.450	0.094	--	0.915
RI	0.373	0.004	0.089	--

accounts for the slightly higher value of I between P. clarkii and P. pictus Cape Cod I (0.699) than for P. clarkii and the other two samples (Texas-Cape Cod II, I = 0.637; Texas-Rhode Island, I = 0.689). The mean genetic similarity and genetic distance for all four samples of Procambarus species is 0.675 ± 0.033 and 0.394 ± 0.049 respectively. These values are very close to the means for the Orconectes species samples (mean I = 0.686 ± 0.070, mean D = 0.381 ± 0.106).

Genetic Distance and Genetic Identity Summary.

Table 20 lists the 153 pairwise comparisons between all eighteen natural populations of crayfish representing three genera and seven species. The identities and distance range from I = 0.171, D = 1.768 between P. clarkii and C. bartoni (Opinicon) to I = 1.00, D = 0 between two O. immunis samples (Stinking Barn I and Stinking Barn II).

Table 21 shows the mean intraspecific genetic similarities and distances for populations where two or more conspecific populations were sampled as well as the interspecific and intergeneric means. As stated previously, the species of Orconectes and Procambarus show almost the same degree of similarity and distance when the identity and distance values from all populations of one genus are averaged. The I for Cambarus species is somewhat lower. The most striking I and D means are the Orconectes and Procambarus comparisons (I = 0.744 ± 0.124, D = 0.313 ± 0.196). These values are considerably higher than those for Orconectes-Cambarus or Cambarus-Procambarus samples.

Table 20. Genetic distance (below diagonal) and genetic identity (above) for all species tested.

	HC	CCR-I	TMC-I	SJ-I	OLP	TOB	SJ-II	SJ-III	SB-I	SB-II	CCR-II	TMC-II	OPIN	GG	TEX	CC-I	CC-II	RI
HC	--	0.888	0.911	0.975	0.884	0.872	0.750	0.704	0.705	0.803	0.450	0.452	0.502	0.456	0.712	0.903	0.848	0,897
CCR-I	0.118	--	0.983	0.969	0.978	0.959	0.744	0.606	0.551	0.737	0.435	0.432	0.456	0.381	0.782	0.838	0.805	0.826
TMC-I	0.093	0.018	--	0.991	0.961	0.941	0.777	0.671	0.748	0.776	0.439	0.466	0.496	0.454	0.778	0.880	0.837	0.871
SJ-I	0.026	0.031	0.009	--	0.965	0.928	0.770	0.674	0.697	0.745	0.524	0.565	0.556	0.523	0.680	0.825	0.808	0.810
OLP	0.123	0.023	0.040	0.036	--	0.987	0.702	0.596	0.577	0.730	0.452	0.450	0.487	0.407	0.725	0.833	0.789	0.816
TOB	0.137	0.042	0.061	0.074	0.013	--	0.669	0.584	0.556	0.695	0.284	0.302	0.364	0.383	0.754	0.841	0.791	0.828
SJ-II	0.288	0.296	0.253	0.261	0.353	0.401	--	0.675	0.684	0.709	0.358	0.408	0.521	0.352	0.411	0.747	0.668	0.750
SJ-III	0.351	0.501	0.399	0.395	0.518	0.538	0.394	--	0.996	0.994	0.259	0.284	0.426	0.369	0.412	0.716	0.566	0.715
SB-I	0.350	0.596	0.434	0.361	0.550	0.586	0.380	0.004	--	1.000	0.255	0.276	0.417	0.357	0.433	0.712	0.583	0.707
SB-II	0.219	0.305	0.253	0.294	0.315	0.364	0.343	0.006	0.000	--	0.287	0.307	0.411	0.268	0.571	0.791	0.698	0.786
CCR-II	0.798	0.833	0.824	0.646	0.794	1.257	1.026	1.349	1.367	1.249	--	0.985	0.531	0.529	0.374	0.500	0.512	0.491
TMC-II	0.795	0.838	0.763	0.572	0.799	1.196	0.896	1.258	1.287	1.182	0.015	--	0.515	0.536	0.407	0.542	0.554	0.535
OPIN	0.688	0.784	0.702	0.587	0.720	1.012	0.652	0.853	0.875	0.889	0.632	0.664	--	0.495	0.171	0.445	0.483	0.446
GG	0.784	0.964	0.791	0.647	0.900	0.960	1.044	0.997	1.030	0.316	0.637	0.624	0.703	--	0.248	0.552	0.562	0.562
TEX	0.340	0.246	0.251	0.385	0.321	0.283	0.888	0.888	0.836	0.560	0.984	0.900	1.768	1.394	--	0.699	0.637	0.689
CC-I	0.102	0.176	0.128	0.193	0.183	0.174	0.292	0.334	0.339	0.235	0.693	0.613	0.809	0.594	0.358	--	0.910	0.996
CC-II	0.165	0.217	0.178	0.214	0.237	0.235	0.404	0.569	0.540	0.360	0.668	0.591	0.729	0.577	0.450	0.094	--	0.915
RI	0.108	0.191	0.138	0.211	0.203	0.189	0.287	0.335	0.346	0.240	0.711	0.626	0.807	0.577	0.373	0.004	0.089	--

TABLE 21

Mean Genetic Similarities and Distances

Populations	N	n	Identity	Distance
O. propinguus	6	15	0.946 ± 0.040	0.056 ± 0.043
O. immunis	3	3	0.997 ± 0.003	0.003 ± 0.003
C. robustus	2	1	0.985	0.015
C. bartoni	2	1	0.495	0.703
P. pictus	3	2	0.912 ± 0.004	0.092 ± 0.004
Species				
Orconectes	3	27	0.686 ± 0.070	0.381 ± 0.106
Cambarus	2	4	0.528 ± 0.009	0.639 ± 0.017
Procambarus	2	3	0.675 ± 0.033	0.394 ± 0.049
Genera				
Orconectes-Cambarus	2	40	0.407 ± 0.086	0.923 ± 0.225
Orconectes-Procambarus	2	40	0.744 ± 0.124	0.313 ± 0.196
Cambarus-Procambarus	2	16	0.462 ± 0.114	0.815 ± 0.329

N is the number of populations or taxa studied; n is the number of comparisons.

Discussion

Genetic Variability in Cambarinae

The results of this study reveal low levels of genetic variation in all populations of Orconectes propinquus, O. virilis, O. immunis, Cambarus robustus, C. bartoni, Procambarus clarkii, and P. pictus examined. O. propinquus was scored for a total of twenty-six loci, O. immunis for a total of nineteen loci, O. virilis for a total of eighteen loci, C. robustus for a total of nineteen loci, C. bartoni for a total of nineteen loci, P. clarkii for a total of fifteen loci, and P. pictus for a total of twenty loci. In genetic variation studies on animals, excluding man, the number of loci scored ranges from one to forty-three with a mean of 17.98 ± 9.98 (Powell, 1975). The number of loci used in this study for each species falls within this range. The sampling requirements (large number of loci, moderate number of organisms) for estimates of heterozygosity and genetic identity have been met, insofar as possible (Nei and Roychoudhury, 1974). Sources of sampling errors in heterozygosity estimates and genetic distances in any genetic variation study are: 1. variation among individuals and among loci, and 2. differences in levels of genetic variability among loci. This effect of the second source can be seen in the large standard deviations of the average heterozygosities in Tables 6, 9, 12, and 15. The number of individuals sampled and the number of loci assayed are, however, adequate for estimating genetic variation within and between the species examined in this study (Avise, 1974).

The mean heterozygosities for each species are given in Table 22. The observed heterozygosities range from 0.080 in C. bartoni down to 0.025 in O. virilis with an overall mean of 0.047. When the heterozygosity values from these species are compared to other invertebrates one finds that they are very low. Powell (1975) lists a heterozygosity estimate of 0.146 ± 0.009 for invertebrates from 58 studies in which ten or more loci were assayed. Vertebrates from 71 studies in which 10 or more loci were assayed, however, give a mean heterozygosity of 0.050 ± 0.004. Levels of genetic variation in crayfish are more comparable to those in vertebrates. However, if one examines heterozygosity in crustaceans for which genetic variation results are available, one sees that these are also low in comparison with other invertebrates (Gooch and Schopf, 1972; Tracey et al., 1975; Hedgecock et al., 1977). Among these, the lobsters show particularly low levels of heterozygosity (0.040) in both the American and European species of Homarus.

The Cambarinae are therefore comparable to other decapod crustaceans with respect to levels of genetic variation. The decapods, when compared to other invertebrates that have been studied electrophoretically, are large, mobile, omnivorous organisms. Large, mobile, omnivors have the ability to alter their environmental circumstances (by moving) and therefore, may have very little need for the highly flexible adaptive strategy which must be pursued by small, immobile organisms that cannot change their habitat. One would expect an organism with a eurytolerant enzyme strategy (Somero and Low, 1977) to evolve to a state of lower heterozygosity since a large number of alleles in its gene pool would no longer be required for physiological adaptation (Levins, 1968; Selander and Kaufman, 1973). The reduced level of heterozygosity reduces the

Table 22. Estimated and actual mean heterozygosities for all species of Orconectes, Cambarus and Procambarus examined.

Mean Heterozygosity	Species						
	O. propinquus	O. virilis	O. immunis	C. robustus	C. bartoni	P. clarkii	P. pictus
Estimated	0.065	0.029	0.043	0.046	0.065	0.049	0.041
Observed	0.060	0.025	0.042	0.040	0.080	0.051	0.032

segregational load associated with polymorphic loci. Valentine (1976) states that homozygosity is generally observed in populations which are subjected to seasonally fluctuating trophic resources. Crayfish, as a group, occupy such a niche and therefore these results tend to reinforce the Ayala-Valentine hypothesis.

Another explanation of the low levels of genetic variation among the Cambarinae is the possibility of a lower mutation rate compared with other invertebrates. They may now have hit upon a near optimum evolutionary strategy which has produced a corresponding drop in mutational pressure (Ohta, 1974).

Large populations will tend to maintain higher levels of heterozygosity than small populations since the probability of loss of alleles from the gene pool through random drift will be less. When a population reaches steady state, heterozygosity, H, can be estimated by:

$$H = 1 - \frac{1}{4N\mu + 1}$$

where N is the effective population size and μ is the mutation rate to neutral alleles (Lewontin, 1974). However, since the mutation rate of a species is, at best, difficult to determine (Auerbach and Kilbey, 1971) as is the effective population size, values of H determined by this method are, at best, rough estimates. The above formula may also be used in the calculation of population size estimates in the form:

$$N = \frac{H}{4\mu(1-H)}$$

if both the heterozygosity and the mutation rate are known. If we assume

a low mutation rate ($\mu = 10^{-5}$), the genetic estimate of N may be compared with a mark-recapture estimate for the Twelve-Mile Creek population of O. propinquus (Tracey, Nemeth, Bradley, Espinet, and Golding, 1976). Heterozygosity in TMC-I equals 0.057; the genetic estimate of N is, therefore 1,511. The mark-recapture estimate for this population is 4100 ± 1894. The estimates are reasonably close suggesting that the mutation rate may, indeed, be low in this population. Note that N and μ are inversely related, so an order of magnitude decrease in μ yields a corresponding N increase.

Genetic Divergence Between Populations, Species, and Genera.

In general, the genetic similarities between populations of the same species are close to the high values (I > 0.90) observed in other studies (Avise, 1976). In examining Table 20, one finds this to be true of all species in which two or more samples were taken with the exception of Cambarus bartoni. Looking at the similarities and distances of Orconectes propinquus, they are observed to range from I = 0.872 and D - 0.137 in the comparison between Hart Creek and Tobermory to I = 0.991 and D = 0.009 in that between Twelve-Mile Creek-I and St. John's-I. The means for all fifteen comparisons are I = 0.946 ± 0.040 and D = 0.056 ± 0.043. The two comparisons noted above are the most widely separated (Hart Creek-Tobermory) and the closest (Twelve-Mile Creek-I-St. John's-I) geographically. If one compares the six samples according to genetic distance and geographic distance, a correlation of 0.75 ($t = 4.12$, $P < 0.001$) is found. With the exception of the Hart Creek-St. John's-I comparison, the general trend appears to be the greater the geographic distance, the greater the genetic

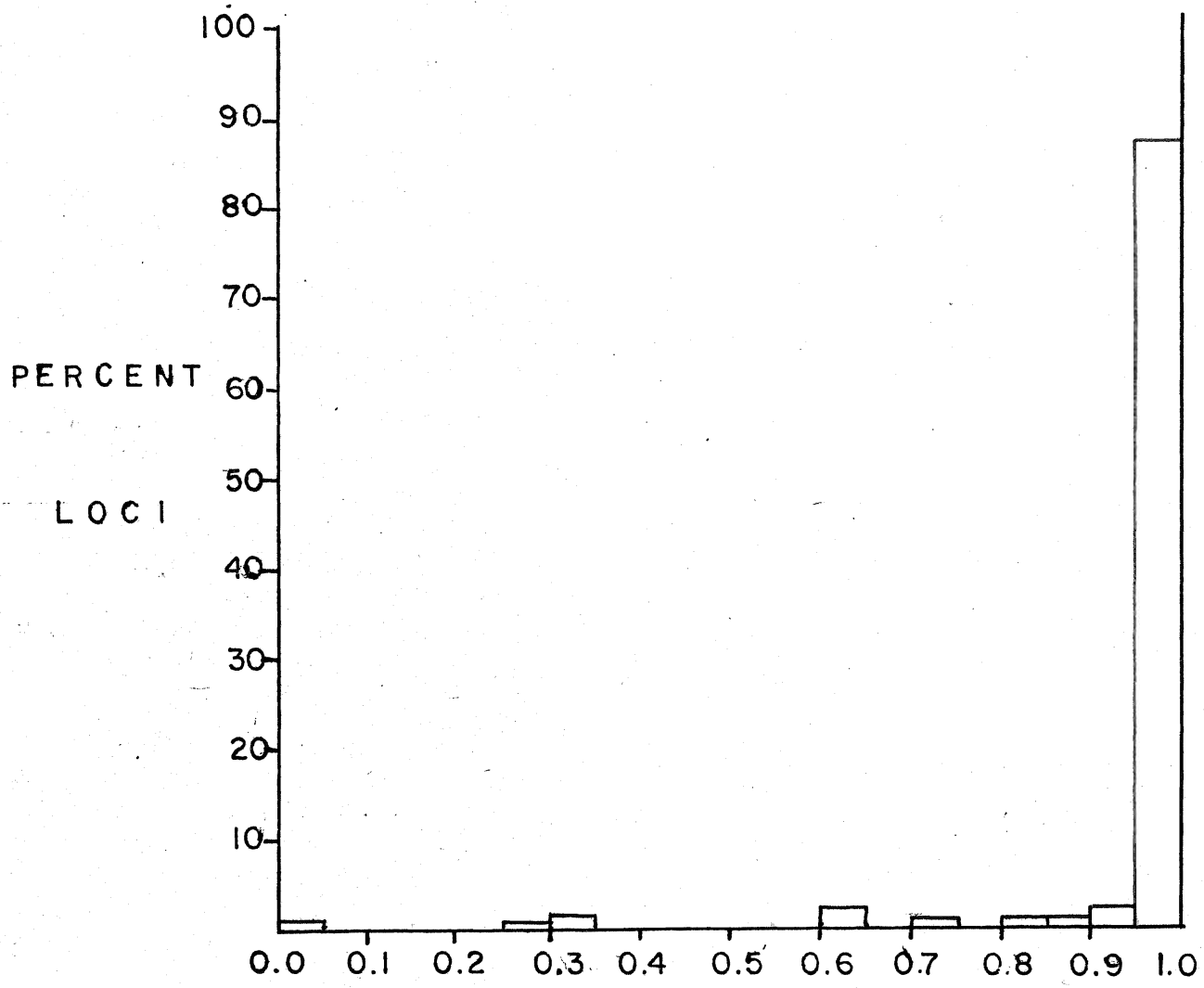
distance (Table 23). The reason that Hart Creek and St. John's-I do not fit the trend may be due to the fact that a smaller number of loci were assayed in the St. John's-I sample than in the others. The correlation does not, by any means prove that homogeneity is maintained by migration. Migration appears to be low in these populations; the mean distance between capture and recapture in the twelve Mile Creek population was 6.91 ± 7.22 meters. On the other hand Jolly estimates of the number of immigrants were high; the overall mean being 1913 ± 3947. This discrepancy is, at present, unresolved (Tracey, et al., 1976). Nevertheless it is clear that the kilometer-genetic distance correlation is explainable on grounds other than migration; habitat may, for example, be correlated with distance.

The mean genetic similarities and distances for the O. immunis samples, C. robustus samples, and P. pictus samples are I = 0.997 ± 0.003, D = 0.003 ± 0.003; I = 0.985, D = 0.015; I = 0.912 ± 0.004, D = 0.092 ± 0.004 respectively (see Table 21). All show the same high degree of similarity as do the samples of O. propinquus. If a locus by locus comparison of genetic similarity is done for each of the above species between each sample in each species one finds that very few of the total number of loci compared show a similarity less than one (Figure 5). The loci primarily responsible for the varying degrees of identiy over the total number of loci compared are Ao-2, Lap, in all species as well as Pgi in O. propinquus. The genetic similarity distributions within each species are therefore consistent with the findings of other genetic variation studies (Avise, 1974).

Table 23. Genetic distance (above diagonal) and distance in km separating any two populations (below diagonal) of Orconectes propinquus.

	HC	CCR-I	TMC-I	SJ-I	OLP	TOB
HC	--	0.118	0.093	0.026	0.123	0.137
CCR-I	525	--	0.018	0.031	0.023	0.042
TMC-I	505	24	--	0.009	0.040	0.061
SJ-I	510	26	5	--	0.036	0.074
OLP	440	290	260	265	--	0.013
TOB	550	400	370	375	80	--

Figure 5. Distribution of loci according to genetic identity observed in 348 locus by locus comparisons pooled from each of *O. propinquus*, *O. immunis*, *C. robustus*, and *P. pictus* samples.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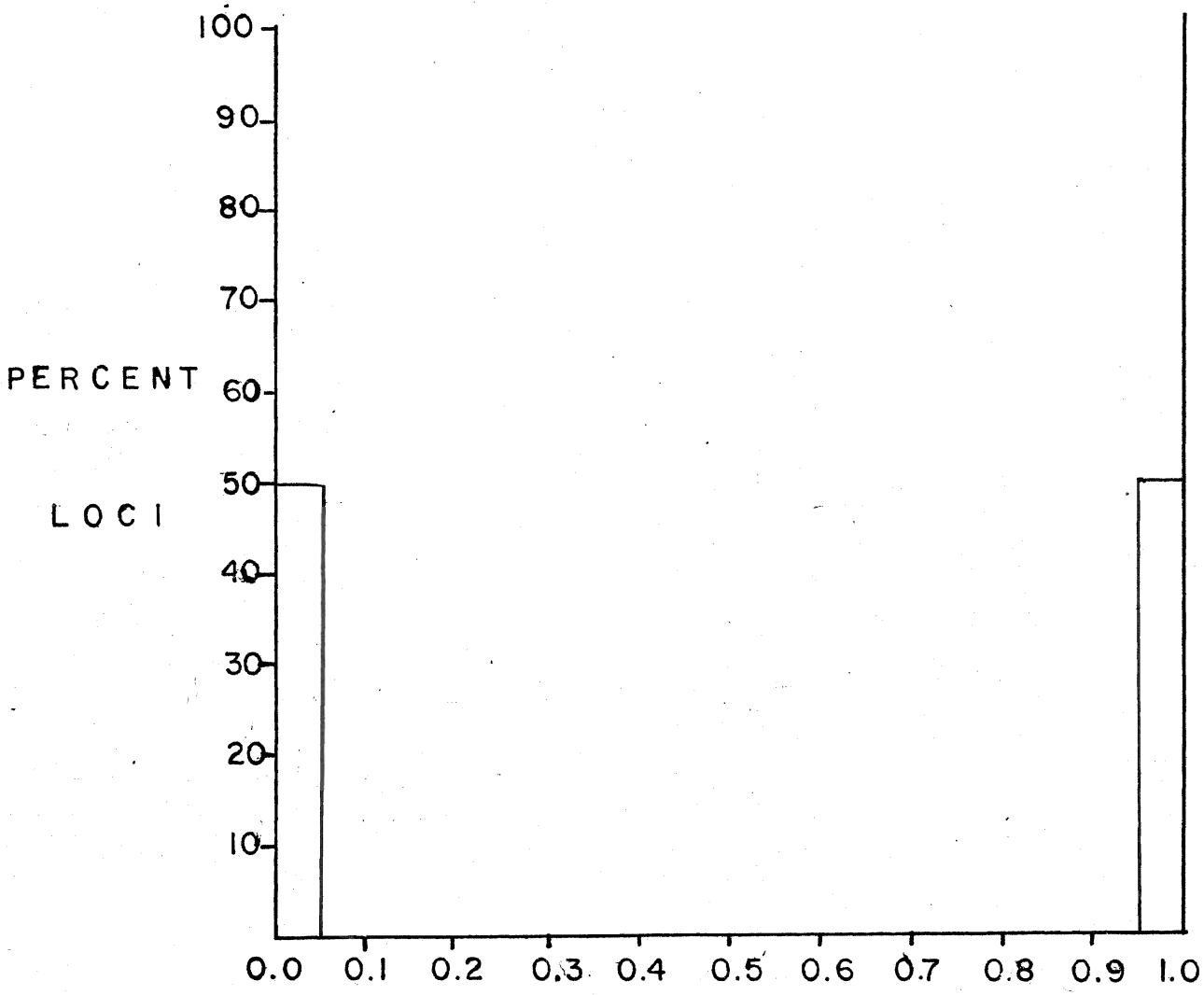
1.0
IDENTITY

(I)

As stated previously, the observed genetic similarity and difference between the two Cambarus bartoni samples is not consistent with other within species comparisons (I = 0.495; D = 0.703). When these two samples are compared locus by locus for genetic similarity, one finds that 50 percent of the loci compared show complete identity and 50 percent show complete dissimilarity (Figure 6). The two populations from which the samples were taken are separated by approximately 2400 km. Presumably the two populations have been effectively separated for a sufficient period of time to allow independent changes in their respective gene pools assuming both came from a common ancestral stock. It is interesting to note however, that a Pgi^{105} allele was detected in the Georgia sample of C. bartoni. This allele was also detected in the Texas sample of P. clarkii, but not in any of the other species examined. Another very interesting and puzzling observation is the fact that the two samples of C. bartoni are more genetically similar to the two C. robustus samples than they are to one another. It may be that hybridization maintains alleles across species lines in some populations. No direct evidence of hybridization was, however, uncovered. No clear explanation for these observations is readily available. At this time, all that can be said is that the level of genetic similarity between the two samples is comparable to that for species comparisons (Ayala, Tracey, Hedgecock, and Richmond, 1974).

If all samples from each species are compared with all samples from each of the other species locus by locus, one discovers that most of the comparisons (93.7 percent) are either highly similar (I > 0.950) or highly dissimilar (I < 0.050) (Figure 7). The intermediate identities between the loci of all of these species are at the Ao-2, Lap, and Pgi loci. The Lap locus was found to show polymorphism in all samples for

Figure 6. Distribution of loci according to genetic identity in 14 locus by locus comparisons between *C. bartoni* populations.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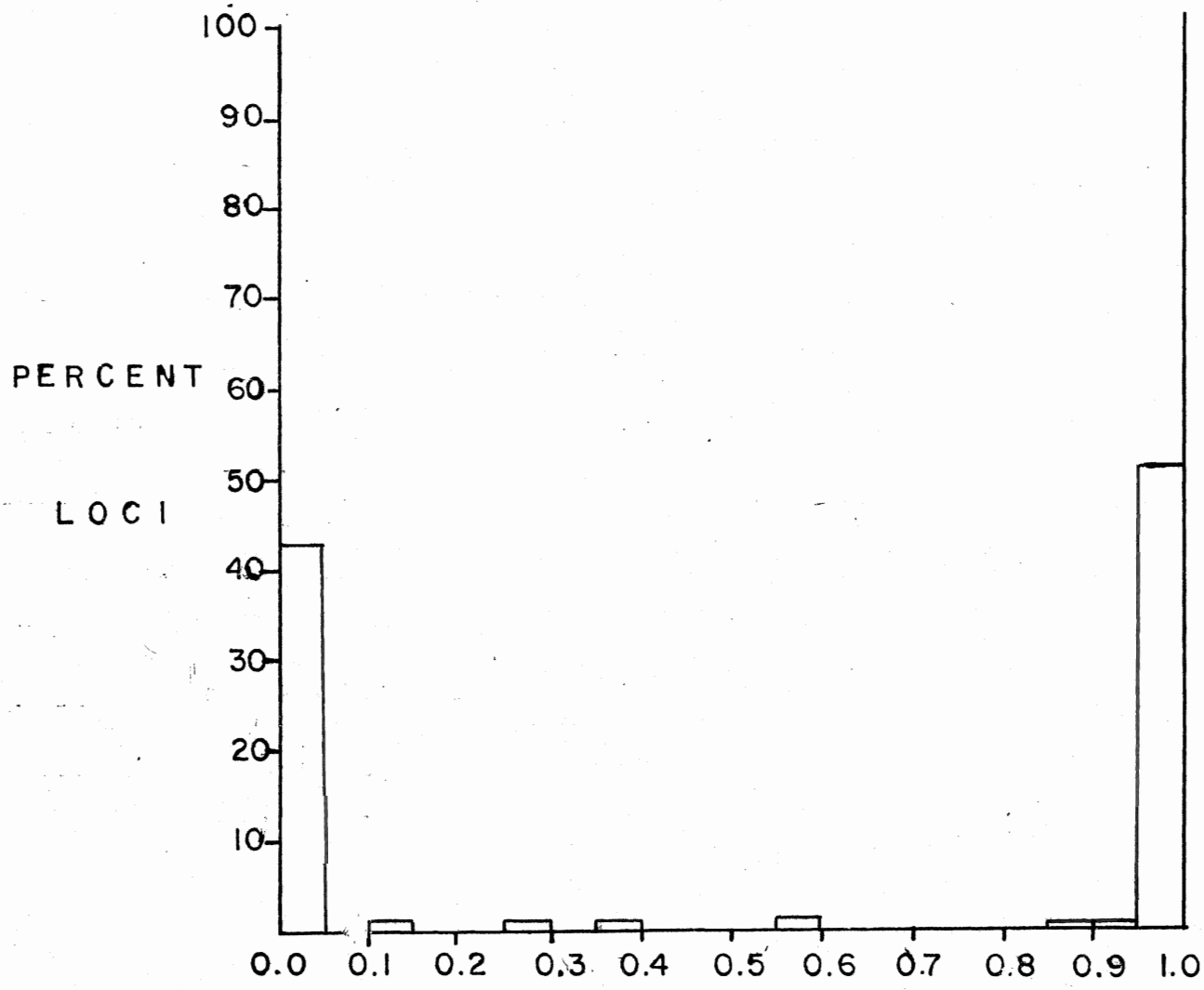
IDENTITY

(I)

Figure 7. Distribution of loci according to genetic identity observed in 1579 locus by locus comparisons between *O. propinquus*, *O. virilis*, *O. immunis*, *C. robustus*, *C. bartoni*, *P. clarkii* and *P. pictus*. All comparisons between *O. propinquus* and *P. pictus* are excluded.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IDENTITY

(I)

which it was assayed (15 samples) and the Ao-2 showed polymorphism in all but four samples. Over all species examined, nine different Lap alleles were detected and eleven different Ao-2 alleles were detected. These two loci have far more alleles than any other loci assayed and would suggest that they are more prone to mutation. Also, the fact that both loci are polymorphic in almost all samples is evidence that the polymorphism is being selected for and maintained by some form of mutational pressure (Ohta, 1974).

Table 21 presents the mean genetic identities and distances of the interspecies comparisons with Orconectes, Cambarus and Procambarus. They are respectively, I = 0.686 ± 0.070, D = 0.381 ± 0.106; I = 0.528 ± 0.009, D - 0.639 ± 0.017; I = 0.675 ± 0.033, D = 0.394 ± 0.049. It is readily evident that interspecific identities and distances for the Orconectes and Procambarus species are similar to one another and quite different from the values given for the Cambarus species. The first two genera are polymorphic for the same enzymes and show complete identity at all five of the non-enzymatic proteins. If all three genera are compared to one another locus by locus the similarity between Orconectes and Procambarus becomes even more striking.

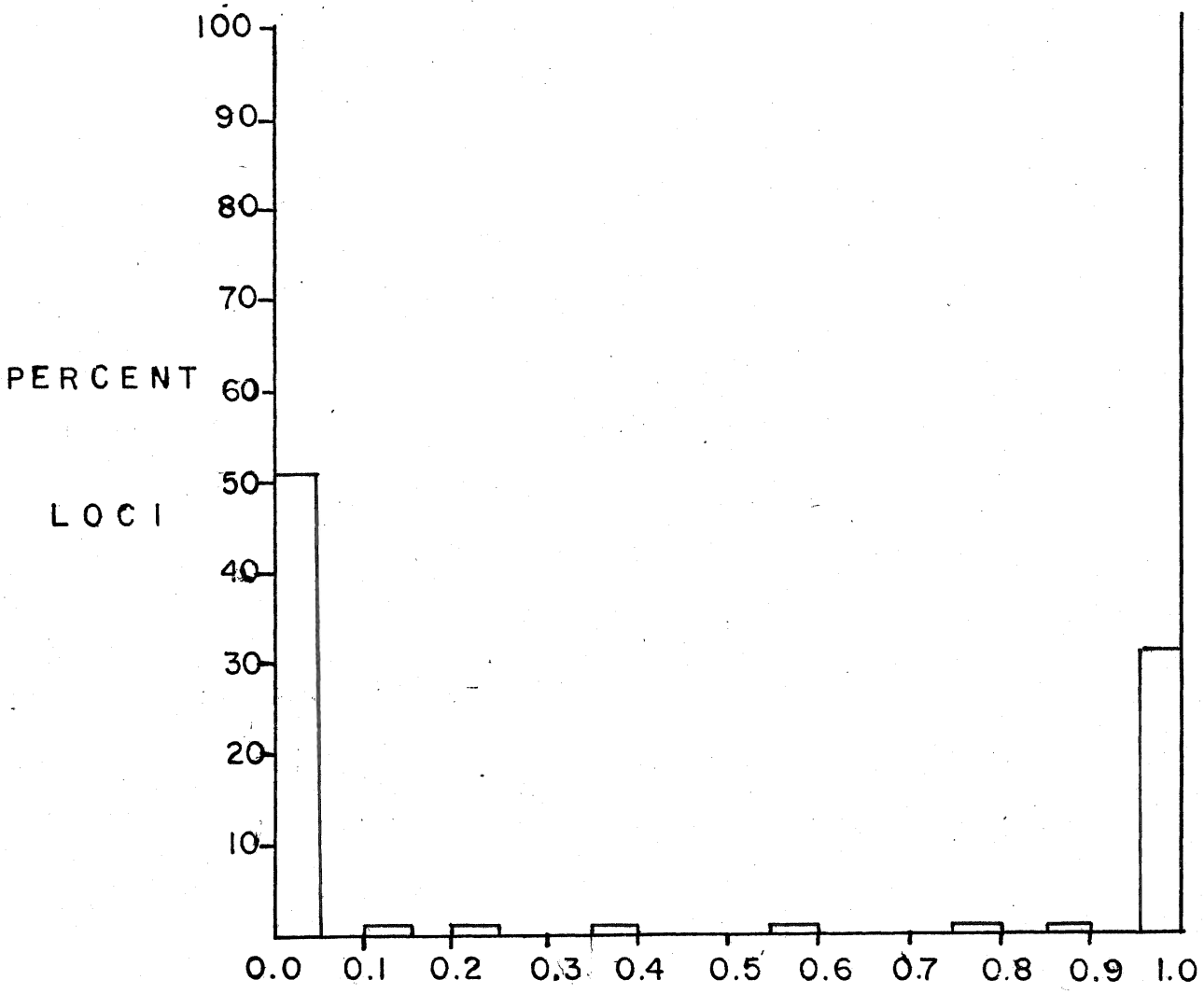
Avise and Ayala (1975) and Avise (1976) have hypothesized larger D values in species-rich (speciose) phylads than in species-poor phylads. The model presumes equivalent evolutionary age and a correlation between number of speciation events and genetic distance. The genera Orconectes (D = 0.38) and Cambarus (D = 0.64) contain approximately fifty species; while Procambarus (D = 0.39) contains approximately one hundred species. Accepted uncritically the data suggest that genetic divergence among the Cambarinae is a function of population size, time and mutation rate; but

not of the number of speciation events. A number of caveats must, however, be listed: 1. The power of this D comparison is related to the magnitude of species number differences among genera; a two-fold difference is quite likely insufficient. 2. The sample size (three genera-seven species), in light of the C. bartoni discrepancy, is small. 3. The loci examined may not be appropriate (Wilson, 1976) if speciation is driven by regulatory changes. An adequate test of the Avise-Ayala model will require more studies.

The intergeneric, locus-by-locus identity distributions are presented in Figures 8, 9 and 10. The Orconectes-Cambarus (Figure 8) and Procambarus-Cambarus (Figure 9) comparisons show approximately the same distributions of identities. The Procambarus-Orconectes comparison (Figure 10), however, shows that 68 percent of all compared loci are highly similar and only 19.6 percent are highly dissimilar. This discrepancy is primarily attributable to the high degree of similarity between O. propinquus and P. pictus illustrated in Figure 11.

The Orconectes-Cambarus and Procambarus-Cambarus I values are 0.41 ± 0.09 and 0.46 ± 0.11 respectively. These values are, as expected, lower than the mean I's for interspecific comparisons in Cambarinae. They are, however, high when compared to other intergeneric comparisons such as asteroids ($I = 0.26$), fish ($I = 0.17$) and newts ($I = 0.31$) (Ayala, 1975). Few such comparisons have been published making it difficult to generalize; if however, the high intergeneric I of Cambarinae is real, it is possibly attributable to low mutation rate in this group. As Nei and Li have shown monomorphic proteins are evolutionarily conservative; these are precisely the loci we have sampled (Nei, 1976; Nei and Li, 1975).

Figure 8. Distribution of loci according to genetic identity observed in 580 locus by locus comparisons between all *Orconectes* and *Cambarus* species.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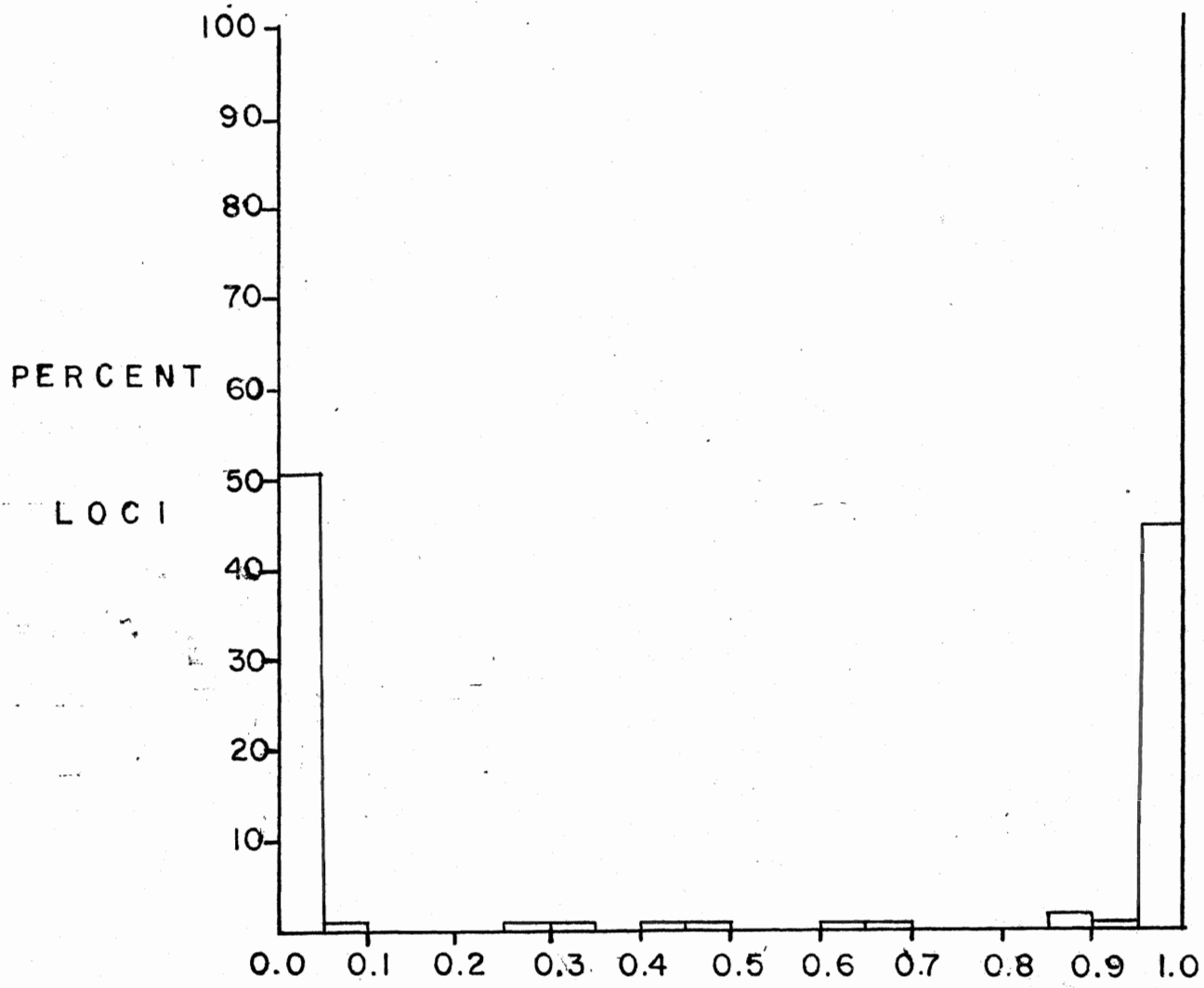
IDENTITY

(I)

Figure 9. Distribution of loci according to genetic identity observed in 219 locus by locus comparisons between all *Cambarus* and *Procambarus* species.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IDENTITY

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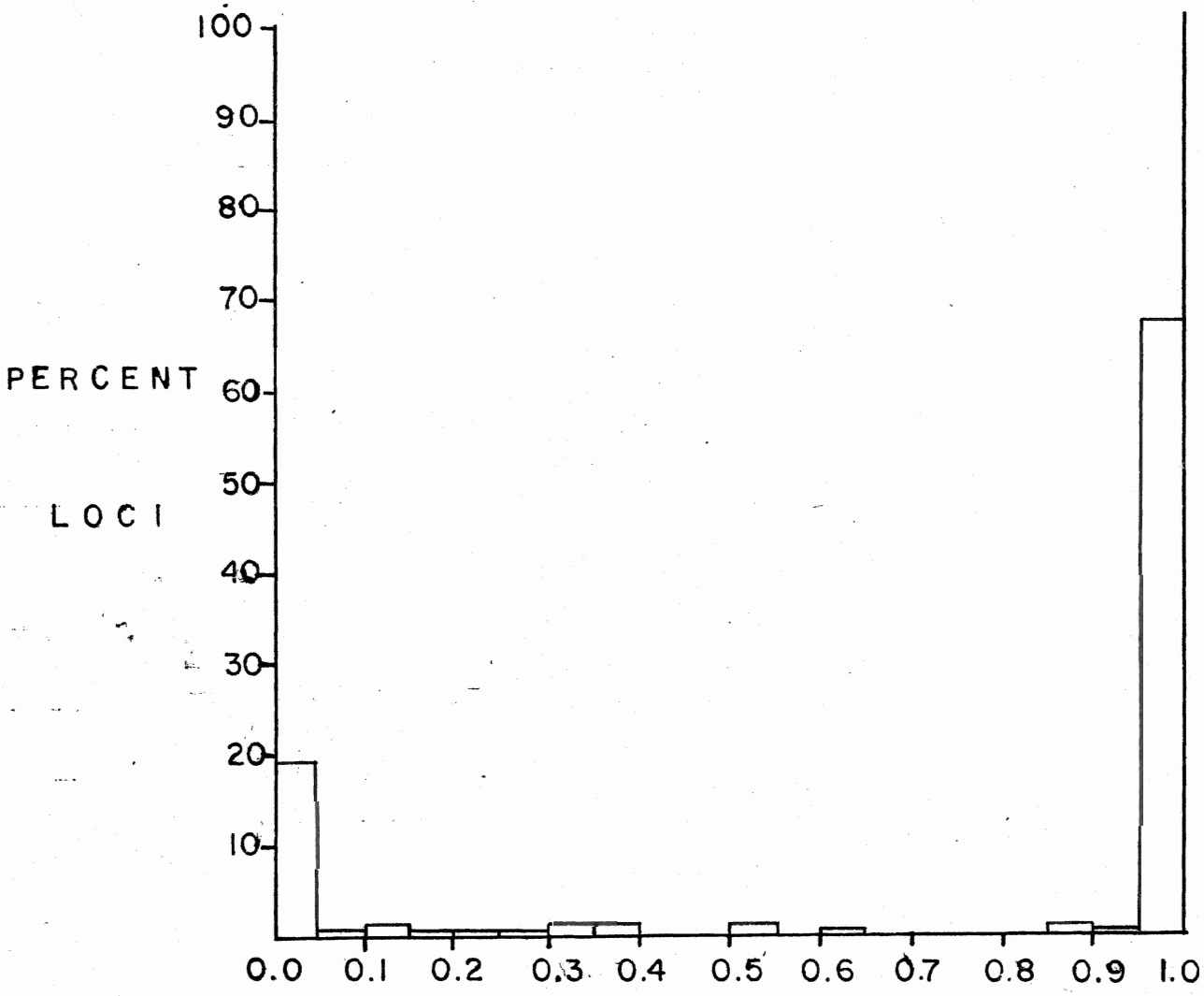
Figure 10. Distribution of loci according to genetic identity observed in 571 locus by locus comparisons between all Orconectes and Procambarus species.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0.6
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0.9
1.0
IDENTITY

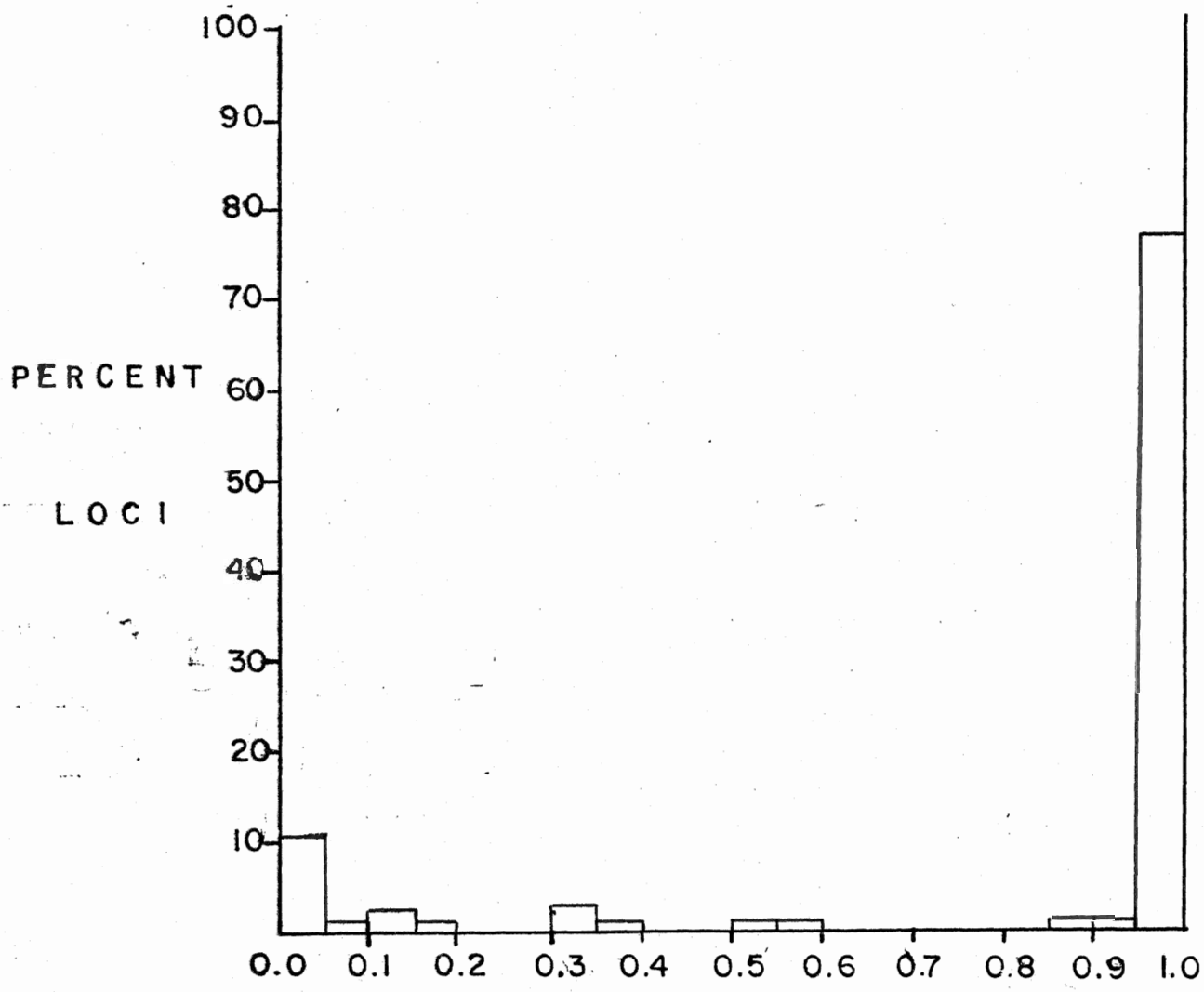
(I)

Phylogenetic Reconstructions

Genetic distance values may be employed to place the species examined in relation to one another phylogenetically (Farris, 1972; see Appendix B for illustration of methods). The dendrogram in Figure 12 was constructed for the Cambarinae using mean D values. The positioning (mispositioning) of P. pictus is striking. If P. pictus is excluded and the dendrogram reconstructed, the phylogeny conforms to the species clustering of the morphological taxonomist. However, the similarity between these two species (from completely different genera!) is very real. The similarity can be illustrated more clearly if one compares the two species locus by locus (Figure 11). The two are found to be highly similar genetically ($I > 0.95$) at 77.4 percent of all loci compared and only 11.5 percent show a high degree of dissimilarity ($I < 0.05$).

One possible explanation of this intergeneric homogeneity is suggested by the hypothesis that speciation involves gene substitutions at regulatory loci which may yield marked morphological divergence, but little structural gene change (Wilson, 1976). King and Wilson (1975) have reported a similarly high value between man (Homo) and chimpanzee (Pan)--members of different taxonomic families.

Figure 11. Distribution of loci according to genetic identity observed in 279 locus by locus comparisons between *O. propinquus* and *P. pictus* samples.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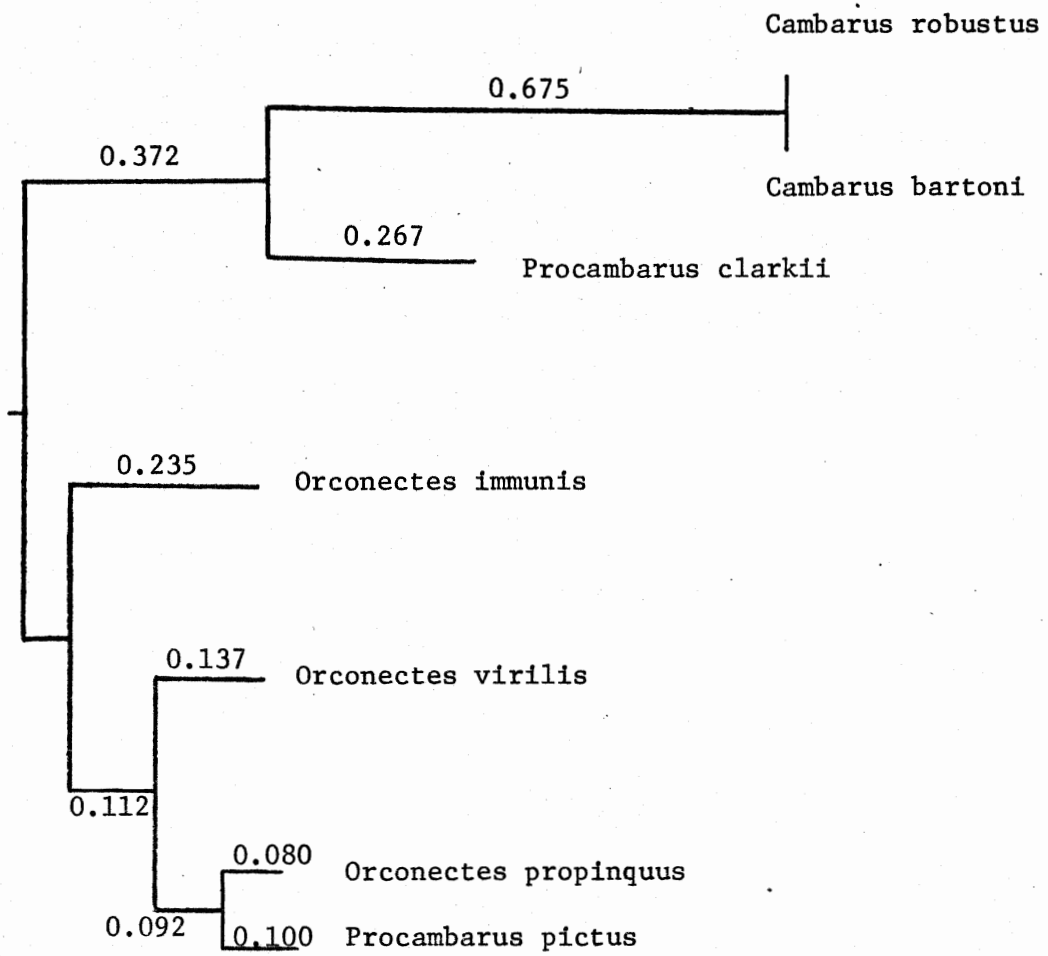
(I)

Figure 12. Cambarinae Wagner tree based on species mean D.



Cambarinae Wagner Tree Based on Species Mean D

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Appendix A

Genotypes of all individuals at all polymorphic loci

Table A-1. Individual genotypes of all Orconectes propinquus studied. Monomorphic loci are not tabulated.[1,2,3]

Population	Animal Number[4]	Locus							
		Acph	AO-2	Est-4	Lap	Mdh-1	Pgi	Pgm-1	Xdh
Hart Creek	481-25	100	102	101	100/102	100	100	100	100
	482-26	--	102	--	100/102	100	--	--	--
	483-25	--	100/102	--	100/102	100/102	--	--	--
	484-24	--	100/102	--	100/102	100	--	--	--
	485-23	--	102	--	100/102	100	--	--	--
	486-25	--		--	100/102	100	--	--	--
	487-32	--	102	--	100	100	--	--	--
	488-27	--	102	--	100/102	100	--	--	--
	489-26	--	102	--	100/102	100	--	--	--
	490-26	--		--	100/102	100/102	--	--	--
	491-24	--	100	--	100/102	100	--	--	--
	492-24	--	100/102	--	100	100	--	--	--
	493-24	--	100/102	--	100	102	--	--	--
	494-22	--	102	--	100/102	100	--	--	--
	495-26	--	102	--	100/102	100/102	--	--	--
	496-22	--	100/102	--	100	102	--	--	--
	497-23	--	100	--	100/102	102	--	--	--
	498-22	--	100	--	100	102	--	--	--
	499-24	--	100/102	--	100	100/102	--	--	--
	500-24	--	100	--	100	100/102	--	--	--
	501-24	--	102	--	100	100/102	--	--	--
	502-24	--	100	--	100	100	--	--	--
	503-22	--	100	--	100	100/102	--	--	--
	504-24	--	102	--	100	100/102	--	--	--
	505-24	--	102	--	100	100	--	--	--
	506-23	--	102	--	100/102	100	--	--	--
	507-22	--	100/102	--	100	100	--	--	--
	508-21	--	100	--	100/102	100/102	--	--	--
	509-20	--	100	--	100/102	100/102	--	--	--
	510-19	100	100	101	100	102	100	100	100
Chippawa Creek I	521-30	100	100	100	100/98	100	100/95	100	100
	522-29	--	--	--	98/95	100	100/95	100	--
	523-28	--	--	--	98	100/102	100/95	100	--
	524-24	--	--	--	100		95	100	--
	525-23	--	--	--	98	100/102	95	100	--
	526-27	--	--	--			100/95	100	--
	527-23	--	--	--	98	100/102	95	100	--
	528-24	--	--	--	98	100/102	100/95	100	--
	529-24	--	--	--	98	100/102	95	100	--
	530-24	--	--	--	98	100	100	100/98	--
	531-24	--	--	--	98	102	100/95	100	--
	532-24	--	--	--	98	100	100	100	--
	533-22	--	--	--	98	100	100	100	--
	534-20	--	--	--	98	100	100	100	--
	535-20	--	--	--	100	100	95	100	--
	536-30	--	--	--	98	100	100	100	--
	537-33	--	--	--		100	100/95	100	--
	538-29	--	--	--	100		95	100	--
	539-28	--	--	--	98	100	100	100	--
	540-28	--	--	--	98	100	95	100	--
	541-28	--	--	--	98	100	95	100	--
	542-28	--	--	--	98	100/102	100/95	100	--
	543-26	--	--	--	98	100	100/95	100	--
	544-27	--	--	--	98	100	95	100	--
	544-25	--	--	--	98	100	100/95	100	--
	546-27	--	--	--	98	100	100/95	100	--
	547-27	--	--	--	98	100	100/95	100	--
	548-27	--	--	--	100/98	100	100/95	100	--
	549-24	--	--	--	100/98	100	100/95	100	--
	550-25	--	--	--	100	100	100/95	100	--
	551-25	--	--	--	100	100	95	100	--
	552-28	--	--	--	100	100	95	100	--
	553-70	--	--	--	98/95	100	95	100	--
	554-31	--	--	--	100/98	100	100/95	100	--
	555-24	--	--	--	100	100	95	100/98	--
	556-26	--	--	--	95	100	95	100	--
	557-26	--	--	--	100	100	100/95	100	--
	558-27	--	--	--	100	100	95	100/98	--
	559-26	--	--	--		100	95	100/98	--
	560-25	--	--	--		100/102	100/95	100	--
	561-23	--	--	--	98	100	100	100	--
	562-25	--	--	--	98	100	100/95	100	--
	563-24	--	--	--	98	100	100	100	--
	564-25	--	--	--	100/98	100	100/95	100	--
	565-22	--	--	--	98	100	100/95	100	--
	566-23	--	--	--	100/98	100	100/95	100/98	--
	567-24	--	--	--	98	100	95	100	--
	568-22	100	100	100	98	100	95	100	100

Table A-1, page 2.

Population	Animal Number	Locus							
		Acph	Ao-2	Est-4	Lap	Mdh-1	Pgi	Pgm-1	Xdh
Twelve Mile Creek	571-25	100/98	100	100	100/98		100	100	100
	572-25	100/98	100	--	100/98		100	--	--
	573-24	100	100	--	98		100	--	--
	574-26	100	100	--	98		100	--	--
	575-24	100	100	--	100/98		100	--	--
	576-20	100	100	--	100/98		100	--	--
	577-26	100/98	100	--	100/98		100	--	--
	578-24	100	100	--	100/98		100/95	--	--
	579-21	100/98	100	--	100/98		100	--	--
	580-20	100	101	--	100		100/95	--	--
	581-20	100	100	--	98		100	--	--
	582-19	100	100/98	--	98		100	--	--
	583-25	100	100	--	100		100/95	--	--
	584-25	100	100	--	100		100/95	--	--
	585-29	100/98	100	--	98		100	--	--
	586-33	100	100	--	98		100/95	--	--
	587-27	100	100/98	--	98		100	--	--
	588-25	100	100	--	100		100	--	--
	589-26	100	100	--	100		100/95	--	--
	590-27	100	100	--	100/98		100	--	--
	591-25	100	100/98	--	100/98		100	--	--
	592-25	100	100	--	100		100	--	--
	593-23	100	100	--	100/98		100/95	--	--
	594-23	100	100/98	--	98		100	--	--
	595-22	100/98	100	--	98		100	--	--
	596-24	100/98	100	--	98		100	--	--
	597-21	100/98	100	--	100/98		100/95	--	--
	598-20	100/98	100/98	--	100		100	--	--
	599-21	100/98	100/98	--	100		100	--	--
	600-19	100	100	100	98/95		100	100	100
St. John's I	341-15	100	100		98		100	100	100
	342-16	--	100		98/95		100	100	--
	343-14	--	100		100		100/95	100/102	--
	344-14	--	100		100		100/95	100/102	--
	345-14	--	100		100		100	100	--
	346-15	--	100		100		100	100	--
	347-15	--	100		100/95		100	100	--
	348-15	--	100		100		100	100	--
	349-12	--	100		98		100	100	--
	350-18	--	100		98/95		100/95	100	--
	351-14	--	100		100/95		100	100	--
	352-13	--	100		100/95		100	100	--
	353-15	--	98		100		100	100	--
	354-12	--	100		98		100/95	100	--
	355-11	--	100		100/95		100	100	--
	356-10	--	100		100		100	100	--
	357-14	--	100		100		100	100	--
	358-14	--	100		100/98		100/95	100	--
	359/14	--	100		100		100	100	--
	360-15	--	100		98		100/95	100	--
	361-13	--	100/98		100/98		100	100	--
	362-10	--	100		100		100	100	--
	363-12	--	100/98		98		100	100	--
	364-11	--	100		98/95		100	100	--
	365-13	--	100		100		100	100	--
	366-14	--	100		100		100	100	--
	367-10	--	100		98		100	100	--
	368-11	--	100		100		100/95	100	--
	369-14	--	100		100		100	100	--
	370-14	--	100		98		100	100	--
	371-14	--	100		100		100/95	100	--
	372-13	--	100		100		100	100	--
	373-10	--	100		100		100	100	--
	374-12	--	100		100/95		100	100	--
	375-12	--	100		100		100	100	--
	376-11	--	100		100		100	100	--
	377-11	--	100		100/98		100	100	--
	378-12	--	100		100/95		100	100	--
	379-14	--	100		100		100	100	--
	380-12	--	100		100		100	100	--
	381-10	--	100		100		100/95	100	--
	382-11	--	100		100		100	100	--
	383-12	--	100		100		100	100	--
	384-12	--	100		100		100/95	100	--
	385-14	--	100		100		95	100	--
	386-14	--	100		100/98		100/95	100	--
	387-13	--	100		100		95	100	--
	388-9	--	100		100		100/95	100	--
	389-11	--	100		100		100	100	--
	390-13	--	100/98		100/98		100/95	100	--

Table A-1, page 3.

Population	Animal Number	Locus							
		Acph	AO-2	Est-4	Lap	Mdh-1	Pgi	Pgm-1	Xdh
St. John's I	391-12	--	100		100/98		100	100	--
	392-13	--	100/98		100/95		100/95	100	--
	393-10	--	100/98		100/95		100/95	100	--
	394-11	--	100/98		100		100	100	--
	395-10	--	100/98		100/98		100	100	--
	396-12	--	100		100		100/95	100	--
	397-11	--	100		100		100	100	--
	398-11	--	100		100		100	100	--
	399-14	--	100		100/95		100	100	--
	400-11	100	100		100		100	100	100
Oliphant	601-17	100	100	100	100/102		95	100	100/102
	602-16	--	--	--	100/102		100/95	--	100/102
	603-16	--	--	--	100/102		95	--	100
	604-14	--	--	--	100/102		100/95	--	100/102
	605-15	--	--	--	100		100	--	100
	606-15	--	--	--	100/102		95	--	100
	607-15	--	--	--	100/102		95	--	100/102
	608-14	--	--	--	100		95	--	100
	609-14	--	--	--	100/102		100/95	--	100
	610-15	--	--	--	100/102		100/95	--	100/102
	611-15	--	--	--	100/102		95	--	100
	612-15	--	--	--	100/98		95	--	100
	613-15	--	--	--	100		100/95	--	100
	614-15	--	--	--	100/102		100/95	--	100
	615-15	--	--	--	100/102		95	--	100
	616-16	--	--	--	100/102		95	--	100
	617-16	--	--	--	100		95	--	100
	618-16	--	--	--	100/102		100/95	--	100
	619-16	--	--	--	100		100/95	--	100
	620-16	--	--	--	100/102		100/95	--	100
	621-15	--	--	--	100/102		100/95	--	100/102
	622-15	--	--	--	100/102		95	--	100
	623-14	--	--	--	100/102		95	--	100
	624-13	--	--	--	100/102		100/95	--	100
	625-11	100	100	100	100/102		100/95	100	100
Tobermory	701-26	100	100/96	100	100	100/102	95	100	100
	702-21	--	100	--	100/102	100	100/95	--	--
	703-21	--	100	--	102	100/102	95	--	--
	704-24	--	100/96	--	102	100	95	--	--
	705-22	--	100/96	--	102	100/102	95	--	--
	706-23	--	100/94	--	102	100/102	95	--	--
	707-19	--	100/96	--	100/102	100	95	--	--
	708-22	--	100/96	--	100/102	100/102	95	--	--
	709-21	--	100	--	102	100	95	--	--
	710-26	--	100	--	100/102	100	95	--	--
	711-26	--	100/96	--	102	100/102	95	--	--
	712-22	--	100/94	--	102	100	95	--	--
	713-22	--	100/94	--	102	100	95	--	--
	714-22	--	100/94	--	100/102	100	95	--	--
	715-23	--	100	--	102	100/102	95	--	--
	716-23	--	100	--	100/102	100	100/95	--	--
	717-22	--	100	--	100/102	100/102	95	--	--
	718-23	--	100/95	--	100/102	100/102	95	--	--
	719-21	--	100/95	--	102	100/102	100	--	--
	720-19	--	100	--	102	100/102	95	--	--
	721-22	--	100	--	100	102	100/95	--	--
	722-20	--	100	--	100	100/102	95	--	--
	723-20	--	100	--	100/102	102	100/95	--	--
	724-22	--	100	--	100/102	102	95	--	--
	725-21	--	100/95	--	100	100/102	100/95	--	--
	726-21	--	100/98	--	100/102	100	95	--	--
	727-25	--	100/94	--	100	100	95	--	--
	728-22	--	100/96	--	102	102	95	--	--
	729-22	--	100/95	--	100/102	100	95	--	--
	730-22	--	100/94	--	100	102	95	--	--
	731-21	--	100/96	--	102	100/102	100	--	--
	732-24	--	100/96	--	102	100/102	95	--	--
	733-24	--	100/95	--	102	100	95	--	--
	734-25	--	100/94	--	100	102	95	--	--
	735-23	--	100/98	--	100/102	100	95	--	--
	736-24	--	100/94	--	102	100	95	--	--
	737-22	--	100/95	--	102	100	95	--	--
	738-22	--	100/96	--	102	100	95	--	--
	739-21	--	100	--	102/104	100/102	95	--	--
	740-21	100	100/94	100	102	102	100/95	100	100

Table A-1, page 4.

[1] Loci monomorphic in a particular population have the genotype recorded for only the first and last individual; all others are dashed.

[2] Where a genotype was not assayed in a particular individual, the genotype space is blank.

[3] O. propinquus was monomorphic when tested at the following loci: Amy-1^{100}, Amy-2^{100}, AO-1^{100}, AO-3^{100}, AO-4^{100}, Est-3^{100}, Est-5^{100}, Mdh-2^{100}, Odh^{100}, Pgm-2^{100}, Pt-1^{100}, Pt-2^{100}, Pt-3^{100}, Pt-4^{100}, Pt-5^{100}, To-1^{100}, and To-2^{100}.

[4] The hyphenated number following the animal number is the individual's carapace length in millimeters.

Table A-2. Individual genotypes of all Orconectes virilis studied. Monomorphic loci are not tabulated.[1]

Population	Animal Number	Locus Amy-1	Lap
St. John's II	401-19	100	98/95
	402-17	100	98/95
	403-19	100	98/95
	404-14	100	98/95
	405-17	100	98/95
	406-14	100	95
	407-14	100	95
	408-19	100	98/95
	409-13	100	95
	410-17	100	95
	411-15	100	98
	412-1[illegible]	100	95
	413-16	100	98
	414-16	100	95
	415-14	100	95
	416-16	100	98/95
	417-14	100	98/95
	418-14	100	95
	419-13	100	98
	420-16	100	98/95
	421-15	100/102	98/95
	422-16	100	98/95
	423-14	100	95
	424-15	100	95
	425-16	100	98/95
	426-14	100	95
	427-16	100	98
	428-16	100	95
	429-18	100	98/95
	430-16	100	95
	431-15	100	98/95
	432-15	100	95
	433-14	100	98/95
	434-19	100	98/95
	435-16	100	98/95
	436-18	100	95
	437-17	100	95
	438-16	100	98/95
	439-16	100	98/95
	440-15	100	95
	441-17	100	98/95
	442-16	100	95
	443-16	100	98/95
	444-16	100	95
	445-16	100	95
	446-16	100	98
	447-12	100	98/95
	448-13	100	95
	449-14	100	95
	450-15	100	98/95
	451-14	100	98/95
	452-14	100	95
	453-13	100	95
	454-14	100	98/95
	455-14	100	98/95
	456-14	100	98/95
	457-14	100	98
	458-15	100	98/95
	459-14	100	98/95
	460-16	100	98/95

[1] O. virilis was monomorphic when tested at the following loci: $Acph^{100}$, $Amy\text{-}2^{100}$, $Ao\text{-}2^{98}$, $Ao\text{-}3^{100}$, $Ao\text{-}4^{100}$, $Est\text{-}1^{100}$, $Mdh\text{-}2^{100}$, Odh^{108}, Pgi^{100}, $Pgm\text{-}1^{100}$, $Pgm\text{-}2^{100}$, $Pt\text{-}1^{100}$, $Pt\text{-}2^{100}$, $To\text{-}2^{100}$, Xdh^{103}.

Table A-3. Individual genotypes of all Orconectes immunis studied. Monomorphic loci are not tabulated.[1]

Population	Animal Number	Locus Ao-2	Est-4	Mdh-2
St. John's III	1-11	102	--	100
	2-13	102		--
	2-10	102		--
	4-12	102		--
	5-12	102		--
	6-13	102		--
	7-11	102		--
	8-12	102		--
	9-11	102		--
	10-13	102		--
	11-12	102		--
	12-14	102		--
	13-12	102		--
	14-12	102		--
	15-14	102		--
	16-13	102		--
	17-12	102		--
	18-17	102		--
	19-13	102		--
	20-12	102		--
	21-14	102		--
	22-10	102		--
	23-13	102		--
	24-14	102		--
	25-11	102		--
	26-10	102/101		--
	27-13	102		--
	28-12	102		--
	29-11	102/101		--
	30-10	102/101		--
	31-12	102/101		--
	32-12	102		--
	33-12	102		--
	34-14	102		--
	35-11	102		--
	36-11	102		--
	37-10	102		--
	38-10	102		--
	39-10	102/101		--
	40-10	102		--
	41-10	102/101		--
	42-11	102		--
	43-11	102		--
	44-11	102/101		--
	45-10	102/101		--
	46-13	102		--
	47-10	102		--
	48-12	102		--
	49-12	102		--
	50-11	102		--
	51-11	102		--
	52-10	102/101		--
	53-10	102/98		--
	54-12	102		--
	55-11	102/100		--
	56-13	102		--
	57-12	102/98		--
	58-9	102/101		--
	59-9	102/100		--
	60-10	102/100		100
Stinking Barn I	61-15	102	101/102	100
	62-13	102	101/102	--
	63-12	102	101/102	--
	64-12	102	101	--
	65-15	102	102	--
	66-13	101	101/102	--
	67-16	102	101/102	--
	68-15	102	101/102	--
	69-11	102	101	--
	70-10	102	101/102	--
	71-11	101	101	--
	72-12	102/100	101/99	--
	73-15	102	101/102	--
	74-12	102	101/102	--
	75-13	102	101	--
	76-11	102	101	--
	77-12	102/101	101	--
	78-16	102	101/102	--

Table A-3, page 2.

Population	Animal Number	Locus Ao-2	Est-4	Mdh-2
Stinking Barn I	79-13	102/100	100	--
	80-12	102/98	102/99	--
	81-13	102/100	101	--
	82-12	102/100	101	--
	83-15	102	101/102	--
	84-16	101	101	--
	85-10	102/101	101	--
	86-12	102/100	101/99	--
	87-15	102	101	--
	88-13	102	101	--
	89-11	102/101	101/99	--
	90-11	102/101	101	--
	91-12	102/101	101/102	--
	92-9	102/99	101/99	--
	93-13	102/99	101/99	--
	94-13	101	101/102	--
	95-11	102/100	101/99	--
	96-13	102/99	101/99	--
	97-12	102/100	101	--
	98-12	102/101	101	--
	99-13	102/100	101	--
	100-15	102/101	101	--
	101-11	102	101	--
	102-14	102/100	101	--
	103-15	102/101	101	--
	104-13	102	101/102	--
	105-13	102/100	101/102	--
	106-11	102/101	101/102	--
	107-10	102/101	101	--
	108-13	102/101	101	--
	109-12	102/101	101/102	--
	110-12	102/100	101/102	--
	111-11	102/101	101	--
	112-16	102/101	101	--
	113-12	102/101	101	--
	114-14	102/101	101/102	--
	115-11	102/100	101	--
	116-13	102/100	101	--
	117-12	102/99	101/102	--
	118-11	102/100	101	--
	119-10	102/99	101	--
	120-10	102/100	101	--
	121-14	102/100	101	--
	122-11	102/99	101/99	--
	123-9	102/100	101	--
	124-10	102/100	101	--
	125-13	101	101	--
	126-14	102	101/102	--
	127-12	101	101	--
	128-14	102	101/102	--
	129-14	102-100	101/99	--
	130-10	102/100	101/99	--
	131-14	102/100	101/99	--
	132-13	102/100	101/99	--
	133-12	102/100	101/99	--
	134-12	102/100	101/99	--
	135-12	102/100	101	--
	136-11	102/100	101	--
	137-12	102/100	101	--
	138-14	102/100	101	--
	139-12	102/100	101	--
	140-13	102/98	102/99	100
Stinking Barn II	141-29	102	--	100
	143-34	102		100
	145-31	102		100
	147-33	100		100
	149-33	101		100
	151-31	100		100
	153-28	102		100/97
	155-30	102		100
	157-29	102		100
	159-27			100
	161-28			100
	163-29	102/101		100
	165-32	102		100
	166-26	102		100
	167-30	102		100
	168-25	100		100
	169-31	102/100		100

Table A-3, page 3.

Population	Animal Number	Locus Ao-2	Est-4	Mdh-2
Stinking Barn II	170-26	102/100		100
	171-27	102/98		100
	172-27	102/100		100
	173-25	102/101		100
	174-28	101		100
	175-26	101		100
	176-24	102/100		100
	177-24	102		100
	178-24	102		100
	179-25	102/100		100
	180-23	101		100
	181-20	102/100		100
	182-24	102/100		100

[1] O. immunis was monomorphic when tested at the following loci: $Acph^{100}$, $Amy\text{-}1^{100}$, $Ao\text{-}3^{100}$, $Ao\text{-}4^{100}$, $Est\text{-}5^{100}$, $Mdh\text{-}1^{102}$, Odh^{100}, Pgi^{100}, $Pgm\text{-}1^{103}$, $Pgm\text{-}2^{100}$, $Pt\text{-}1^{100}$, $Pt\text{-}3^{100}$, $Pt\text{-}4^{100}$, $Pt\text{-}5^{100}$, $To\text{-}2^{100}$, Xdh^{101}.

Table A-4. Individual genotypes of all Cambarus robustus studied. Monomorphic loci are not tabulated.[1]

Population	Animal Number	Locus Ao-2	Locus Lap
Chippawa Creek II	201-39	100	105
	202-41	100	105
	203-37	98	105
	204-41	100/98	105
	205-41	100/98	105
	206-36	100/97	105
	207-35		103
	208-41	100	105
	209-38	100/97	103
	210-31	100	105
	211-41	100/97	105
	212-34	100/98	105
	213-38	100/97	105
	214-33	100/97	103/105
	215-36	100/97	105
	216-32	97	105
	217-34	100/97	105
	218-35		105
	219-29	100/97	105
	220-29	100/97	105
	221-35	100	103
	222-31	100/98	105
	223-29	100	103/105
	224-28	100/98	105
	225-25	100/98	105
	226-32	100/101	105
	227-35	100/97	105
	228-37	100/98	105
	229-37	100/98	105
	230-32	100	105
	231-31	100/98	105
	232-31	100	105
	233-35	100/98	105
	234-32	100	105
	235-34	100	105
	236-30	100	105
	237-27	100	105
	238-28	100	105
	239-27	100	105
	240-27	100	105
Twelve-Mile Creek II	631-25	100	105
	632-24	100	105
	633-28	100/98	105
	634-27	100/98	105
	635-25	100/98	105
	636-24	100	103
	637-28	100/98	103
	638-26	100/98	105
	639-22	100	103
	640-32	98	103/105
	641-39	100/98	103
	642-38	100/98	105
	643-25	100/98	105
	644-25	98	103
	645-23	100/98	103/105
	646-25	100/97	103/105
	647-26	100	103
	648-38	100/98	103
	649-22	100/98	103/105
	650-21	100	103/105
	651-23	98	103
	652-22	100/98	103
	653-40	100/98	103/105
	654-23	100/98	103/105
	655-23	100/98	103
	656-25	100	103
	657-20	98	105
	658-28	100/98	103/105
	659-24	100/98	103
	660-21	100	103/105

[1] C. robustus was monomorphic when tested at the following loci: $Acph^{101}$, $Amy\text{-}1^{102}$, $Ao\text{-}3^{100}$, $Ao\text{-}4^{100}$, $Est\text{-}3^{105}$, $Est\text{-}4^{105}$, $Mdh\text{-}2^{100}$, Odh^{100}, Pgi^{100}, $Pgm\text{-}1^{100}$, $Pgm\text{-}2^{102}$, $Pt\text{-}1^{97}$, $Pt\text{-}2^{98}$, $Pt\text{-}3^{96}$, $Pt\text{-}4^{96}$, $To\text{-}2^{101}$, Xdh^{100}.

Table A-5. Individual genotypes of all Cambarus bartoni studied. Monomorphic loci are not tabulated.[1]

Population	Animal Number	Locus			
		Ao-2	Lap	Pgi	To-2
Opinicon	461-29	98	102/104	100	100/97
	462-26	98	102/104	--	100
	463-27	98/96	102/104	--	100/97
	464-25	98/96	102/104	--	100
	465-20	98/96	102	--	100
	466-25	98	102/104	--	100
	467-19	98/96	102/104	--	100
	468-27	96	102/104	--	100
	469-25	98/96	104	--	100
	470-27	96	104	--	100
	471-22	96	104	--	100
	472-26	98/96	102/104	--	100/97
	473-28	98/96	102/104	--	100
	474-24	98/96	102/104	--	100
	475-28	96	102/104	--	100
	476-17	98/96	102/104	100	100
Georgia	271-28	102	104	100	100
	272-28	102	104	100	--
	273-24	102	104	100	--
	274-26	102	102/104	100	--
	275-21	102/104	102/104	100	--
	276-31	102	102/104	100/105	--
	277-26	102	104	100	--
	278-28	102	104	100	--
	279-25	102/104	102/104	100	--
	280-23	102	104	100	--
	281-22	104	104	100	--
	282-21	102/104	102/104	100	--
	283-20	102/104	102/104	100	--
	284-22	102/104	102/104	100	--
	285-20	102/104	104	100	--
	286-22	103/104	102/104	100	--
	287-21	102/104	104	100	--
	288-21	102/104	102/104	100	--
	289-20	102/104	102/104	100	--
	290-20	102	104	100	--
	291-18	102/104	104	100	--
	292-21	102	102/104	100	--
	293-21	102	104	100	--
	294-20	102	102	100	--
	295-20	102/104	104	100	--
	296-18	102/104	102	100	--
	297-20	102/104	104	100	--
	298-20	102/104	102/104	100	--
	299-20	102	102/104	100	--
	300-19	102/104	102/104	100	--
	301-18	102/104	102/104	100	--
	302-19	102/104	102/104	100	--
	303-18	102/104	102/104	100	--
	304-18	102/104	102/104	100	100

[1] C. bartoni (Opinicon) was monomorphic when tested at the following loci: $Acph^{101}$, $Amy\text{-}1^{102}$, $Ao\text{-}3^{100}$, $Ao\text{-}4^{100}$, $Est\text{-}4^{102}$, $Mdh\text{-}2^{100}$, Odh^{104}, $Pgm\text{-}1^{100}$, $Pgm\text{-}2^{100}$, $Pt\text{-}1^{102}$, $Pt\text{-}2^{98}$, $Pt\text{-}3^{98}$, $Pt\text{-}4^{98}$, Xdh^{100}.

C. bartoni (Georgia) was monomorphic when tested at the following loci: $Acph^{101}$, $Mdh\text{-}2^{100}$, Odh^{100}, $Pgm\text{-}1^{100}$, $Pgm\text{-}2^{102}$, $Pt\text{-}1^{96}$, $Pt\text{-}2^{95}$, $Pt\text{-}3^{86}$, $Pt\text{-}4^{85}$, $Pt\text{-}5^{85}$, Xdh^{100}.

Table A-6. Individual genotypes of all Procambarus clarkii studied. Monomorphic loci are not tabulated.[1]

Population	Animal Number	Locus	
		Ao-2	Lap
Texas	661-29	100	98/97
	662-28	100	98/97
	663-27	100/99	97
	664-24	100	98/97
	665-25	100	97
	666-21	100/98	97
	667-22	100	98
	668-19	100	98
	669-18	100	98/97
	670-20	100	98
	671-21	100	98/97
	672-20	100	98
	673-22	100	98/97
	674-19	100	98/97
	675-19	100	98/97
	676-19	100	98/97
	677-19	100	98/97
	678-18	100/96	98
	679-18	100/98	98
	680-20	100/98	98/97
	681-18	100	98/97
	682-17	100	98
	683-19	100	98/97
	684-16	100	98
	685-16	100/98	98
	686-17	100	97
	687-17	100/98	98/97
	688-17	100	97
	689-17	100	98
	690-16	100/98	98/97

[1] P. clarkii was monomorphic when tested at the following loci: $Acph^{100}$, $Amy\text{-}1^{100}$, $Mdh\text{-}2^{100}$, Odh^{100}, Pgi^{105}, $Pgm\text{-}1^{104}$, $Pt\text{-}1^{100}$, $Pt\text{-}3^{100}$, $Pt\text{-}4^{100}$, $Pt\text{-}5^{100}$, $To\text{-}1^{100}$, $To\text{-}2^{101}$, Xdh^{100}.

Table A-7. Individual genotypes of all Procambarus pictus studied. Monomorphic loci are not tabulated.[1]

Population	Animal Number	Locus			
		Ao-2	Lap	To-2	Xdh
Cape Cod I	306-41	100/102	98	100	--
	307-43	100/102	102	--	
	308-37	102	102	--	
	309-31	102	102	--	
	310-37	100/102	102/98	--	
	311-29	102	102	--	
	312-34	100/102	102	--	
	313-29	100	102/98	--	
	314-29	102	102	--	
	315-37	102	102	--	
	316-31	102	102	--	
	317-27	100/102	102/98	--	
	318-27	102	102	--	
	319-25	100/102	102	--	
	320-40	102	102	--	
	321-28	102	102	100	
Cape Cod II	741-26	101	102/104	100	100/102
	742-26	--	104	--	100
	743-28	--	104	--	100
	744-28	--	102/104	--	100/102
	745-26	--	102/104	--	100/102
	746-27	--	102	--	100
	747-77	--	102/104	--	100
	748-34	--	102/104	--	100/102
	749-28	--	102/104	--	100
	750-24	--	100/104	--	100
	751-25	--	104	--	100
	752-21	--	104	--	100
	753-22	--	104	--	100
	754-22	--	104	--	100
	755-23	--	104	--	102
	756-22	--	104	--	100
	757-31	--	102/104	--	100
	758-31	--	102/104	--	100
	759-23	--	102/104	--	100
	760-21	--	104	--	100
	761-21	--	104	--	100/102
	762-20	--	102/104	--	100
	763-18	--	104	--	100
	764-18	--	104	--	100
	765-17	101	104	100	100
Rhode Island	323-38	102	102	100	--
	324-43	102	102	100	
	325-36	102	102	100	
	326-37	102/104	102/104	100	
	327-27	102	102	100	
	328-39	102	102	100	
	329-31	102	102/98	100	
	330-35	102		100	
	331-32	102/104		100	
	332-31	102	98	100/97	
	333-30	102/104		100	
	334-31	104		100	
	335-28	104		100	
	336-25	102/104		100	
	337-28	102		100	
	338-27	102		100	
	339-26	102		100	

[1] P. pictus was monomorphic when tested at the following loci: $Acph^{100}$, $Amy\text{-}1^{100}$, $Amy\text{-}2^{100}$, $Ao\text{-}3^{100}$, $Ao\text{-}4^{100}$, $Mdh\text{-}2^{100}$, Odh^{100}, Pgi^{100}, $Pgm\text{-}1^{100}$, $Pgm\text{-}2^{102}$, $Pt\text{-}1^{100}$, $Pt\text{-}2^{100}$, $Pt\text{-}3^{100}$, $Pt\text{-}4^{100}$, $Pt\text{-}5^{100}$, $To\text{-}1^{100}$.

Appendix B

Wagner tree calculations

Appendix B

The estimation technique is that of James S. Farris (1972). It is a distance Wagner procedure which produces a most parsimonious tree for the data matrix used. For any set of phenetic differences the Wagner algorithm permits computation of an approximate Wagner tree. Once the tree has been constructed, the patristic distances may be added to produce a matrix of patristic distances. These may be compared with the original data matrix to provide a relative measure of goodness-of-fit for various possible trees, a homoplasy matrix.

The algorithmic steps and their application to the Cambarinae genetic distance matrix follow.

1. Select the minimum genetic distance from Table B1 and use it to construct an initial tree with two nodes and one branch by connecting the taxa. For this set we select 0.180 the distance between O. propinquus and P. pictus.

2. Select the next taxon to be added to the tree by locating the taxon with the minimum distance to O. propinquus and P. pictus. O. virilis has the minimum distances $(\frac{0.309 + 0.328}{2}) = 0.3185$.

3. Identify the branch (Op, Pp; Pp, Ov or Op, Ov) which minimizes the distance between node or taxon and the various possible branches. Use Farris' equations (5), (6) and (7). All the taxon of minimal distance to the appropriate branch.

$$D[Ov, (Op,Pp)] = \frac{1}{2} [D(Ov, Op) + D(Ov, Pp) - D (Op, Pp)]$$

$$= \frac{1}{2} [0.309 + 0.328 - 0.180]$$

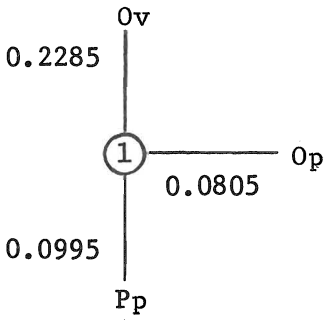
$$= 0.2285$$

$$D[Op, (Ov,Pp)] = \frac{1}{2}[D(Op,Ov) + D(Op,Pp) - D(Ov,Pp)]$$
$$= \frac{1}{2}[0.309 + 0.180 - 0.328]$$
$$= 0.0805$$
$$D[Pp, (Op,Ov)] = \frac{1}{2}[D(Pp,Op) + D(Pp,Ov) - D(Op,Ov)]$$
$$= \frac{1}{2}[0.180 + 0.328 \_ 0.309]$$
$$= 0.0995$$

The minimum D is 0.0805; O. propinquus is added to the O. virilis-P. pictus branch.

4. Add an HTU (hypothetical taxonomic unit) to this branch, and use the following equational forms to position the taxon on the branch. The HTU is labelled 1.

$$D(Ov,1) = D(Ov,Op) - D(Op,1)$$
$$= 0.309 - 0.0805$$
$$= 0.2285$$
$$D(Pp,1) = D(Pp,Op) - D(Op,1)$$
$$= 0.180 - 0.0805$$
$$= 0.0995$$



Note that for three taxa all elements of the homoplasy matrix equal zero, $H = P - D = 0$.

5. Establish the value of D(1,z) for all taxa not on the tree, that is, using Farris' (18) to approximate the triangle inequality, compute the distances for all unused taxa to 1.

$$D(Oi,1) = \sup\{[D(Oi,Op) - P(Op,1)] = 0.3265$$
$$[D(Oi,Pp) - P(Pp,1)] = 0.2665$$
$$[D(Oi,Ov) - P(Ov,1)]\} = 0.1435$$
$$= \sup\{\cdot\} = \text{largest number}$$

Table B1. Mean Genetic Distances Between the Cambarinae. The numbers in parentheses are the number of comparisons averaged.

	Op	Ov	Oi	Pp	Pc	Cb	Cr
Op	--	0.309 (6)	0.407 (18)	0.180 (18)	0.304 (6)	0.795 (12)	0.843 (12)
Ov		--	0.372 (3)	0.328 (3)	0.888 (1)	0.848 (2)	0.961 (2)
Oi			--	0.366 (9)	0.761 (3)	0.993 (6)	1.282 (6)
Pp				--	0.394 (3)	0.682 (6)	0.650 (6)
Pc					--	1.581 (2)	0.942 (2)
Cb						--	0.639 (4)
Cr							--

$$= \sup\{[0.407 - 0.0805] = 0.3265$$

$$[0.366 - 0.0995] = 0.2665$$

$$[0.372 - 0.2285]\} = 0.1435$$

$D(Oi,1) = 0.3265$

$D(Pc,1) = \sup\{[D(Pc,Op) - 0.0805] = 0.2235$

$[D(Pc,Pp) - 0.0995] = 0.2945$

$[D(Pc,Ov) - 0.2285]\} = 0.6595$

$D(Pc,1) = 0.6595$

In a similar manner we find

$D(Cb,1) = \sup\{\cdot\} = 0.7145$

and

$C(Cr,1) = \sup\{\cdot\} = 0.7625$

Using these values for taxa-branchpoint distances we next use Farris' (5), (6) and (7) to compute Table B2 of branch to taxa distances for all taxa not on the tree.

For example,

$$D(Oi,(Op,1)) = \frac{1}{2}[D(Oi,Op) + D(Oi,1) - D(Op,1)]$$

$$= \frac{1}{2}[0.407 + 0.3265 - 0.0805]$$

$$= 0.3265$$

$$D(Oi,(Pp,1)) = \frac{1}{2}[D(Oi,Pp) + D(Oi,1) - D(Pp,1)]$$

$$= 0.2965$$

$$D(Oi,(Ov,1)) = \frac{1}{2}[D(Oi,Ov) + D(Oi,1) - D(Ov,1)]$$

$$= 0.2350$$

Distances are calculated similarly for $D(Pc(Op,1))$, $D(Pc,(Pp,1))$... $D(Cr,(Ov,1))$.

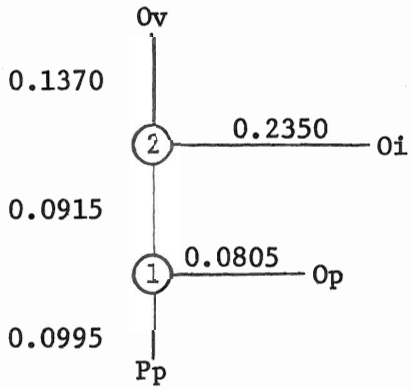
Table B2. Taxa-Branch Distances

Branch	Taxon			
	Oi	Pc	Cb	Cr
Op,1	0.3265	0.4415*	0.7145	0.7625
Pp,1	0.2965	0.4770	0.6485*	0.6565*
Ov,1	0.2350**	0.6595	0.6670	0.7475

Examining the table we see that the distance O. immunis to the O. virilis-1 branch is the minimum distance. Add O. immunis to the tree and position it by using the equation analogous to step 4 above.

$D(Ov,2) = D(Ov,Oi) - D(Oi,2) = 0.372 - 0.2350 = 0.137$

$D(1,2) = D(Oi,1) - D(Oi,2) = 0.3265 - 0.2350 = 0.0915$



If any taxa remain unconnected to the tree, return to step 5 above and add the remaining taxa one at a time.

$D(Pc,2) = \sup\{\cdot\} = 0.7510$

$D(Cb,2) = \sup\{\cdot\} = 0.7580$

$D(Cr,2) = \sup\{\cdot\} = 1.0470$

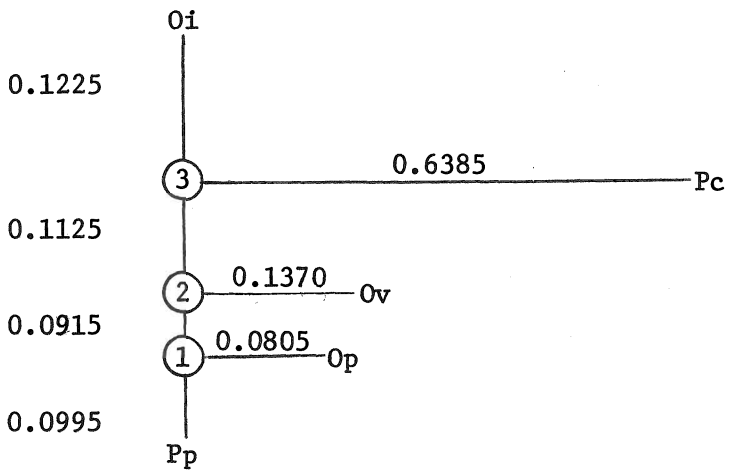
Table B3. Taxa-Branch Distances

	Taxon		
Branch	Pc	Cb	Cr
Ov,2	0.7510	0.7345	0.9355
Oi,2	0.6385**	0.7590	1.0470
1,2	0.6595	0.6905*	0.8590*

Examining the table we see that the distance P. clarkii to the O. immunis-2 branch is the minimum distance. Add P. clarkii to the tree and position it by using the equations analogous to step 4 above.

D(Oi,3) = D(Oi,Pc) - D(Pc,3) = 0.1225

D(2,3) = D(Pc,2) - D(Pc,3) = 0.1125



If any taxa remain unconnected to the tree, return to step 5 above and add the remaining taxa one at a time.

D(Cb,3) = sup{•} = 0.9425

D(Cr,3) = sup{•} = 1.0470

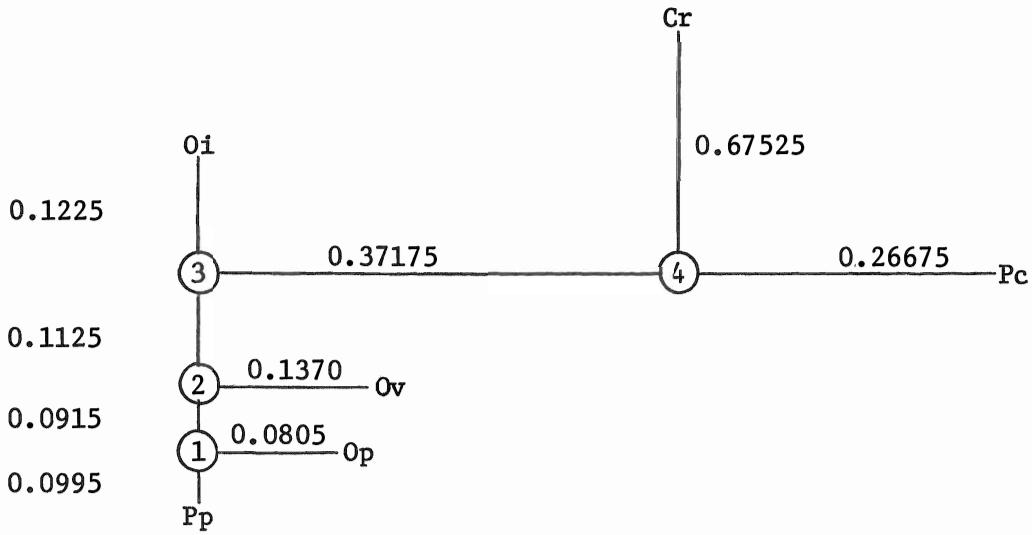
Table B4. Taxa-Branch Distances

	Taxon	
Branch	Cb	Cr
Pc,3	0.9425	0.67525**
Oi,3	0.85025	1.0470
2,3	0.7940*	0.99075

Examining the table we see that C. robustus is to be added to the branch P. clarkii-3.

D(Pc,4) = D(Pc,Cr) - D(Cr,4) = 0.26675

D(3,4) = D(Cr,3) - D(Cr,4) = 0.37175



Add the remaining taxon to the tree by returning to step 5 above.

D(Cb,4) = sup{•} = 1.31425

Table B5. Taxon-Branch Distances

	Taxon
Branch	Cb
Pc,4	0.31425
Cr,4	0.6390**
3,4	0.9425

Examining the table we see that C. bartoni is to be added to the branch C. robustus-4.

D(Cr,5) = D(Cb,Cr) - D(Cb,5) = 0.0000

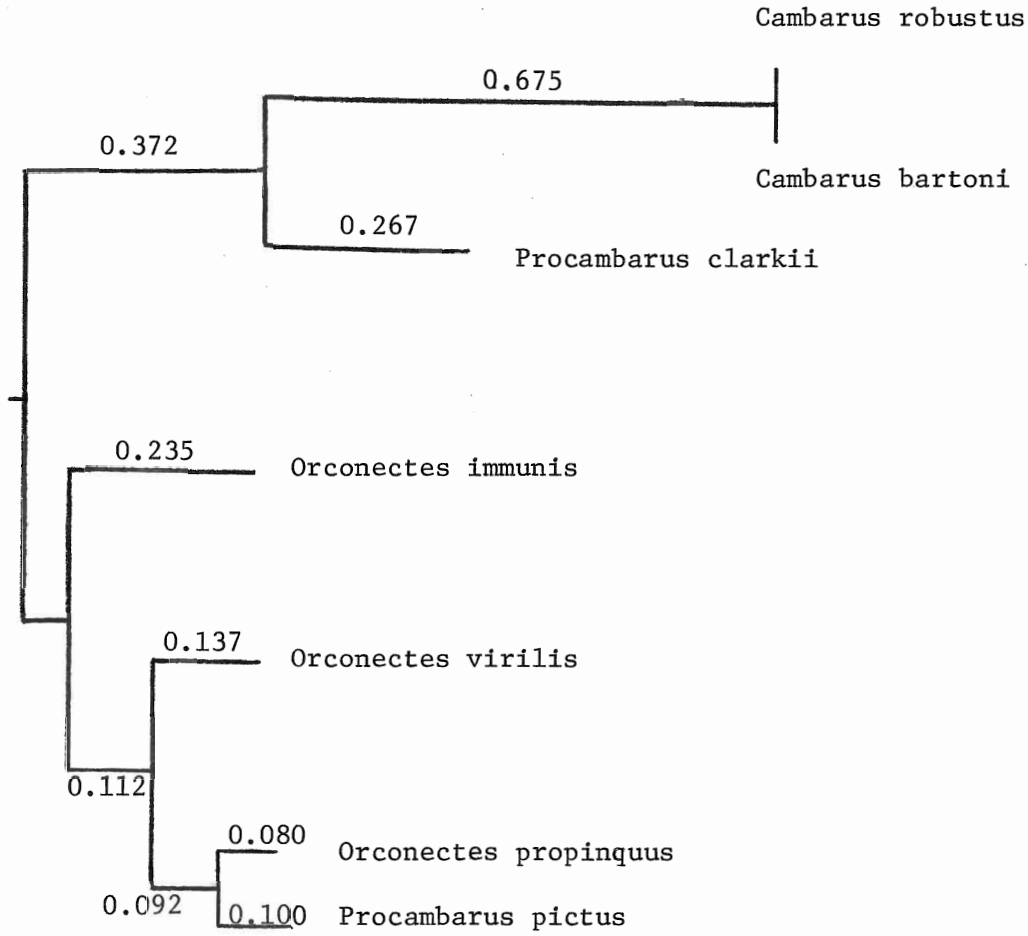
D(4,5) = D(Cb,4) - D(Cb,5) = 0.67525

By subtracting the matrix of genetic distances (Table B1) from the patristic distance matrix (Table B6) we can compute a homoplasy matrix (Table B6) and a value of homoplasy. This value can be used to compare the goodness-of-fit of different trees.

Table B6. Patristic Distance Matrix (upper diagonal) and Homoplasy matrix (lower diagonal)

	O.p.	O.v.	O.i.	P.p.	P.c.	C.b.	C.r.
O.p.	--	0.3090	0.5195	0.1800	0.9230	1.3315	1.3315
O.v.	0.00	--	0.4845	0.3280	0.888	1.2965	1.2965
O.i.	0.1125	0.1125	--	0.5385	0.9835	1.2820	1.2820
P.p.	0.00	0.00	0.1725	--	0.942	1.3505	1.3505
P.c.	0.6190	0.00	0.1125	0.5480	--	0.9420	0.9420
C.b.	0.5365	0.4485	0.2890	0.6685	-0.639	--	0.00
C.r.	0.4885	0.3355	0.00	0.7005	0.00	-0.639	--

$$H(\cdot) = |P(\cdot) - D(\cdot)| = 6.4220$$



Cambarinae Wagner Tree Based on Species Mean D

Appendix C

Genetic identity (I) and genetic distance (D) values for all sample comparisons listing loci compared.

POPULATION 1 COMPARED TO POP. 2 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, EST-4, LAP, MDH-1, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, PROT3, PROT4, PROT5, TO-1, TO-2, XDH,
I= .8882539680079 D= .1184975768712

POPULATION 1 COMPARED TO POP. 3 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, EST-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, PROT5, TO-1, TO-2, XDH,
I= .9110243472424 D= 9.31856562E-02

POPULATION 1 COMPARED TO POP. 4 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, EST-5, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, TO-2, XDH,
I= .9747296819998 D= 2.55950956E-02

POPULATION 1 COMPARED TO POP. 5 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, EST-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, PROT5, TO-2, XDH,
I= .884400311416 D= .1228454778777

POPULATION 1 COMPARED TO POP. 6 LOCI COMPARED=ACPH, AO-2, EST-4, LAP, MDH-1, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, PROT3, PROT4, PROT5, TO-1, TO-2, XDH,
I= .8718170695031 D= .1371756597606

POPULATION 1 COMPARED TO POP. 7 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP , MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, TO-2, XDH,
I= .7500509962485 D= .2876140797651

POPULATION 1 COMPARED TO POP. 8 LOCI COMPARED=ACPH, AO-2, AO-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, XDH,
I= .7040696977633 D= .3508779252197

POPULATION 1 COMPARED TO POP. 9 LOCI COMPARED=ACPH, AO-2, AO-4, EST-4, MDH-1, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, XDH,
I= .7047258672777 D= .3499463925287

POPULATION 1 COMPARED TO POP. 10 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, EST-5, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, PROT5, TO-2, XDH,
I= .8030156398946 D= .2193810883949

POPULATION 1 COMPARED TO POP. 11 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, EST-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, PROT3, PROT4, TO-2, XDH,
I= .4502884403625 D= .7978669229734

POPULATION 1 COMPARED TO POP. 12 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, EST-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, PROT3, PROT4, TO-2, XDH,
I= .4517536703503 D= .794618224805

POPULATION 1 COMPARED TO POP. 13 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, EST-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, PROT3, PROT4, TO-2, XDH,
I= .5025082819058 D= .6881431577801

POPULATION 1 COMPARED TO POP. 14 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, PROT3, PROT4, PROT5, TO-2, XDH,
I= .4563991541442 D= .784387514317

POPULATION 1 COMPARED TO POP. 15 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH, PGI, PGM-1, PROT1, PROT3, PROT4, PROT5, TO-1, TO-2, XDH,
I= .7117265280922 D= .3400615311096

POPULATION 1 COMPARED TO POP. 16 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, PROT5, TO-1, TO-2
,
I= .9027633904654 D= .1022947859627

POPULATION 1 COMPARED TO POP. 17 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT2, PROT3, PROT4, PROT5, TO-2, XDH
,
I= .8482657053433 D= .1645613604956

POPULATION 1 COMPARED TO POP. 18 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, PROT5, TO-1, TO-2
,
I= .8973611523011 D= .1082968755447

POPULATION 2 COMPARED TO POP. 3 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, EST-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, PROT5, TO-1, TO-2, XDH,
I= .9825699549942 D= 1.75837367E-02

POPULATION 2 COMPARED TO POP. 4 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP , MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, TO-2, XDH,
I= .969118294973 D= 3.13685951E-02

POPULATION 2 COMPARED TO POP. 5 LOCI COMPARED=ACPH, AO-1, AO-2, AO-3, AO-4, EST-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, PROT5, TO-2, XDH,
I= .9777296000327 D= 2.25221297E-02

POPULATION 2 COMPARED TO POP. 6 LOCI COMPARED=ACPH, AO-2, EST-4, LAP, MDH-1, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, PROT3, PROT4, PROT5, TO-1, TO-2, XDH,
I= .9590018375485 D= 4.18622879E-02

POPULATION 2 COMPARED TO POP. 7 LOCI COMPARED=ACPH, AO-1, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, TO-2, XDH,
I= .74403828672 D= .2956627848278

POPULATION 2 COMPARED TO POP. 8 LOCI COMPARED=ACPH, AO-2, AO-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, XDH,
I= .6060016999837 D= .500872487663

POPULATION 2 COMPARED TO POP. 9 LOCI COMPARED=ACPH, AO-2, AO-4, EST-4, MDH-1, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, XDH,
I= .5509272841697 D= .596152449192

POPULATION 2 COMPARED TO POP. 10 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, PROT5, TO-2, XDH,
I= .736898944294 D= .3053045138414

POPULATION 2 COMPARED TO POP. 11 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, EST-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, PROT3, PROT4, TO-2, XDH,
I= .4349304734736 D= .8325690917628

POPULATION 2 COMPARED TO POP. 12 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, EST-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, PROT3, PROT4, TO-2, XDH,
I= .4324400995868 D= .8383114602669

POPULATION 2 COMPARED TO POP. 13 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, EST-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, PROT3, PROT4, TO-2, XDH,
I= .4564920535572 D= .7841839864211

POPULATION 2 COMPARED TO POP. 14 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, PROT3, PROT4, PROT5, TO-2, XDH,
I= .3812131104125 D= .9643967153156

POPULATION 2 COMPARED TO POP. 15 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH, PGI, PGM-1, PROT1, PROT3, PROT4, PROT5, TO-1, TO-2, XDH,
I= .7823065107533 D= .245508657747

POPULATION 2 COMPARED TO POP. 16 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, PROT5, TO-1, TO-2
,
I= .8384095756148 D= .1762485441843

POPULATION 2 COMPARED TO POP. 17 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT2, PROT3, PROT4, PROT5, TO-2, XDH
,
I= .8050892701188 D= .2168021131543

POPULATION 2 COMPARED TO POP. 18 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, PROT5, TO-1, TO-2 ,
I= .8260580484078 D= .1910902314076

POPULATION 3 COMPARED TO POP. 4 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP , MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, XDH, AMY-1,
I= .9906512943368 D= 9.39267908E-03

POPULATION 3 COMPARED TO POP. 5 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, EST-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, PROT5, TO-2, XDH,
I= .9608748003466 D= 3.99111590E-02

POPULATION 3 COMPARED TO POP. 6 LOCI COMPARED=ACPH, AO-2, EST-3, EST-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, PROT5, TO-1, TO-2 , XDH, AMY-1,
I= .9409187855325 D= 6.08984496E-02

POPULATION 3 COMPARED TO POP. 7 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP , MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, XDH, AMY-1, AMY-2,
I= .7766741107131 D= .2527344365272

POPULATION 3 COMPARED TO POP. 8 LOCI COMPARED=ACPH, AO-2, AO-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, XDH, AMY-1,
I= .6709143275261 D= .3991138289606

POPULATION 3 COMPARED TO POP. 9 LOCI COMPARED=ACPH, AO-2, AO-4, EST-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, XDH, AMY-1,
I= .6480884937818 D= .4337280274757

POPULATION 3 COMPARED TO POP. 10 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, PROT5, TO-2, XDH, AMY-1,
I= .7761847203332 D= .2533647454597

POPULATION 3 COMPARED TO POP. 11 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, EST-3, EST-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, TO-2, XDH, AMY-1,
I= .4386098744396 D= .8241449297095

POPULATION 3 COMPARED TO POP. 12 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, EST-3, EST-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, TO-2, XDH,
I= .4662734719913 D= .7629829672187

POPULATION 3 COMPARED TO POP. 13 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, EST-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, TO-2, XDH , AMY-1,
I= .495564245404 D= .7020582758894

POPULATION 3 COMPARED TO POP. 14 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, PROT5, TO-2, XDH,
I= .4536036015043 D= .7905315867756

POPULATION 3 COMPARED TO POP. 15 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH, PGI, PGM-1, PROT1, PROT3, PROT4, PROT5, TO-1, TO-2, XDH, AMY-1,
I= .7783629425622 D= .250562356435

POPULATION 3 COMPARED TO POP. 16 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, PROT5, TO-1, TO-2, AMY-1, AMY-2,
I= .8800579901496 D= .1277674757837

POPULATION 3 COMPARED TO POP. 17 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT3, PROT4, PROT5, TO-2, XDH, AMY-1,
I= .8365328090052 D= .1784895375609

POPULATION 3 COMPARED TO POP. 18 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, PROT5, TO-1, TO-2, AMY-1, AMY-2,
I= .870924237806 D= .138200288914

POPULATION 4 COMPARED TO POP. 5 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, XDH,
I= .9647964490238 D= 3.58381335E-02

POPULATION 4 COMPARED TO POP. 6 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, TO-2, XDH, AMY-1,
I= .9282410030343 D= 7.44638783E-02

POPULATION 4 COMPARED TO POP. 7 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, TO-2, XDH, AMY-1,
I= .7699892334999 D= .2613787466997

POPULATION 4 COMPARED TO POP. 8 LOCI COMPARED=ACPH, AO-2, AO-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, XDH, AMY-1,
I= .6739348382379 D= .3946218519158

POPULATION 4 COMPARED TO POP. 9 LOCI COMPARED=ACPH, AO-2, AO-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, XDH, AMY-1,
I= .6967282234242 D= .3613598676127

POPULATION 4 COMPARED TO POP. 10 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, EST-5, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, XDH, AMY-1,
I= .7451549481379 D= .2941630981504

POPULATION 4 COMPARED TO POP. 11 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, TO-2, XDH, AMY-1,
I= .5240624449086 D= .6461444320881

POPULATION 4 COMPARED TO POP. 12 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, TO-2, XDH,
I= .5646133224883 D= .5716141671104

POPULATION 4 COMPARED TO POP. 13 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, TO-2, XDH, AMY-1,
I= .5557879305447 D= .5873684773741

POPULATION 4 COMPARED TO POP. 14 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, TO-2, XDH,
I= .5234582496322 D= .6472980042258

POPULATION 4 COMPARED TO POP. 15 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH, PGI, PGM-1, PROT1, TO-2, XDH, AMY-1,
I= .6805030977484 D= .3849229047357

POPULATION 4 COMPARED TO POP. 16 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, AMY-1,
I= .8246970327104 D= .1927391931723

POPULATION 4 COMPARED TO POP. 17 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT2, TO-2, XDH, AMY-1,
I= .8077071093647 D= .2135557745859

POPULATION 4 COMPARED TO POP. 18 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, AMY-1,
I= .8097199248222 D= .2110668629329

POPULATION 5 COMPARED TO POP. 6 LOCI COMPARED=ACPH, AO-2, EST-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, PROT5, TO-2, XDH,
I= .9866274700622 D= 1.34627474E-02

POPULATION 5 COMPARED TO POP. 7 LOCI COMPARED=ACPH, AO-1, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, XDH,
I= .702471858985 D= .3531499369608

POPULATION 5 COMPARED TO POP. 8 LOCI COMPARED=ACPH, AO-2, AO-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, XDH,
I= .5958015103429 D= .517847703723

POPULATION 5 COMPARED TO POP. 9 LOCI COMPARED=ACPH, AO-2, AO-4, EST-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, XDH,
I= .5770797789927 D= .5497747568797

POPULATION 5 COMPARED TO POP. 10 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, PROT5, TO-2, XDH,
I= .7295615870168 D= .3153114909818

POPULATION 5 COMPARED TO POP. 11 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, EST-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, TO-2, XDH
,
I= .452204941742 D= .7936197909757

POPULATION 5 COMPARED TO POP. 12 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, EST-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, TO-2, XDH
,
I= .4497146789034 D= .7991419441917

POPULATION 5 COMPARED TO POP. 13 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, EST-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, TO-2, XDH
,
I= .4867706292295 D= .7199622540572

POPULATION 5 COMPARED TO POP. 14 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, PROT5, TO-2, XDH,
I= .406572959055 D= .8999918850287

POPULATION 5 COMPARED TO POP. 15 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH, PGI, PGM-1, PROT1, PROT3, PROT4, PROT5, TO-2, XDH,
I= .725065373019 D= .3214934585109

POPULATION 5 COMPARED TO POP. 16 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, PROT5, TO-2,
I= .8330141029204 D= .182704706682

POPULATION 5 COMPARED TO POP. 17 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT3, PROT4, PROT5, TO-2, XDH,
I= .788719066948 D= .2373450837113

POPULATION 5 COMPARED TO POP. 18 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, PROT5, TO-2,
I= .8160195919744 D= .2033169145337

POPULATION 6 COMPARED TO POP. 7 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH , PGI, PGM-1, PGM-2, PROT1, PROT2, TO-2, XDH, AMY-1,
I= .6693226577624 D= .4014890365239

POPULATION 6 COMPARED TO POP. 8 LOCI COMPARED=ACPH, AO-2, MDH-2, ODH, PGI , PGM-1, PGM-2, PROT1, TO-2, XDH, AMY-1,
I= .5836747102198 D= .5384114543858

POPULATION 6 COMPARED TO POP. 9 LOCI COMPARED=ACPH, AO-2, EST-4, MDH-1, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, XDH, AMY-1,
I= .5564094710536 D= .5862507970545

POPULATION 6 COMPARED TO POP. 10 LOCI COMPARED=ACPH, AO-2, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, PROT5, TO-2, XDH, AMY-1,
I= .6949021838426 D= .363984185995

POPULATION 6 COMPARED TO POP. 11 LOCI COMPARED=ACPH, AO-2, EST-3, EST-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, PROT3, PROT4, TO-2, XDH, AMY-1,
I= .2844390451396 D= 1.257236300955

POPULATION 6 COMPARED TO POP. 12 LOCI COMPARED=ACPH, AO-2, EST-3, EST-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, PROT3, PROT4, TO-2, XDH,
I= .3022509847302 D= 1.196497531493

POPULATION 6 COMPARED TO POP. 13 LOCI COMPARED=ACPH, AO-2, EST-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, PROT3, PROT4, TO-2, XDH, AMY-1,
I= .3639484582414 D= 1.010743019609

POPULATION 6 COMPARED TO POP. 14 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, PROT3, PROT4, PROT5, TO-2, XDH,
I= .3828159587018 D= .9602009308736

POPULATION 6 COMPARED TO POP. 15 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH, PGI, PGM-1, PROT1, PROT3, PROT4, PROT5, TO-1, TO-2, XDH, AMY-1,
I= .7537137572233 D= .2827426153824

POPULATION 6 COMPARED TO POP. 16 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, PROT5, TO-1, TO-2, AMY-1,
I= .8406569652512 D= .1735715913372

POPULATION 6 COMPARED TO POP. 17 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT2, PROT3, PROT4, PROT5, TO-2, XDH, AMY-1,
I= .7905638267567 D= .2350088833319

POPULATION 6 COMPARED TO POP. 18 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, PROT5, TO-1, TO-2, AMY-1,
I= .8278059494634 D= .188976512615

POPULATION 7 COMPARED TO POP. 8 LOCI COMPARED=ACPH, AO-2, AO-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, XDH, AMY-1,
I= .6746674396281 D= .3935353915495

POPULATION 7 COMPARED TO POP. 9 LOCI COMPARED=ACPH, AO-2, AO-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, XDH, AMY-1,
I= .6838610431744 D= .3800005352517

POPULATION 7 COMPARED TO POP. 10 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, XDH, AMY-1,
I= .7094300415041 D= .3432933897746

POPULATION 7 COMPARED TO POP. 11 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, TO-2, XDH, AMY-1,
I= .3583139108146 D= 1.026345830935

POPULATION 7 COMPARED TO POP. 12 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, TO-2, XDH,
I= .408328646526 D= .8956829226327

POPULATION 7 COMPARED TO POP. 13 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, TO-2, XDH, AMY-1,
I= .5208857653418 D= .6522245216502

POPULATION 7 COMPARED TO POP. 14 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, TO-2, XDH,
I= .351961604621 D= 1.044233187114

POPULATION 7 COMPARED TO POP. 15 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH, PGI, PGM-1, PROT1, TO-2, XDH, AMY-1,
I= .4113387167666 D= .8883382755073

POPULATION 7 COMPARED TO POP. 16 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, AMY-1, AMY-2,
I= .7468546721144 D= .2918846614853

POPULATION 7 COMPARED TO POP. 17 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT2, TO-2, XDH, AMY-1,
I= .6678262956483 D= .4037271757154

POPULATION 7 COMPARED TO POP. 18 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, AMY-1, AMY-2,
I= .7503483848191 D= .2872176672124

POPULATION 8 COMPARED TO POP. 9 LOCI COMPARED=ACPH, AO-2, AO-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, XDH, AMY-1,
I= .9955117371746 D= 4.49836531E-03

POPULATION 8 COMPARED TO POP. 10 LOCI COMPARED=ACPH, AO-2, AO-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, XDH, AMY-1,
I= .9939638953977 D= 6.05439501E-03

POPULATION 8 COMPARED TO POP. 11 LOCI COMPARED=ACPH, AO-2, AO-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, XDH, AMY-1,
I= .259408290235 D= 1.349352048339

POPULATION 8 COMPARED TO POP. 12 LOCI COMPARED=ACPH, AO-2, AO-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, XDH,
I= .2841386016668 D= 1.258293125832

POPULATION 8 COMPARED TO POP. 13 LOCI COMPARED=ACPH, AO-2, AO-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, XDH, AMY-1,
I= .4259250465643 D= .8534918952252

POPULATION 8 COMPARED TO POP. 14 LOCI COMPARED=ACPH, AO-2, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, XDH,
I= .3690211787671 D= .9969012415558

POPULATION 8 COMPARED TO POP. 15 LOCI COMPARED=ACPH, AO-2, MDH-2, ODH, PGI, PGM-1, PROT1, TO-2, XDH, AMY-1,
I= .4115391526366 D= .8878511172511

POPULATION 8 COMPARED TO POP. 16 LOCI COMPARED=ACPH, AO-2, AO-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, AMY-1,
I= .7159775128146 D= .3341065191982

POPULATION 8 COMPARED TO POP. 17 LOCI COMPARED=ACPH, AO-2, AO-4, MDH-2, ODH, PGI, PGM-1, PGM-2, TO-2, XDH, AMY-1,
I= .5658536851403 D= .5694197410168

POPULATION 8 COMPARED TO POP. 18 LOCI COMPARED=ACPH, AO-2, AO-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, AMY-1,
I= .7151356522936 D= .3352830307957

POPULATION 9 COMPARED TO POP. 10 LOCI COMPARED=ACPH, AO-2, AO-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, XDH, AMY-1,
I= .9997393389945 D= 2.60694983E-04

POPULATION 9 COMPARED TO POP. 11 LOCI COMPARED=ACPH, AO-2, AO-4, EST-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, XDH, AMY-1,
I= .2549974605823 D= 1.366501692374

POPULATION 9 COMPARED TO POP. 12 LOCI COMPARED=ACPH, AO-2, AO-4, EST-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, XDH,
I= .2761056734213 D= 1.286971611827

POPULATION 9 COMPARED TO POP. 13 LOCI COMPARED=ACPH, AO-2, AO-4, EST-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, XDH, AMY-1,
I= .416951176393 D= .8747861470282

POPULATION 9 COMPARED TO POP. 14 LOCI COMPARED=ACPH, AO-2, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, XDH,
I= .3568380722996 D= 1.030473179093

POPULATION 9 COMPARED TO POP. 15 LOCI COMPARED=ACPH, AO-2, MDH-2, ODH, PGI, PGM-1, PROT1, TO-2, XDH, AMY-1,
I= .4334465099014 D= .8359868816055

POPULATION 9 COMPARED TO POP. 16 LOCI COMPARED=ACPH, AO-2, AO-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, AMY-1,
I= .7122144798015 D= .3393761772562

POPULATION 9 COMPARED TO POP. 17 LOCI COMPARED=ACPH, AO-2, AO-4, MDH-2, ODH, PGI, PGM-1, PGM-2, TO-2, XDH, AMY-1,
I= .5826379982419 D= .5401892147472

POPULATION 9 COMPARED TO POP. 18 LOCI COMPARED=ACPH, AO-2, AO-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, TO-2, AMY-1,
I= .707257112843 D= .3463610118088

POPULATION 10 COMPARED TO POP. 11 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, TO-2, XDH, AMY-1,
I= .2867275238035 D= 1.249222908595

POPULATION 10 COMPARED TO POP. 12 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, TO-2, XDH,
I= .3065498516141 D= 1.182374888824

POPULATION 10 COMPARED TO POP. 13 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, TO-2, XDH, AMY-1,
I= .4109736875169 D= .8892260871753

POPULATION 10 COMPARED TO POP. 14 LOCI COMPARED=ACPH, AO-2, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, PROT5, TO-2, XDH,
I= .2682914031408 D= 1.315681564028

POPULATION 10 COMPARED TO POP. 15 LOCI COMPARED=ACPH, AO-2, MDH-2, ODH, PGI, PGM-1, PROT1, PROT3, PROT4, PROT5, TO-2, XDH, AMY-1,
I= .5714598047037 D= .5595611311976

POPULATION 10 COMPARED TO POP. 16 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, PROT5, TO-2, AMY-1,
I= .7908180677739 D= .2346873404834

POPULATION 10 COMPARED TO POP. 17 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT3, PROT4, PROT5, TO-2, XDH, AMY-1,
I= .6976101010867 D= .3600949267233

POPULATION 10 COMPARED TO POP. 18 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, PROT5, TO-2, AMY-1,
I= .7864674461717 D= .2402039480946

POPULATION 11 COMPARED TO POP. 12 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, EST-3, EST-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, PROT3, PROT4, TO-2, XDH,
I= .9853452842817 D= 1.47631568E-02

POPULATION 11 COMPARED TO POP. 13 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, EST-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, PROT3, PROT4, TO-2, XDH, AMY-1,
I= .5313022654377 D= .6324241815821

POPULATION 11 COMPARED TO POP. 14 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, PROT3, PROT4, TO-2, XDH,
I= .5291355620606 D= .6365106189688

POPULATION 11 COMPARED TO POP. 15 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH, PGI, PGM-1, PROT1, PROT3, PROT4, TO-2, XDH, AMY-1,
I= .3738841568331 D= .9838092706351

POPULATION 11 COMPARED TO POP. 16 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, TO-2, AMY-1,
I= .4998888039115 D= .6933695974697

POPULATION 11 COMPARED TO POP. 17 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT2, PROT3, PROT4, TO-2, XDH, AMY-1
,
I= .5126079015077 D= .6682440506026

POPULATION 11 COMPARED TO POP. 18 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, TO-2, AMY-1,
I= .4912444242711 D= .7108134659471

POPULATION 12 COMPARED TO POP. 13 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, EST-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, PROT3, PROT4, TO-2, XDH,
I= .5149080863884 D= .6637668672788

POPULATION 12 COMPARED TO POP. 14 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, PROT3, PROT4, TO-2, XDH,
I= .5357072174024 D= .624167503342

POPULATION 12 COMPARED TO POP. 15 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH, PGI, PGM-1, PROT1, PROT3, PROT4, TO-2, XDH,
I= .4066790563628 D= .899730963921

POPULATION 12 COMPARED TO POP. 16 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, TO-2,
I= .5419533798503 D= .6125752962782

POPULATION 12 COMPARED TO POP. 17 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT2, PROT3, PROT4, TO-2, XDH,
I= .5539440452114 D= .5906915987591

POPULATION 12 COMPARED TO POP. 18 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, TO-2,
I= .5348828703847 D= .6257074899158

POPULATION 13 COMPARED TO POP. 14 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT2, PROT3, PROT4, TO-2, XDH,
I= .4949517745156 D= .7032949463804

POPULATION 13 COMPARED TO POP. 15 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH, PGI, PGM-1, PROT1, PROT3, PROT4, TO-2, XDH, AMY-1,
I= .1706010608958 D= 1.768427425328

POPULATION 13 COMPARED TO POP. 16 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, TO-2, AMY-1,
I= .4454127185632 D= .8087539691932

POPULATION 13 COMPARED TO POP. 17 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT2, PROT3, PROT4, TO-2, XDH, AMY-1
,
I= .4825254642774 D= .728721583891

POPULATION 13 COMPARED TO POP. 18 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, TO-2, AMY-1,
I= .4460001899183 D= .8074359011363

POPULATION 14 COMPARED TO POP. 15 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH, PGI, PGM-1, PROT1, PROT3, PROT4, PROT5, TO-2, XDH,
I= .2481182183169 D= 1.393849959634

POPULATION 14 COMPARED TO POP. 16 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, PROT5, TO-2,
I= .5523596592753 D= .5935558882176

POPULATION 14 COMPARED TO POP. 17 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT2, PROT3, PROT4, PROT5, TO-2, XDH,
I= .5616594858563 D= .576859509771

POPULATION 14 COMPARED TO POP. 18 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, PROT5, TO-2,
I= .5617543511696 D= .5766906222064

POPULATION 15 COMPARED TO POP. 16 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH, PGI, PGM-1, PROT1, PROT3, PROT4, PROT5, TO-1, TO-2, AMY-1,
I= .6990608895464 D= .3580174310336

POPULATION 15 COMPARED TO POP. 17 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH, PGI, PGM-1, PROT3, PROT4, PROT5, TO-2, XDH, AMY-1,
I= .6373177236404 D= .4504869665347

POPULATION 15 COMPARED TO POP. 18 LOCI COMPARED=ACPH, AO-2, LAP, MDH-2, ODH, PGI, PGM-1, PROT1, PROT3, PROT4, PROT5, TO-1, TO-2, AMY-1,
I= .6886145168168 D= .3730736466605

POPULATION 16 COMPARED TO POP. 17 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT3, PROT4, PROT5, TO-2, AMY-1,
I= .909881999358 D= 9.44403589E-02

POPULATION 16 COMPARED TO POP. 18 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT1, PROT3, PROT4, PROT5, TO-1, TO-2, AMY-1, AMY-2,
I= .996115632601 D= 3.89193114E-03

POPULATION 17 COMPARED TO POP. 18 LOCI COMPARED=ACPH, AO-2, AO-3, AO-4, LAP, MDH-2, ODH, PGI, PGM-1, PGM-2, PROT3, PROT4, PROT5, TO-2, AMY-1,
I= .9152518030777 D= 8.85560569E-02

Appendix D

Basic computer program listings used in data analysis

```
10 REM NEIS GEN ID
20 COM X(40),Y(40):INPUT "NO. OF ALLELES OVER ALL LOCI ([40) ",
N
30 FOR I=1 TO N
40 INPUT "X AND Y", X(I), Y(I)
50 NEXT I
60 FOR I=1 TO N
70 PRINT "X(";I;")"; X(I);" Y(";I;")"; Y(I);
80 NEXT I: STOP
90 FOR I=1 TO N
100 S1=S1+ X(I)*Y(I)
110 S2=S2 + X(I)!2 : S3=S3 + Y(I)!2
120 NEXT I : S4=SQR(S2 * S3): I1=S1/S4
130 PRINT "I=";I1, "D="; -1*LOG(I1):END
```

```
10REM "COMPUTE":DIM A$(4)64,X(107),Y(107),B$5:N=1:DATA LOAD "DAT
A":FOR I=1TO 17
20FOR J=1TO I:DATA LOAD A$():NEXT J:UNPACK(#.###)A$()TO X():READ
B$,P
30FOR J=I+1TO 18:SELECT PRINT 215(40):PRINT "POPULATION ";I;"COM
PARED TO POP.";J,"LOCI COMPARED=";:DATA LOAD A$():UNPACK(#.###)A
$()TO Y()
40IF X(N)=9.999THEN 60:IF Y(N)=9.999THEN 60
50FOR K=NTO N+P-1:S1=S1+X(K)*Y(K):S2=S2+X(K)!2:S3=S3+Y(K)!2:NEXT
K:PRINT B$;", ";
60N=N+P:IF N]=108THEN 70:READ B$,P:GOTO 40
70S4=SQR(S2*S3):I1=S1/S4:PRINT :PRINT "I=";I1:PRINT "D=";-1*LOG(
I1):PRINT :RESTORE :S1,S2,S3,S4=0:N=1:READ B$,P:NEXT J
80BACKSPACE BEG :RESTORE :NEXT I:SELECT PRINT 005:END
90DATA "ACPH",4,"AO-1",2,"AO-2",11,"AO-3",2,"AO-4",2,"EST-1",2
100DATA "EST-3",5,"EST-4",8,"EST-5",4,"LAP",10,"MDH-1",3,"MDH-2"
,5,"ODH",5,"PGI",3,"PGM-1",6,"PGM-2",3,"PROT1",4,"PROT2",3
110DATA "PROT3",4,"PROT4",4,"PROT5",3,"TO-1",1,"TO-2",3,"XDH",6,
"AMY-1",3,"AMY-2",1," ",5
```

```
10REM "DATASAVE":COM A(107),B(11),A$(4)64,B$5,K$3,L$3:PRINT HEX(
03);"THIS PROGRAM ASSUMES A FILE LABELLED 'DATA' EXISTS FOR 18 P
OPU- LATIONS."
20INPUT "NO. OF FIRST POPULATION FOR TODAY",H:DATA LOAD "DATA":S
KIP END
30FOR I=1TO 107:A(I)=0:NEXT I:N=1
40READ B$,P:K=0:FOR I=1TO 11:B(I)=0:NEXT I:PRINT :PRINT "FOR POP
ULATION";H;" LOCUS  ";B$;"INPUT THE NO. OF ALLELES TO BE ENTERED
";
50INPUT K:IF K[]0THEN 60:B(1)=9.999:GOTO 70
60FOR I=1TO K:INPUT "VALUE OF ALLELE",B(I):NEXT I
70K=0:FOR I=NTO N+P-1:K=K+1:A(I)=B(K):NEXT I:N=N+P:IF N]=108THEN
80:GOTO 40
80RESTORE :N=0
90READ B$,P:PRINT HEX(03);"POPULATION ";H;" LOCUS  ";B$:FOR J=1T
O P:PRINT "A(";N+J;")",A(N+J):NEXT J:INPUT "IS DATA CORRECT (Y=Y
ES)",K$:IF K$="Y"THEN 100:STOP "MAKE CORRECTIONS"
100N=N+P:IF N[107THEN 90:PACK(#.###)A$()FROMA():DATA SAVE A$():R
ESTORE :INPUT "ARE YOU FINISHED FOR TODAY (Y=YES)",L$:IF L$="Y"T
HEN 120
110H=H+1:IF H[19THEN 30
120DATA SAVE END :END
130DATA "ACPH",4,"AO-1",2,"AO-2",11,"AO-3",2,"AO-4",2,"EST-1",2
140DATA "EST-3",5,"EST-4",8,"EST-5",4,"LAP",10,"MDH-1",3,"MDH-2"
,5,"ODH",5,"PGI",3,"PGM-1",6,"PGM-2",3,"PROT1",4,"PROT2",3
150DATA "PROT3",4,"PROT4",4,"PROT5",3,"TO-1",1,"TO-2",3,"XDH",6,
"AMY-1",3,"AMY-2",1," ",5
```

Appendix E

Regulated power supply schematic

Designed and constructed by John Rustenberg

Brock University Technical Services

Regulator Reference Supply

Theory of Operation

The LM304 device is a fixed voltage regulator chip with 0.01% line regulation and 1 mV stability with load regulation. However, since load is constant, because it supplies only a fixed reference voltage, the only variable is line regulation and ripple noise, essentially giving 4 figure accuracy with no warmup.

The 304 has a separate bias supply (10 volts) Zener regulated across pins 2 and 6 with respect to the output buss to get the specified stability for the 304.

To get the positive buss voltage Zener diode D_2 is in series with the 304 regulator supplied from the same unfiltered supply (consisting of a 660 μF capacitor) at 25 V.

Since a constant I flows through Zener D_2, it also yields a good positive buss stability of about 3 figure accuracy.

Note: For the current limit circuit to work, Resistor R_2 is installed on the chassis of the power supply, as for a high current supply it must be on a heat sink.

Also to regulate high voltages, resistor R_{v2} is not of sufficient resistance so an additional external limit resistor (of good stability) must be mounted on the chassis to limit the voltage on the non-inverting input of amplifier A_1 to the maximum voltage supplied by the reference buss, ≃6.0 volts.

OCT. 75 REGULATED POWER SUPPLY

GENERAL INFORMATION

The basic regulator circuit has the capacity to give both constant current and constant voltage control. The output current and voltage limit is dependant upon the capacity of the basic supply and the limitation of the series pss transistor (s).

THEORY OF OPERATION

The reference supply , nominally 8 V. out is stablized by a LM304 regulator and 2N2904 buffer.

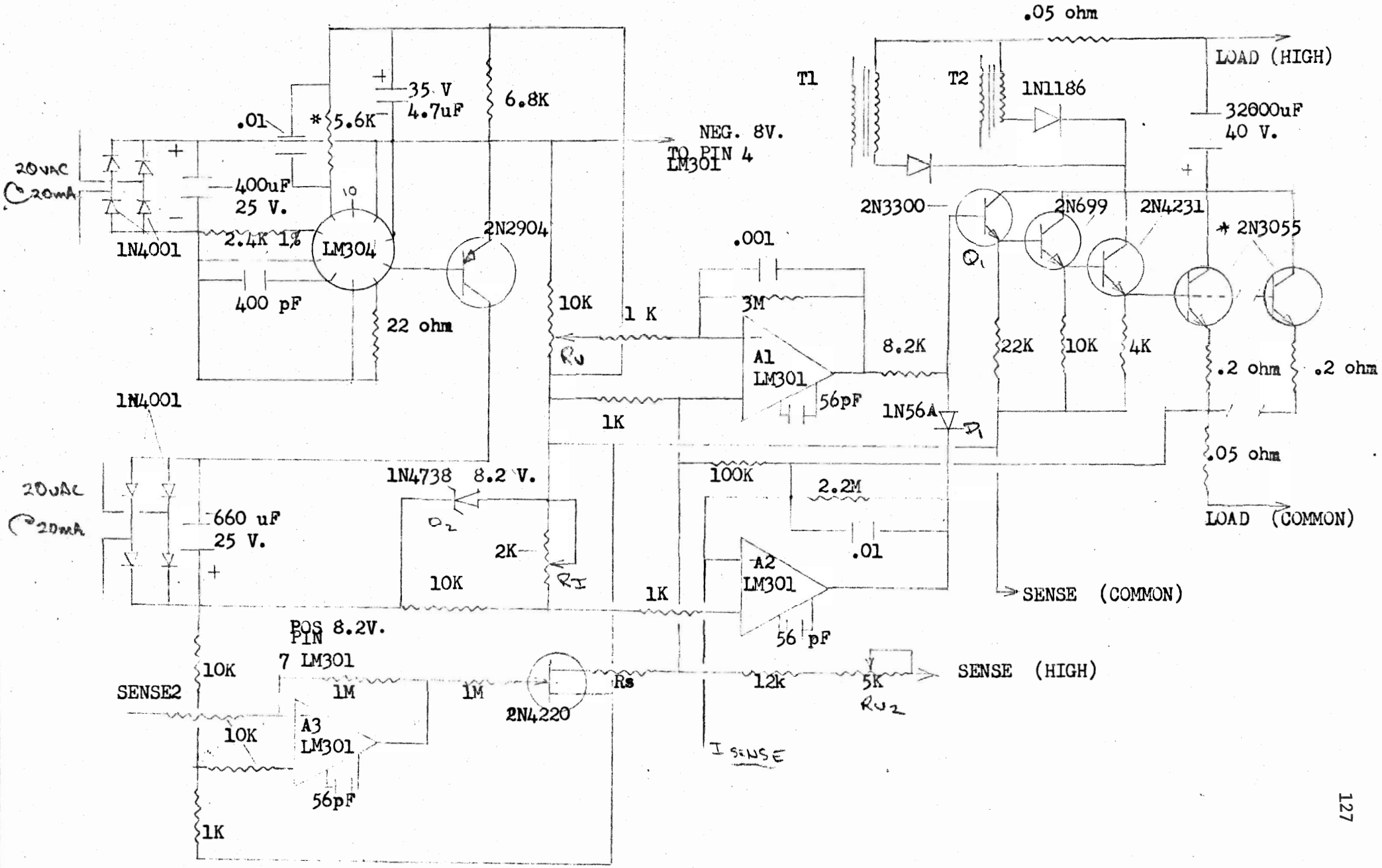
The voltage control pot R_V supplys the inverting input of A1 which is compared to thescalled output of the supply (sense high)terminal. Trim pot R_{V2} is part of this resistor network.

A reduction in supply voltage therefore increases the base drive to transistor Q_1 and the following transistors to increase the output and maintain it at ascaled fraction of the reference input.

The current limit amplifier A_2 samples the voltage drop across the .05 ohm resistor in the common output terminal, where the drop in volts is proportional to the output current. When this voltage is equal to or greater than the reference voltageon the non-inverting input of A_2 a negative voltage is applied to diode D_1 and hence reduces the drive to Q_1

The reference voltage that limits the current is set by potentiometer R_1

SCHEMATIC


.05 ohm
LOAD (HIGH)
T1
T2
1N1186
32000uF
40 V.
.01
*5.6K
35 V
4.7uF
6.8K
NEG. 8V.
TO PIN 4
LM301
20VAC
20mA
400uF
25 V.
2N3300
2N699
2N4231
* 2N3055
1N4001
2.4K 1%
LM304
2N2904
.001
400 pF
22 ohm
10K
1 K
3M
Q1
8.2K
22K
10K
4K
.2 ohm
.2 ohm
A1
LM301
56pF
1N56A
R1
1K
1N4001
1N4738 8.2 V.
100K
2.2M
.05 ohm
LOAD (COMMON)
20VAC
20mA
660 uF
25 V.
Q2
2K
10K
R1
.01
A2
LM301
SENSE (COMMON)
1K
POS 8.2V.
PIN 7 LM301
56 pF
10K
SENSE2
1M
1M
Rs
12k
5K
RV2
SENSE (HIGH)
2N4220
10K
A3
LM301
I SENSE
56pF
1K