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(Dr. Jefferson D. Gonor)

Allozyme variation was studied electrophoretically for twelve presumptive gene loci within and between four species in the genus Limnoria and one species in the cofamilial genus Phycolimnoria. The species studied were L. tripunctata, L. tuberculata, L. quadripunctata, L. lignorum, and P. algarum. All of these species were clearly differentiated on the bases of allozymes. Populations of L. tripunctata from the west coast of North America exhibited a latitudinal allelic cline at three loci, Mdh-2, Got-2 and Gpi. It is hypothesized that temperature related selection at one or more of these loci, or at another linked locus we have not studied, is responsible for the allelic cline. Geographically separated populations of L. tripunctata not from the west coast of North America had allelic frequencies at these three loci consistent with this hypothesis. Populations of L. lignorum from the Atlantic and Pacific Oceans, and from opposite sides of the Pacific Ocean, were genetically differentiated using allozymes. This differentiation could be due to a combination of random genetic processes and natural selection. The two populations of L. quadripunctata studied, both from the northern Californian coast, were essentially identical at the twelve loci studied.

Biochemical Genetics of Geographically Separated Populations of Wood Boring Isopods of the Genus *Limnoria* (Flabellifera, Isopoda)

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Introduction

Among the 21 known species of the wood boring isopod genus Limnoria and the 9 known algal boring species of the genus Phycolimnoria (Flabellifera, Limnoridae), a small number have strikingly broad geographical distributions (Menzies, 1957,1959, 1972). The dispersion methods by which the present distributions were achieved is not definitely known but the role of human transport has probably been a major one (Menzies, 1968, 1972; Carlton, 1979; Quayle, 1964). Limnoria females brood developing young and there is no planktonic larval stage. Migratory young adults, the short distance dispersive stage, are poor swimmers. The transport by currents of floating wood containing colonies is the only known natural longer distance dispersal mechanism.

Limnoria tripunctata Menzies, 1951, one of the most widespread species, is present at a large number of tropical to warm-temperate locations in all oceans (Menzies, 1957, 1959, 1972; Kuhne, 1976) and has also been recorded from fewer, more cool-temperate locations. It has been indentified from many locations in England (Jones, 1963) and north of San Francisco to 50°N latitude in British Columbia (Quayle, 1964). We here confirm Quayle's records for locations north of its originally known distributional limit (Menzies, 1957) on the Pacific Coast of North America and add other Washington and Oregon locations. The problems associated with dispersion of this species between oceans and over large

latitudinal ranges have been previously defined by Menzies (1972). He showed experimentally that widely separated Atlantic, Carribean and Pacific populations have remained interfertile and produce viable second generation progeny when crossed. In that study Menzies also found that Massachusetts populations previously identified as L. tripunctata (Menzies and Beckman, 1958) produced only non-viable first generation progeny when crossed with known L. tripunctata from other locations. He concluded that the Massachusetts population and others from southern England thought to be L. tripunctata were a separate species, Limnoria tuberculata Sowinski, 1884, rediscovered in the Black Sea and synonymized with L. tripunctata by Esakova (1965).

Limnoria quadripunctata Holthius, 1949 is also world-wide in its distribution but usually confined to more temperate, cooler regions than L. tripunctata. It is known from fewer, more widely separated locations, such as New Zealand, Chile, Europe and South Africa and is distributed from Humboldt Bay (40°N) to La Jolla (33°N) on the Pacific Coast of North America (Menzies, 1957, 1959; Kuhne, 1976). Like that of L. tripunctata its original distribution before the advent of extensive circumglobal navigation by wooden ships is unknown. However, Jones (1963) considers that Limnoria lignorum is the only endemic species in England and that the other two are recent introductions still extending their ranges. Carlton (1979) considers that both L. tripunctata and L. quadripunctata were introduced into California in the mid-nineteenth century.

Limnoria lignorum (Rathke, 1799) is a cold-temperate, circumboreal species widely distributed on both eastern and western

sides of the North Atlantic and North Pacific. On the Pacific coast of North America, it extends from the Aleutian Islands (Richards and Belmore, 1976) to Point Arena, California (Menzies, 1957, 1959). Since adults die quickly at 20°C (Becker and Kampf, 1955; Becker, 1959; Anderson and Reish, 1967), it apparently has never survived human transport to southern hemisphere locations. Kussakin (1963) added more records of L. lignorum from the northeastern Pacific but recorded other species from the Arctic. Atlantic and Pacific populations of L. lignorum are apparently disjunct and the present separation is presumably of long duration.

Limnoria species are considered to be limited in their geographic distributions by temperature levels and durations required for reproduction (Menzies, 1957; Beckman and Menzies, 1960). On the Pacific coast of North America, L. quadripunctata is found on the open California coast or within the entrance of embayments while L. tripunctata is found only within the warmer regions of embayments (Menzies, 1958; Quayle, 1964). Limnoria lignorum is capable of reproducing under the hydrographic regimes of both embayment entrances and the open coastal ocean. These temperature requirements provide a potential mechanism for reproductive isolation in geographically disjunct populations, which may differ in its effectiveness with latitude within species.

This study investigated the degree of genetic isolation between geographically separated populations of Limnoria, particularly L. tripunctata, and compares such interpopulational differences along the Pacific coast latitudinal gradient with differences between populations in different oceans. Because nothing

was known about the genetics of Limnoria, we also determined the nature of the genetic differences between Limnoria species and between them and a species in the cofamilial genus Phycolimnoria, for comparison with intraspecific differences.

Collection location data for the populations studied are given in Table 1. We compare populations of L. tripunctata ranging from British Columbia to Los Angeles with populations from Japan, Florida and North Carolina. Comparisons are also made to a population obtained from Massachusetts which is similar to L. tripunctata but uniformly shows the telsonal sculpturing illustrated by Esakova (1965). It is here designated Limnoria tuberculata Sowinski, 1884, according to the designation used by Menzies (1972) for such Massachusetts material. All other populations were identified by use of diagnostic characters given in Menzies, 1957. The northernmost Pacific coast population of L. quadripunctata in Humboldt Bay is compared to the population from Bodega Bay. Limnoria lignorum populations from Washington and Oregon are compared with others from Japan and Massachusetts.

Table 1. Collection locations and sampling dates for the limnorid species studied.

Localities		Date	Species
Ladysmith Harbor, British Columbia, Canada	48°59'N, 123°49'W	25 May 1981	*L. tripunctata*
Oyster Bay (Dyes Inlet), Puget Sound, Wash.	47°36'N, 122°42'W	1 Nov 1980	*L. lignorum*
Tillamook Bay, Oregon	45°32'N, 124°26'W	28 Aug 1980	*L. tripunctata*
Boiler Bay, Oregon	44°53'N, 124°10'W	27 Apr 1982	*Phycolimnoria algarum*
Yaquina Bay, Oregon	44°38'N, 124°02'W	19 Apr 1979	*L. lignorum*
Yaquina Bay, Oregon	44°38'N, 124°02'W	22 Mar 1979	*L. tripunctata*
Coos Bay, Oregon	43°19'N, 124°40'W	24 Mar 1979	*L. tripunctata*
Humboldt Bay, California	40°48'N, 124°25'W	6 May 1981	*L. quadripunctata*
Bodega Bay, California	38°19'N, 123°05'W	28 Apr 1982	*L. quadripunctata*
Tiburon, San Francisco Bay, California	37°53'N, 122°27'W	27 Apr 1982	*L. tripunctata*
Los Angeles-Long Beach Harbor, California	33°46'N, 118°15'W	11 May 1982	*L. tripunctata*
Manomet, Massachusetts	41°55'N, 70°31'W	25 Jun 1982	*L. lignorum*
Woods Hole, Massachusetts	41°32'N, 70°39'W	24 Apr 1981	*L. tuberculata*
Beaufort, North Carolina	34°43'N, 76°40'W	11 Jun 1981	*L. tripunctata*
Miami Beach, Florida	25°47'N, 80°07'W	26 Jun 1982	*L. tripunctata*
Otsuchi, Japan	39°22'N, 141°54'E	5 Jun 1981	*L. tripunctata* and *L. lignorum*

Methods

Genetic relationships between the populations studied were examined using gel electrophoresis methods for characterizing allozymes. Separation of soluble proteins on an acrylamide matrix occurs by size and charge (Brewer, 1970), and, followed by specific staining, allows estimates of minimum genetic distance to be made. This technique detects 27% to 33% of the DNA base substitutions present per gene (Lewontin, 1974; Selander, 1976). Gel electrophoresis of isoenzymes has been used successfully to investigate taxonomic relationships in a wide variety of animals (Ayala, 1975). It is especially useful at the level of genera or below (Avise, 1975) with which we are concerned in this study.

Before use, population samples were kept at 15°C for at least one week. Individuals placed in separate wells of a plastic tray on ice in 10 µl w/v glycerin in 0.05 M Tris-HCl buffer (pH 7.5) were homogenized whole with a glass pestle. The entire aqueous extract was placed with a Hamilton syringe into one well in the gel. The small volume of extract from one individual proved sufficient for detection of only one enzyme or protein system. Samples from various populations were juxtaposed on the same gel to allow comparison of electromorphs.

Vertical polyacrylamide gel electrophoresis was performed using modifications of the techniques of Selander et al. (1971) and Ayala et al. (1972). Gels 1.5 or 0.75 mm thick were used, the latter giving more sensitivity but less resolution. The acrylamide monomer concentration and buffer system used and the number of loci

scored for each enzyme system assayed are listed in Table 2. The buffer systems used were: (A). Electrode, 300 mM boric acid, 60 mM NaOH, gel, 76 mM Tris, 5 mM citric acid, pH 8.65; (B). Electrode and gel, 87 mM tris, 8.7 mM boric acid, pH 8.6; (C). Electrode, 135 mM Tris, 30 mM citric acid, pH 8.0, gel, 1:15 dilution of the electrode buffer; (D). Electrode, same as A., gel, same as B; (E). Electrode, 0.1 M Tris, 0.1 M maleic acid, 10 mM EDTA, 10 mM $MgCl_2$, pH 7.4, gel, 1:9 dilution of the electrode buffer.

Only allozymes migrating toward the anode using the specified buffer systems were scored. The following enzymes were also examined, but gave patterns which were difficult to interpret, gave inconsistent results, or were not detected: EST, α-GPDH, IDH, GDH, FUM, APH, ACPH, ADH, HEX (abbreviations as in Ayala et al. (1972)). In the following recipies for the stains used, the abbreviations are: NAD = β-diphosphopyridine nucleotide, oxidized form; NADP = triphosphopyridine nucleotide, oxidized form; MTT = MTT tetrazolium; PMS = phenazine methosulfate; Tris = tris(hydroxymethyl)aminomethane; NBT = nitroblue tetrazolium.

GOT: 500 mg l-aspartic acid, 75 mg α-ketoglutaric acid, 100 mg pyridoxal 5′-phosphate, 200 mg Fast Violet B salt, in 100 ml 0.1 M phosphate buffer, pH 7.5.

GP: 110 mls of 23% isopropanol, 9% acetic acid; 100 mg Serva Blue-R 250.

LDH: 600 mg L,+- lactate, lithium salt, 75 mg NAD, 20 mg MTT, 3 mg PMS, in 100 ml 0.05 Tris-HCl, pH 8.5.

MDH: 100 mg malic acid, 40 mg NAD, 20 mg MTT, 3 mg PMS, in 100 ml 0.05 M Tris-NCl, pH 7.8.

GPI: 100 mg fructose-6-phosphate (with 1-2% fructose-1, 6-diphosphate), 30 mg NADP, 80 mg $MgCl_2 \cdot 6H_2O$, 2 mg PMS, 15 mg MTT, 50 units glucose-6-phosphate dehydrogenase in 100 ml 0.1 M Tris-Hcl, pH 7.8.

PGM: 600 mg glucose-1-phosphate (disodium salt), 200 mg $MgCl_2 \cdot 6H_2O$, 40 mg NADP, 20 mg MTT, 80 units glucose-6-phosphate dehydrogenase, 4 mg PMS in 100 ml 0.05 M Tris-HCl, pH 7.8.

TO: 25 mg NBT, 5 mg PMS, 20 mg NAD in 100 ml 0.05 M Tris-HCl, pH 8.0.

Table 2. Enzyme systems assayed, buffer type, concentration of acrylamide monomer and number of loci scored.

System (abbreviation)	Buffers used-see text	Monomer concentration	No. of loci scored
Glutamate-oxaloacetate transaminase (GOT)	C,E	7%	2
General protein (GP)	D	10%	2
Lactate dehydrogenase (LDH)	A,D	10%	2
Malate dehydrogenase (MDH)	B	10%	2
Glucose phosphate isomerase (GPI)	D	10%	1
Phosphoglucomutase (PGM)	D	10%	2
Tetrazolium oxidase (TO)	B	10%	1

Results

Interspecific Comparisons

Allelic frequencies for the twelve presumptive gene loci studied are presented in Table 3, along with the observed heterozygosities for the loci. For each pair of species, the percentage of twelve loci differing significantly in allelic frequency and the percentage of these loci fixed for different alleles in the pair, are given in Table 4. The percentages with frequency differences are high for all pairs, and the percentage fixed at different alleles is also high for most pairs. By these criteria, each species including Limnoria tuberculata can be clearly distinguished from all the others at the $p<0.01$ level, with each possessing several diagnostic loci in the sense of Ayala and Powell (1972).

Inter and intrapopulational genic variations in the entire genome were estimated using Nei's (1972, 1973, 1975) genic diversity statistics. Nei (1973, 1975) defined a measure of total gene diversity, H_T, as

$$H_T = 1 - \sum_{i=1}^{k} \bar{x}_i^2,$$

where $\bar{x}_i$ is the mean frequency of the i^{th} of k alleles at a locus. This measure is an estimate of the mean heterozygosity expected under random mating. Total gene diversity can be partitioned into average gene diversities within (H_S) and between (D_{ST}) populations. H_S is estimated by

$$H_S = 1 - \sum_{i} \sum_{k} x_{ik}^2/s,$$

where x_{ik} is the frequency of the k^{th} allele of the i^{th} population,

Table 3. Gene frequency data and observed heterozygosities for the 12 loci studied. Gene symbols as in Table 2. Alleles listed by relative mobility, with the most common allele in L. tripunctata defined as 1.00. A dash indicates the allele was not detected in the population sample.

		L. tripunctata									L. quadripunctata		L. lignorum				L. tuberculata	P. algarum
Locus	Allele	Lady	Till	Yaq	Coos	Tib	LA	Beau	Miami	Otsu	Humb	Bod	Oyst	Yaq	Mano	Otsu	Woods Hole	Boiler Bay
	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-	-	1.00	-	1.00	1.00
Got-1	5.00	-	-	-	-	-	-	-	-	-	-	-	1.00	.80	-	1.00	-	-
	6.00	-	-	-	-	-	-	-	-	-	-	-	-	.20	-	-	-	-
H_o		0/20	0/20	0/19	0/21	0/15	0/15	0/20	0/15	0/5	0/20	0/20	0/5	4/20	0/16	0/5	0/20	0/4
	.82	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.80	-	-
	.87	-	-	-	-	-	-	-	-	-	-	-	1.00	1.00	1.00	.20	.08	-
Got-2	.92	.35	.10	.45	.43	.20	-	.05	.03	.20	1.00	1.00	-	-	-	-	.90	-
	1.00	.65	.90	.55	.57	.80	1.00	.95	.97	.80	-	-	-	-	-	-	.02	-
	1.08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.00
H_o		6/20	5/25	5/19	8/21	4/15	0/15	1/20	1/15	2/5	0/20	0/20	0/5	0/24	0/16	2/5	4/20	0/4
	.20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	.84	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.02	-
Mdh-1	.92	-	-	-	-	-	-	.06	.60	-	-	-	-	-	-	-	.98	-
	1.00	1.00	1.00	1.00	1.00	.97	1.00	.88	.40	.90	1.00	1.00	1.00	1.00	1.00	1.00	-	1.00
	1.04	-	-	-	-	.03	-	.06	-	.10	-	-	-	-	-	-	-	-
H_o		0/18	0/20	0/18	0/17	1/15	0/21	4/16	6/14	1/5	0/25	0/15	0/5	0/25	0/16	0/5	1/23	0/4
	1.00	1.00	.90	.92	.91	.50	.19	.09	.30	.30	-	-	.62	-	1.00	.75	-	-
	1.04	-	-	-	-	-	-	-	-	-	-	-	.38	.98	-	.25	-	-
Mdh-2	1.09	-	.10	.08	.09	.50	.81	.91	.70	.70	-	-	-	.02	-	-	1.00	1.00
	1.11	-	-	-	-	-	-	-	-	-	1.00	1.00	-	-	-	-	-	-
H_o		0/18	4/20	3/18	3/17	11/25	6/21	3/16	12/20	1/5	0/19	0/15	1/4	1/25	0/16	2/4	0/23	0/4

Table 3 (Continued)

Locus	Allele	Lady	Till	Yaq	Coos	Tib	LA	Beau	Miami	Otsu	Humb	Bod	Oyst	Yaq	Mano	Otsu	Woods Hole	Boiler Bay
	.63	-	-	-	-	-	-	-	-	-	.06	-	-	-	1.00	.80	-	-
	.80	.87	.67	.80	.83	.47	.03	.13	-	-	-	-	-	-	-	.20	-	-
	.89	-	-	-	-	-	-	-	-	-	.94	.97	-	-	-	-	-	-
	.92	-	-	-	-	-	-	-	-	-	-	.03	-	-	-	-	-	-
Gpi	.96	-	-	-	-	-	-	-	-	-	-	-	1.00	1.00	-	-	-	-
	1.00	.13	.33	.20	.17	.53	.97	.87	1.00	1.00	-	-	-	-	-	-	-	-
	1.32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.41	-
	1.42	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.59	-
	1.63	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.00
H_o		2/15	9/20	2/15	3/15	6/15	1/18	4/15	0/16	0/5	5/40	1/15	0/5	0/34	0/18	2/5	24/58	0/5
	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-	-	-	-	1.00	-
Gp-1	2.00	-	-	-	-	-	-	-	-	-	-	-	1.00	1.00	1.00	1.00	-	-
	2.50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.00
H_o		0/20	0/20	0/20	0/19	0/15	0/20	0/20	0/15	0/5	0/20	0/15	0/5	0/30	0/15	0/5	0/16	0/5
	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-	-	-	-	1.00	-
	1.04	-	-	-	-	-	-	-	-	-	-	-	1.00	1.00	1.00	-	-	-
Gp-2	1.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.00	-	-
	1.06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.00
H_o		0/15	0/15	0/15	0/13	0/15	0/15	0/15	0/15	0/5	0/20	0/15	0/5	0/25	0/15	0/5	0/16	0/5
	.60	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-	-	-	-	1.00	-
To	1.00	-	-	-	-	-	-	-	-	-	-	-	1.00	1.00	1.00	1.00	-	-
	1.30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.00
H_o		0/15	0/20	0/20	0/20	0/15	0/15	0/20	0/15	0/5	0/33	0/15	0/5	0/25	0/15	0/5	0/16	0/6
	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-	-	-	-	-	-	-	-
	1.39	-	-	-	-	-	-	-	-	-	-	-	1.00	1.00	1.00	1.00	-	-
Ldh-1	1.78	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.00
	1.83	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.00	-
	1.94	-	-	-	-	-	-	-	-	-	1.00	1.00	-	-	-	-	-	-
H_o		0/23	0/27	0/18	0/25	0/15	0/26	0/21	0/17	0/5	0/60	0/15	0/5	0/60	0/15	0/5	0/19	0/5

Table 3 (Continued)

Locus	Allele	Lady	Till	Yaq	Coos	Tib	LA	Beau	Miami	Otsu	Humb	Bod	Oyst	Yaq	Mano	Otsu	Woods Hole	Boiler Bay
	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-	-	-	-	-	-	-	-
	1.24	-	-	-	-	-	-	-	-	-	-	-	1.00	1.00	1.00	1.00	-	-
Ldh-2	1.56	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.00	-
	1.60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.00
	1.64	-	-	-	-	-	-	-	-	-	1.00	1.00	-	-	-	-	-	-
H_o		0/23	0/27	0/18	0/25	0/15	0/26	0/21	0/17	0/5	0/60	0/15	0/5	0/60	0/15	0/5	0/19	0/5
	.40	.11	-	-	-	-	.10	-	-	-	-	-	-	-	-	-	-	-
Pgm-1	.50	-	-	-	-	-	-	-	-	-	-	-	1.00	1.00	1.00	1.00	-	-
	.75	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.00
	1.00	.89	1.00	1.00	1.00	1.00	.90	1.00	1.00	1.00	1.00	1.00	-	-	-	-	1.00	-
H_o		2/18	0/20	0/20	0/20	0/16	2/20	0/24	0/15	0/5	0/26	0/18	0/5	0/23	0/17	0/5	0/16	0/6
	.72	-	-	-	-	-	-	-	-	-	.04	.06	-	-	-	-	-	-
	.76	-	-	-	-	-	-	-	-	-	.79	.78	.80	.96	-	-	.38	-
	.84	-	-	-	.03	-	.03	-	.07	-	.17	.14	.20	.04	-	.20	.44	.67
	.93	.06	.05	.05	.03	-	.03	.05	.10	-	-	.03	-	-	1.00	.80	.06	-
Pgm-2	.98	.09	.20	.15	.12	.25	.18	.37	.23	1.00	-	-	-	-	-	-	.06	-
	1.00	.34	.45	.40	.30	.50	.50	.32	.47	-	-	-	-	-	-	-	-	.33
	1.02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1.04	.37	.20	.25	.30	.03	.18	.15	.13	-	-	-	-	-	-	-	-	-
	1.06	.14	.10	.15	.22	.22	.03	.12	-	-	-	-	-	-	-	-	-	-
H_o		12/18	14/20	13/20	12/20	9/16	14/19	12/19	11/15	0/5	7/26	6/18	2/5	2/25	0/17	2/5	10/17	0/6

Table 4. Percentage of loci diagnostic for the species studied, in a pair by pair comparison.

Species Pair	Per cent of 12 loci with significantly different frequencies ($p<0.01$)	Per cent of 12 loci fixed at different alleles
L. tripunctata vs. *L. quadripunctata*	50.0	33.3
L. tripunctata vs. *L. tuberculata*	58.3	25.0
L. tripunctata vs. *L. lignorum*	91.7	58.3
L. tripunctata vs. *Phycolimnoria algarum*	83.3	75.0
L. quadripunctata vs. *L. tuberculata*	50.0	41.7
L. quadripunctata vs. *L. lignorum*	83.3	66.7
L. quadripunctata vs. *P. algarum*	91.7	83.3
L. tuberculata vs. *L. lignorum*	100.0	58.3
L. tuberculata vs. *P. algarum*	83.3	75.0
L. lignorum vs. *P. algarum*	100.0	75.0

and s is the number of populations. D_{ST} is estimated by

$$D_{ST} = (\Sigma_i \Sigma_j D_{ij})/s^2, \text{ where}$$

$$D_{ij} = \Sigma_k (x_{ik} - x_{jk})^2/2,$$

where k refers to the k^{th} allele at the locus, and x_{ik} and x_{jk} are the frequency of the alleles in the populations i and j. These statistics are related in an additive manner, with

$$H_T = H_S + D_{ST} .$$

Minimum net codon differences between populations, $\overline{D}_m$, are estimated as (Nei 1973, 1975)

$$\overline{D}_m = sD_{ST}/(s-1) ,$$

where s is the number of populations studied. Interpopulational gene diversity relative to intrapopulational gene diversity is given by Nei (1975) as

$$R_{ST} = \overline{D}_m / H_S .$$

The gene diversity statistics for each locus,
along with their averages over the loci, are given in Table 5. Among the species studied, total gene diversity ranges from 0.236 at Got-1 to 0.860 at Gpi, with a mean of 0.644. This is partitioned into a mean intrapopulational diversity of 0.117, and a mean interpopulational diversity of 0.527. Thus, nearly all of the total gene diversity is attributable to interspecific variation. The mean of $\overline{D}_m$ is 0.805, and this may be viewed as the average net codon differences between species. $\overline{R}_{ST}$ was calculated as

$$\overline{R}_{ST} = (\Sigma_{\ell=1}^{L} \overline{D}_m/L)/\overline{H}_S ,$$

Table 5. Interspecific gene diversity statistics based on pooled population data of the five Limnorid species studied. H_T, total gene diversity; H_S, Intrapopulational gene diversity; D_{ST}, Interpopulational gene diversity; $\overline{D}_m$, Minimum net codon differences between populations; R_{ST}, Interpopulational gene diversity relative to intrapopulational gene diversity. (undef = undefined due to division by zero)

Locus	H_T	H_S	D_{ST}	$\overline{D}_m$	R_{ST}
Got-1	.236	.109	.127	.158	1.456
Got-2	.753	.115	.638	.788	10.103
Mdh-1	.591	.073	.518	.685	32.619
Mdh-2	.666	.197	.469	.711	6.837
Gp-1	.560	.000	.560	.700	undef
Gp-2	.567	.036	.531	.700	undef
To	.560	.000	.560	.700	undef
Gpi	.860	.313	.547	.684	2.185
Ldh-1	.800	.000	.800	1.000	undef
Ldh-2	.800	.000	.800	1.000	undef
Pgm-1	.556	.010	.546	.683	68.3
Pgm-2	.779	.548	.231	.289	.527
AVERAGES	.644	.117	.527	.790	6.75

where 1 refers to the 1^{th} locus and L is the total number of loci. The value of $\bar{R}_{ST}$ is 6.75, indicating that there is nearly seven times as much variation between species as there is within species.

Several measures of genetic distance (eg. Cavalli-Sforza and Edwards, 1967; Balakrishnan and Sanghvi, 1968; Hedrick, 1971; Rogers, 1972; Latter, 1972) as a function of gene frequencies have been proposed. That of Nei (1971, 1972) has the advantage of measuring a biologically interpretable parameter, the average number of codon differences per locus, denoted by D. Since electrophoretic techniques may not reveal all alleles at a locus, D is a minimum estimate of genetic distance. Nei also defines genetic identity, I, related to D by

$$D = -\log_e(I) \ .$$

Values for I and D for the species studied, calculated using weighted averages, are given in Table 6. Because only twelve loci were scored, sampling errors in D are potentially large, but would be systematic, affecting all species similarly, hence values of D may be used as relative measures (Nei, 1975). The definitions of I and D make no assumptions concerning evolutionary forces, measuring relationships as they exist at the time of sampling. Values of D range from 0.548 between L. tripunctata and L. quadripunctata to 2.9 between L. lignorum and Phycolimnoria algarum. Overall, the observed values for the gene diversity and genetic distance statistics show that all of the species are genetically distinct and identifiable using electrophoretic techniques.

Table 6. Genetic identity (I), above diagonal, and genetic distance (D), below diagonal, (Nei, 1971a, 1972) for all limnorid species studied.

	L. tripunctata	L. quadripunctata	L. tuberculata	L. lignorum	P. algarum
L. tripunctata		.578	.507	.155	.134
L. quadripunctata	.548		.539	.155	.087
L. tuberculata	.680	.619		.078	.207
L. lignorum	1.9	1.9	2.6		.057
P. algarum	2.0	2.4	1.6	2.9	

Intraspecific Comparisons

L. tripunctata populations were monomorphic for the same alleles at six of the twelve loci (Table 3). No alleles unique to a population were detected at any locus. Several populations were differentiated at three loci , Mdh-2, Gpi, and Got-2.

In the Miami Beach population the allelic frequencies at the Mdh-1 locus differed significantly from that of other L. tripunctata populations studied (Table 3). For the other loci in L. tripunctata only Pgm-2 showed significant heterogeneity ($p<0.005$, genic homogeneity chi-square, Workman and Niswander, 1970). The distribution of alleles at this locus for the populations studied did not fit any obvious geographical pattern.

At six of the twelve loci, L. lignorum populations were identical and monomorphic, but these loci were in a different combination than in L. tripunctata. In the Yaquina Bay population, at both the Got-1 and Mdh-2 loci, there was an allele in low frequency which was not found in the other three populations of L. lignorum. No unique alleles were detected in the Ladysmith Harbor population; the Otsuchi population had unique alleles at three loci, Got-2, Gpi and Gp-2, and the Manomet population was fixed for a unique allele at the Got-1 locus (Table 3).

Each of the two L. quadripunctata populations had an allele at the Gpi locus in low frequencies not found in the other population; in the Bodega Bay sample one heterozygous individual had an allele at Pgm-2 not found in the Humboldt Bay population. The two L. quadripunctata populations were fixed for the same alleles at the

remaining ten loci (Table 3).

The expected proportion of heterozygotes (H_e) at a locus was calculated as

$$H_e = 1 - \sum_{i=1}^{k} x_i^2,$$

where x_i is the frequency of the i^{th} allele, and k is the total number of alleles. The average expected and observed heterozygosities over the twelve loci studied for each population sampled, as well as species averages for observed heterozygosities, are given in Table 7. The expected and observed heterozygosities do not differ significantly in any population studied. Observed heterozygosities for each locus in each population are included in Table 3. In only one case, the Got-2 locus for L. tripunctata from Yaquina Bay, was there deviation from Hardy-Weinberg expectations ($p \sim 0.05$), but since no other locus for this population showed significant deviation it is concluded that this is a sampling artifact.

Genetic distances (D) and identities (I) calculated by the method of Nei (1971, 1972) for the conspecific populations are given in Table 8. The Pacific Northwest populations of L. tripunctata (Tillamook Bay, Ladysmith Harbor, Yaquina Bay, and Coos Bay) are nearly identical, with an average genetic distance of 0.010 between them. The average genetic distance between these Pacific Northwest populations and the population from Tiburon is 0.040, while the average genetic distance between the Pacific Northwest populations and the remaining four populations is 0.173. The genetic distances between Tiburon, Los Angeles, Beaufort, Miami Beach and Otsuchi

Table 7. Mean number of animals per locus ($\bar{N}$), percent polymorphic loci (95% criterion), mean observed ($\bar{H}_o$) and expected ($\bar{H}_e$) heterozygosities for each population studied, the standard error (SE) of $\bar{H}_e$, and the average observed heterozygosity for each species.

Population	$\bar{N}$	Percent Polymorphic loci	$\bar{H}_o$	$\bar{H}_e$	SE($\bar{H}_e$)
L. tripunctata					
Ladysmith Harbor	18.6	33	.101	.119	.067
Tillamook Bay	21.2	25	.133	.125	.065
Yaquina Bay	18.3	33	.101	.141	.071
Coos Bay	19.4	33	.113	.140	.071
Tiburon	16.0	42	.152	.168	.071
Los Angeles	19.3	33	.098	.124	.062
Beaufort	18.9	42	.120	.131	.069
Miami Beach	14.9	33	.184	.138	.067
Otsuchi	5.0	25	.067	.077	.071
Weighted Average			.119		
L. quadripunctata					
Humboldt Bay	30.8	17	.037	.039	.030
Bodega Bay	16.3	17	.033	.036	.031
Weighted Average			.035		
L. lignorum					
Oyster Bay	4.9	17	.142	.108	.046
Yaquina Bay	31.3	25	.027	.036	.027
Manomet	15.9	0	.000	.000	.000
Otsuchi	4.9	33	.142	.108	.046
Weighted Average			.031		
L. tuberculata					
Woods Hole	21.9	25	.102	.114	.064
Phycolimnoria algarum					
Boiler Bay	4.9	8	.028	.037	.037

Table 8. Interpopulational genetic identity (I), above diagonal, and genetic distance (D), below diagonal, (Nei, 1971a, 1972) for L. tripunctata, L. lignorum and L. quadripunctata.

L. tripunctata

	Lady	Till	Yaq	Coos	Tib	LA	Beau	Miami	Otsu
Lady		.988	.997	.997	.948	.826	.823	.828	.829
Till	.012		.985	.983	.977	.841	.826	.885	.881
Yaq	.003	.015		.999	.962	.842	.823	.845	.853
Coos	.003	.017	.001		.959	.842	.824	.840	.848
Tib	.054	.023	.039	.042		.965	.971	.913	.935
LA	.191	.173	.172	.173	.036		.991	.962	.950
Beau	.195	.191	.193	.194	.030	.009		.963	.965
Miami	.188	.122	.168	.175	.091	.038	.037		.929
Otsu	.188	.126	.159	.165	.067	.051	.035	.073	

L. lignorum

	Oyst	Yaq	Mano	Otsu
Oyst		.961	.744	.714
Yaq	.040		.679	.653
Mano	.296	.387		.756
Otsu	.337	.426	.280	

L. quadripunctata

Humboldt and Bodega Bays

I = 0.9996

D = 0.0004

populations range from 0.009 to 0.091, with an average of 0.047. The low genetic distance (D = 0.009) between the geographically separated Los Angeles and Beaufort populations is noteworthy.

The genetic distances between the Pacific Northwest populations of L. tripunctata and those from Tiburon, Los Angeles, Miami Beach, Beaufort and Otsuchi are primarily the result of differences in allele frequencies at three of the 12 loci examined; Mdh-2, Gpi and Got-2. Using an a posteriori simultaneous testing procedure (Sokal and Rohlf, 1981, p. 729) maximal nonsignificantly different groups of populations were constructed on the basis of these allelic frequency differences. For the Mdh-2 locus, the Pacific Northwest populations formed one group, the populations from Los Angeles, Beaufort, Miami Beach and Otsuchi formed another, and the population from Tiburon, intermediate in allelic frequencies, did not fit with either group. The result for the Gpi locus was similar; the Pacific Northwest populations formed one group, and any three of the populations from Los Angeles, Beaufort, Miami Beach and Otsuchi could be used to construct another group. Again, the population from Tiburon did not fall into either group. At the Got-2 locus, allelic frequencies for the Tillamook Bay and Tiburon populations fell into neither the Pacific Northwest group nor the other population group.

These comparisons suggest that the Pacific Northwest populations as a group can be considered distinct from the other populations. In the following genic diversity analysis, the populations from Los Angeles, Beaufort, Miami Beach and Otsuchi will be treated as another, less homogeneous group.

On the west coast of North America there is a clinal variation in allelic frequencies at the Mdh-2, Gpi and Got-2 loci. The population from Tiburon has allelic frequencies nearly identical to the mean frequency of the Pacific Northwest population group and the Los Angeles population. Geographically, Tiburon, in San Francisco Bay, is nearly halfway between Coos Bay and Los Angeles, and the gene frequency data suggest that the Tiburon population lies near the midpoint of the allelic cline.

The Oyster Bay and Yaquina Bay populations of L. lignorum have a genetic distance of 0.040, while the genetic distances between these and the other, widely separated populations from Manomet and Otsuchi are fairly large, averaging 0.345 (Table 8). The two populations of L. quadripunctata from Humboldt Bay and Bodega Bay are essentially identical, with a genetic distance of 0.0004.

Gene diversity in L. tripunctata was analyzed using two different hierarchical schemes. First, the total gene diversity of the species (H_T) was split into gene diversity within (H_S) and between (D_{ST}) populations, with the results given in Table 9. Total gene diversity for L. tripunctata averaged 0.190, with an interpopulational diversity average of 0.062 and an intrapopulational diversity average of 0.128. The mean value of R_{ST} is 0.539, indicating that a significant proportion of the gene diversity is due to differences between populations. This is consistent with the genetic distances given above. Second, on the basis of genetic distances, the populations of L. tripunctata were split into two groups for analysis, the Pacific Northwest populations in one and all other populations in another group. The results of a genic

Table 9. Interpopulational gene diversity statistics for *L. tripunctata*, statistics as in Table 6.

Locus	H_T	H_S	D_{ST}	$\overline{D}_m$	R_{ST}
Got-1	.000	.000	.000	.000	undef
Got-2	.332	.268	.064	.072	.269
Mdh-1	.177	.105	.072	.081	.771
Mdh-2	.482	.257	.225	.253	.984
Gpi	.497	.238	.259	.291	1.223
Gp-1	.000	.000	.000	.000	undef
Gp-2	.000	.000	.000	.000	undef
To	.000	.000	.000	.000	undef
Ldh-1	.000	.000	.000	.000	undef
Ldh-2	.000	.000	.000	.000	undef
Pgm-1	.049	.042	.007	.008	.190
Pgm-2	.740	.627	.113	.127	.203
AVERAGES	.190	.128	.062	.069	.539

diversity analysis based on this dichotomy are given in Table 10. In this analysis total gene diversity (H_T) was partitioned into gene diversity between the two major groups (D_{ST}), gene diversity between the population of a given group (D_{CS}), and gene diversity within the populations (H_C) of a group. The parameter H_S is a measure of the total gene diversity within a group, and $\overline{D}_m$ estimates minimum net codon differences within a group. The relationships between these statistics are:

$$H_T = H_C + D_{CS} + D_{ST}, \text{ with}$$

$$H_S = H_C + D_{CS} .$$

Interpopulational gene diversity relative to intrapopulational gene diversity within a group, G_{CS}, is given by

$$G_{CS} = \overline{D}_m / H_C .$$

Total gene diversity (H_T) is again 0.190, the average gene diversity between populations in a given group ($\overline{D}_{CS}$) is 0.023, and the average gene diversity within a population ($\overline{H}_C$) is 0.128. Therefore, gene diversity between the two major groups (D_{ST}) is estimated to be 0.049. Thus, 67.3% ($\overline{H}_C/H_T$) of the total gene diversity exists within the populations, 12.1% ($\overline{D}_{CS}/H_T$) of the total gene diversity exists between populations in a given group, and 25.8% (D_{ST}/H_T) of the total gene diversity exists between the population groups.

Genic diversity analysis for the four populations of L. lignorum, given in Table 11, indicates that there has been significant divergence between these L. lignorum populations, a result consistent with the estimated genetic distances. The average total gene

Table 10. Gene diversity statistics for two groups of L. tripunctata populations.

H_S, Total population group gene diversity;
H_C, Intrapopulational gene diversity within the population group;
D_{CS}, Interpopulational gene diversity within the population group;
$\bar{D}_m$, Minimum net codon differences between populations;
G_{CS}, Interpopulational gene diversity relative to intrapopulational gene diversity within a group;
D_{ST}, Intergroup gene diversity; H_T, Total gene diversity.

	Pacific Northwest Populations (Tillamook Bay, Ladysmith Harbor, Yaquina Bay, and Coos Bay).					Other Populations (Tiburon, Los Angeles, Beaufort, Miami Beach, and Otsuchi).				
Locus	H_S	H_C	D_{CS}	$\bar{D}_m$	G_{CS}	H_S	H_C	D_{CS}	$\bar{D}_m$	G_{CS}
Got-1	.000	.000	.000	.000	undef	.000	.000	.000	.000	undef
Got-2	.442	.404	.038	.048	.119	.175	.159	.016	.019	.119
Mdh-1	.000	.000	.000	.000	undef	.297	.184	.113	.136	.739
Mdh-2	.126	.123	.003	.004	.033	.401	.364	.037	.044	.121
Gpi	.342	.317	.025	.031	.098	.290	.174	.116	.139	.799
Gp-1	.000	.000	.000	.000	undef	.000	.000	.000	.000	undef
Gp-2	.000	.000	.000	.000	undef	.000	.000	.000	.000	undef
To	.000	.000	.000	.000	undef	.000	.000	.000	.000	undef
Ldh-1	.000	.000	.000	.000	undef	.000	.000	.000	.000	undef
Ldh-2	.000	.000	.000	.000	undef	.000	.000	.000	.000	undef
Pgm-1	.054	.049	.005	.006	.122	.039	.036	.003	.004	.111
Pgm-2	.737	.725	.012	.015	.021	.690	.548	.142	.170	.310
Averages	.142	.135	.007	.009	.065	.158	.122	.036	.043	.353
Overall Averages and Totals	H_T = .190; $\bar{H}_C$ = .128; $\bar{D}_{CS}$ = .023; D_{ST} = .049									
	Undef = not defined due to division by zero.									

diversity is 0.227, partitioned into an average intrapopulational diversity of 0.053 and an average interpopulational diversity of 0.174. There is over four times as much variation between populations of L. lignorum as there is within populations ($\overline{R}_{ST}$ = 4.38).

Table 11. Interpopulational gene diversity statistics for *L. lignorum*, statistics as in Table 6.

Locus	H_T	H_S	D_{ST}	$\overline{D}_m$	R_{ST}
Got-1	.408	.080	.328	.437	5.46
Got-2	.320	.080	.240	.320	4.00
Mdh-1	.000	.000	.000	.000	undef
Mdh-2	.486	.221	.265	3.53	1.60
Gpi	.545	.080	.465	.620	7.75
Gp-1	.000	.000	.000	.000	undef
Gp-2	.000	.000	.000	.000	undef
To	.000	.000	.000	.000	undef
Ldh-1	.000	.000	.000	.000	undef
Ldh-2	.000	.000	.000	.000	undef
Pgm-1	.000	.000	.000	.000	undef
Pgm-2	.592	.179	.413	.551	3.08
AVERAGES	.227	.053	.174	.232	4.38

Discussion

The relative divergence of the species studied was estimated by a statistic which also allows the scaling of intraspecific differences. In summarizing many multi-locus electrophoretic studies, Avise (1975) observed that conspecific populations are usually nearly identical in allelic content at 85 percent of more of their loci, and that congeneric species pairs are often completely distinct at one-fifth to four-fifths of their loci. Some of our samples had only a few animals available; however, estimates of genetic relatedness based upon small numbers of individuals are usually accurate (Gorman and Renzi, 1979).

An important result of this study is the clear separation of L. tripunctata and L. tuberculata based on allozymes. These taxa are significantly different ($p<0.01$) in allelic frequency for seven of the 12 loci studied, and fixed for different alleles at three of these seven (Table 4). The genetic distance between L. tripunctata and L. tuberculata (0.680, Table 6) is very close to the distance between each of them and the morphologically more distinct L. quadripunctata ($\overline{D}$ = 0.616). This distance is similar to the divergence found between sibling species or species of other taxa (Ayala and Tracy, 1974; Nei, 1975; Ayala, 1975). The lack of extensive morphological divergence between genetically closely related but distinct species has been observed in a variety of taxa (eg. Ayala and Tracy, 1974, in Drosophila; Parker et al., in Cyathura; Hedgecock, 1979, in Chthamalus, and Skibinski et al., 1978, in Mytilus.)

The genetic distance between L. lignorum and the three congeners studied averaged 2.13, similar to the average genetic distance of 2.33 between the confamilial Phycolimnoria algarum and the four Limnoria species (Table 7). Values of D greater than one have a large standard error, and possibly grossly underestimate the actual gene codon differences (Nei, 1975).

The absolute distances and even the relative position of L. lignorum and P. algarum from the other three Limnoria species based on allozyme differences is subject to high uncertainty. The genetic distances indicate an old divergence of both P. algarum and L. lignorum from the remaining three Limnoria species. The distance values between L. lignorum and the other three Limnoria species are of the order found between non-sibling species or between genera in other taxa (Nei, 1975). The organization of the genic diversity was consistent with the genetic distances above, with an average of 82% (D_{ST}/H_T) of the total diversity being among the species (Table 5). The species studied are well differentiated from each other by electrophoretic techniques and this level of divergence can be distinguished from the intraspecific populational differentiation discussed below.

The average observed heterozygosities of the species range from 3.1% to 11.9% (Table 7) and are similar to those found in other invertebrate species (Valentine, 1976; Nevo, 1978). With only twelve loci the standard error of average heterozygosities is high. Four populations have an excess of heterozygotes and twelve a deficit, a result not significant (Wilcoxon sign rank test, Hollander and Wolfe, 1973, p. 27). This, along with the locus by locus analysis

given earlier, leads to the conclusion that the populations sampled approximate Hardy-Weinberg conditions.

Populations of L. tripunctata along the west coast of North America were studied to determine if there was significant differentiation of the populations. Differences may result from isolating mechanisms, leading to random genetic drift, or result from selection along a latitudinal environmental gradient.

North of 34°N on the Pacific Coast of North America populations of L. tripunctata are geographically isolated because bays with hydrographic conditions suitable for reproduction are relatively few and discontinuous, with no open coast habitat suitable for permanent colonies in between. Seasonal swarming will keep the gene pool of a given bay panmictic (Maruyama, 1970) but because swarming results in only limited dispersal adjacent bays will not be mixed by this means. Since within the Pacific Northwest, populations of L. tripunctata are not strongly differentiated, they may presently share a common gene pool or did so in the recent past. Natural selection or gene flow through natural dispersal mechanisms or through human transport, may be maintaining similar allelic frequencies in the Pacific Northwest populations. If the populations are reproductively isolated by the dispersal barriers mentioned above, this could eventually permit genetic differentiation through random drift.

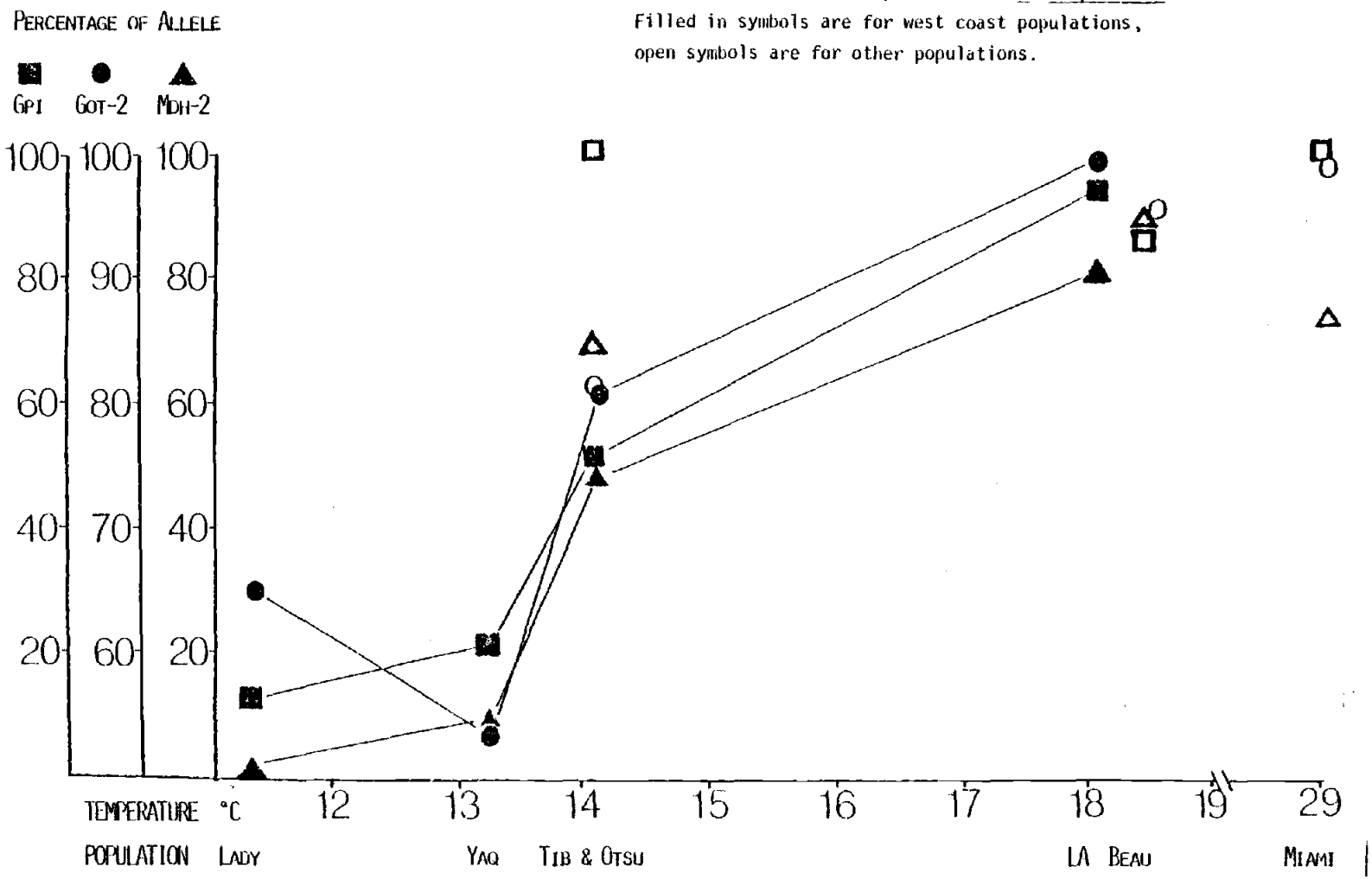
Based on indirect evidence, it has been suggested that L. tripunctata is an introduced species on the Pacific coast of North America. Carlton (1979) proposed that L. tripunctata was introduced into San Francisco Bay by human transport in the mid-nineteenth

century. Quayle (1964), proposed that since L. tripunctata in the Pacific Northwest is found in places where Japanese oysters had been planted, it may have been introduced from Japan with oyster seed no earlier than 1900. Limnoria tripunctata was found in Sendai, Japan, (38°16'N), which was the major source for oyster seed for North America, and is also known from many other Japanese locations (Menzies, 1957; Kuhne, 1976). The importation of oysters has been responsible for the introduction of other marine species in British Columbia (Carl and Guiget, 1958) and San Francisco Bay (Carlton, 1979). The population from Otsuchi (39°22'N) was examined to test Quayle's suggestion that L. tripunctata was introduced into the Pacific Northwest from Japan. The pattern of allelic differentiation does not support Quayle's suggestion. At the three loci useful in differentiating L. tripunctata populations, Got-2, Mdh-2, and Gpi, allelic frequencies in the Otsuchi population sample were more similar to those in the Los Angeles population than those in the Pacific Northwest populations (Table 3). The average genetic distances between the Pacific Northwest populations and the Otsuchi population was 0.160, a value similar to the average genetic distance of 0.172 between the Pacific Northwest populations and the population from Los Angeles (Table 8).

The clines in allele frequencies at Got-2, Mdh-2, and Gpi in L. tripunctata on the west coast of North America could have arisen in several ways. One possibility is that the cline has arisen through selectively neutral processes, i.e., migration between

previously differentiated northern and southern populations. Alternatively, natural selection may be important in maintaining the observed clines. Selection may be acting on any of these loci if they are linked or upon other linked loci which we have not studied. The polymorphic Pgm-2 locus is differentiated among populations but does not show clinal variation with latitude. Thus, the factors(s) responsible for the clinal patterns at Got-2, Mdh-2 and Gpi is not also affecting the pattern of differentiation at the Pgm-2 locus. This observation, coupled with the observation that geographically distant populations have allele frequencies at these three loci appropriate for selection by a latitudinally related environmental parameter, lead us to favor the hypothesis that natural selection acting at these or linked loci is responsible for the cline. Water temperature is the most obvious environmental factor correlating with the latitudinal gradient. Figure 1 shows the relationship between allelic frequencies and mean water temperature at the location the L. tripunctata were collected. For clarity not all the Pacific Northwest populations are shown. If temperature is the factor selecting allozymes at these loci, then allelic frequencies in populations which are not from the west coast of North America, and are genetically isolated from them, would be expected to demonstrate the same temperature response. The data from the Miami Beach, Beaufort, and Otsuchi populations are included in Figure 1. There is good agreement of allelic frequencies in these populations predicted on the basis of the temperature/allelic frequency correlations, from the latitudinal cline. Thus temperature related selection for allelic differences at these three loci found between the populations

Figure 1. Allelic frequencies at three loci versus mean annual environmental temperature for L. tripunctata. Filled in symbols are for west coast populations, open symbols are for other populations.



of L. tripunctata is a viable hypothesis. Other studies have found correlations between gene frequency and environmental temperature in Drosophila (Johnson and Schaffer, 1973), bryozoans (Schopf and Gooch, 1971) fish (Koehn, 1969; Johnson, 1977; Powers and Powers, 1975; Powers and Place, 1978) and anemones (Hoffmann, 1981a, b). The biochemical basis for possible temperature related selective advantage of allozymes has been demonstrated in a number of cases, including allozymes of lactate dehydrogenase in Fundulus heteroclitus (Powers and Place, 1977; Place and Powers, 1979) and Pimephales promelas (Merrit, 1972) phosphoglucose isomerase in Metridium senile (Hoffmann, 1981a) and esterase in Catostomus clarki (Koehn, 1969).

Because Limnoria lignorum cannot survive transport through equatorial waters, the populations in the Atlantic and Pacific Oceans are presently isolated. Unlike the situation for L. tripunctata, L. quadripunctata and possibly other species, interoceanic transport by wooden ships is not possible for L. lignorum. Much of the wooden ship traffic across both the North Atlantic and North Pacific also passed through warm waters, so that the human transport of L. lignorum across these oceans was probably not as important as it was for L. tripunctata or L. quadripunctata, both of which have wider temperature tolerances. Successful, long distance transoceanic transport by floating infested wood will be rare and the populations of L. lignorum in the two oceans and on different sides of oceanic basins could have genetically differentiated due to genetic drift.

Our results on L. lignorum are consistent with these considerations. The average genetic distance between the Manomet population and the populations from the Pacific Ocean is 0.321, and the average

genetic distance between the Otsuchi population and the populations from Yaquina and Oyster Bays is 0.383 (Table 9). These genetic distances are of the magnitude found for subspecies to sibspecies for other taxa (Ayala and Tracy, 1974; Ayala, 1975; Nei, 1975). The genetic distance between the Yaquina Bay and Oyster Bay populations (0.040) is primarily due to an electromorph in the Oyster Bay population at the Mdh-2 locus which was not found in the Yaquina Bay population, although it was found in the Manomet and Otsuchi populations. The results of the gene diversity analysis (Table 12) are similar; 77% (D_{ST}/H_T) of the total gene diversity is between the populations studied.

The genetic differentiation of these populations could be due to either genetic drift as developed above, or to natural selection. The populations of L. lignorum sampled experience different hydrographic regimes, and as hypothesized for L. tripunctata these hydrographic differences are potentially large enough for selection to operate on allozymes. The greater differentiation among L. lignorum populations than that found among L. tripunctata populations is consistent with the lower dispersal potential of L. lignorum, and might be due to a combination of random genetic drift and selection.

The two populations of L. quadripunctata are essentially identical at the twelve loci studied. Humboldt Bay represents the northern extreme of this species' reported distribution (Menzies, 1957), and we could not find this species north of there although a careful search was made of all potential locations. Bodega Bay is nearer the center of the distribution of the species on the west coast of North America. The possibility of genetic differentiation between

the two populations studied seems lessened by the extensive wooden ship transport along the west coast of the United States in the last century and hydrographic conditions on the open coast throughout its range more suitable for sustained populations of L. quadripunctata than those of L. tripunctata throughout its range.

Summary and Conclusions

The five species studied were well differentiated by gel electrophoresis of allozymes, with 82% of the total gene diversity of these species being diversity among the species. *Limnoria tuberculata* and *L. tripunctata* were clearly separated by this technique; the degree of divergence between them is at the same level as the divergence between either of them and the morphologically more distinct *L. quadripunctata*. The average genetic distance among these taxa, based upon twelve presumptive gene loci, is 0.616. *Limnoria lignorum* was differentiated at over 90% of the loci studied from the three congeners above, with an average genetic distance of 2.13, indicating an older divergence of this species, a conclusion consistent with morphological considerations. The average genetic distance between *Phycolimnoria algarum* and the four *Limnoria* species is 2.33, also indicative of an old divergence.

The populations of *L. tripunctata* on the west coast of North America exhibited a latitudinal allelic cline at three loci, Mdh-2, Got-2, and Gpi. It is hypothesized that selection acting on any of the loci if they are linked, or upon other linked loci not studied, is responsible for the allelic cline. Water temperature is the most obvious environmental factor correlating with latitude, and widely separated populations not from the west coast of North America have allelic frequencies close to those predicted on the basis of temperature related selection.

Populations of *L. lignorum* from the Atlantic and Pacific Oceans and from opposite sides of the Pacific Ocean are differentiated at several loci. The differentiation could be due to a combination of

random genetic processes and natural selection. The two populations of L. quadripunctata studied were essentially identical at the twelve loci studied.

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