ALLOZYME SURVEY AND RELATIONSHIPS OF *LIMNOPORUS* STÅL SPECIES (HETEROPTERA: GERRIDAE)

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Abstract

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Five species of *Limnoporus* Stål (*L. canaliculatus* [Say], *L. dissortis* [Drake and Harris], *L. nearcticus* [Kelton], *L. notabilis* [Drake and Hottes], and *L. rufoscutellatus* [Latreille]) were each sampled at 20 electrophoretic loci. Twofold differences among species in mean heterozygosity appear to be unrelated to presence of wing dimorphism. Low heterozygosity in some populations within species may reflect geographic isolation. There were substantial differences in allele frequency among, but not within, species. *Limnoporus rufoscutellatus* from western Europe and *L. nearcticus* from Alaska were the most similar pair of species, with a Nei's standard genetic identity that is generally found only between populations of the same species. *Limnoporus canaliculatus* was the most divergent species, and the relationship among *L. dissortis*, *L. notabilis*, and the *L. rufoscutellatus* – *L. nearcticus* pair is resolved as a trichotomy.

Résumé

Cinq espèces de *Limnoporus* Stål (*L. canaliculatus* [Say], *L. dissortis* [Drake et Harris], *L. nearcticus* [Kelton], *L. notabilis* [Drake et Hottes] et *L. rufoscutellatus* [Latreille]) ont été échantillonnées à 20 loci. Des différences interspécifiques de l'ordre du double existant au niveau de l'hétérozygosité n'ont pas pu être imputées au dimorphisme alaire. Au niveau intraspécifique, l'isolation géographique de certaines populations pourrait expliquer leur faible degré d'hétérozygosité. On a noté des différences substantielles concernant la fréquence de certains allèles au niveau interspécifique, mais pas au niveau intraspécifique. *Limnoporus rufoscutellatus* d'Europe occidentale et *L. nearcticus* d'Alaska constituent la paire d'espèces les plus semblables, avec une valeur du standard d'identité génétique de Nei caractéristique de populations d'une même espèce *Limnoporus canaliculatus* était l'espèce la plus divergente, et la parenté entre *L. dissortis*, *L. notabilis*, et la paire *L. rufoscutellatus* – *L. nearcticus* se résout à une trichotomie.

Introduction

The waterstrider genus *Limnoporus* contains six species and has an exclusively Holarctic distribution (Calabrese 1980; Andersen 1982). Four species are Nearctic: *L. canaliculatus, L. dissortis, L. notabilis*, and *L. nearcticus*. The remaining two species, *L. rufoscutellatus* and *L. esakii* Myamoto, are Palearctic. Subspecies are currently recognized only for *L. rufoscutellatus*, where *L. r. genitalis* Myamoto was described from northern Japan, to distinguish it from *L. r. rufoscutellatus* from northern Europe. Until Andersen's (1975) generic reclassification of the Gerrinae, *Limnoporus* was usually considered a subgenus of *Gerris*. However, the exact placement of *Limnoporus* within the tribe Gerrini is still in question (Calabrese 1980; Andersen 1982).

Several recent studies of *Limnoporus* species have provided comparative information about their ecology, behavior, and genetics. Mating behavior has been investigated in *L. dissortis, L. notabilis*, and *L. rufoscutellatus* (Vepsäläinen and Nummelin 1985; Wilcox and Spence 1986; Spence and Wilcox 1986; and Nummelin 1987). Spence (1990) has cross-bred *L. notabilis* and *L. dissortis* in the laboratory, and the cytogenetics and meiotic behavior of both species and their hybrids have been described by Spence and Maddison (1986). We have also done an electrophoretic survey of protein variation across a hybrid zone between these two species (unpublished data).

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Table 1. Species of *Limnoporus*, with species ranges and location of populations sampled in this study

L. canaliculatus	Range: Southern Ontario (unpublished data) and eastern half of United States (Drake and Harris 1934). Sample: Canada, Ontario, Chaffey's Locks, 28 Aug. 1984.
L. esakii	Range: Japan (Myamoto 1958) and China (N.M. Andersen pers. comm.). Sample: None.
L. notabilis	Range: United States west of the Mississippi River (Drake and Harris 1930, 1934), British Columbia (Scudder 1977), and Rocky Mountains and foothills of Alberta (Brooks and Kelton 1967). Samples: Canada, British Columbia: (1) Coombs, Vancouver Island, 6 Sept. 1984; (2) Marion Lake, Maple Ridge, 14 Sept. 1983, 4 Sept. 1984; (3) Misty Lake, Queen Charlotte Islands, 5 June 1986.
L. dissortis	Range: United States and Canada east of the Rocky Mountains (Drake and Harris 1934), interior British Columbia (Scudder 1977). Samples: Canada: (1) Alberta, Swan Hills, 16 Aug. 1983, 12 Sept. 1984; (2) Quebec, Mont St. Marie, 8 May 1985.
L. rufoscutellatus	Range: across Eurasia, from approx. 42 to 62°N (Andersen 1982), Ireland (Murray 1986). Sample: Finland, Hanko, Tvarminne, May 1985.
L. nearcticus	Range: United States, south-central Alaska; Canada, Yukon Territory (Kelton 1961). Sample: United States, Alaska, nr. Fairbanks, 16 June 1986.

The phylogenetic relationships of species within *Limnoporus* are uncertain, and no analysis of the genus has been undertaken. The species most closely allied to *L. rufoscutellatus* has been suggested to be either *L. nearcticus* (Kelton 1961) or *L. dissortis* (Schaefer and Calabrese 1980). Also, *L. dissortis* hybridizes with *L. notabilis* in western Canada (Spence 1981; Spence and Maddison 1986; Spence 1990) and the confusing similarity of these two species to each other, as well as to *L. rufoscutellatus*, has been noted since their earliest descriptions (Drake and Hottes 1925; Drake and Harris 1930, 1934). For convenience of discussion, we refer informally to these four species, characterized by large body size and constant macroptery, as the "*L. rufoscutellatus group*." *Limnoporus canaliculatus* and *L. esakii* are similarly characterized by small size and their relationships are thought to be more basal within the genus (N.M. Andersen personal communication).

Before detailed evolutionary interpretations of comparative studies are possible, character differences must be placed in a systematic context that clarifies the extent of genetic variation within and between species, as well as the phylogenetic relationships between species. We attempt here to provide this context for *Limnoporus*, with a survey of electrophoretic character variation in five of the six species in the genus.

Methods and Materials

Table 1 contains range information for the species of *Limnoporus*, and also specific collection data for the five taxa and eight populations studied with electrophoresis. We studied samples of three populations of *L. notabilis*, two from major islands and one from the mainland on the west coast of British Columbia. *Limnoporus dissortis* was represented by samples from different ends of the species range, in central Alberta and Quebec. Each of the other three species was represented by a sample from a single population. We were unable to obtain usable samples of *L. esakii* and *L. rufoscutellatus genitalis*, both known only from China and Japan.

Adult gerrids were netted off ponds and stored at 4–6°C on damp *Sphagnum* moss until electrophoresis. Whole specimens were homogenized live, in 0.3 mL of 0.03 *M* Tris-H_3PO_4, pH 6.7, containing 1.6 m*M* nicotinamide adenine dinucleotide phosphate (NADP), 8.0 m*M* DL-dithiothreitol, and 3.5% polyvinylpyrolidine. Subsamples of 0.01–0.03 mL homogenate were electrophoresed in 9 or 11% polyacrylamide gels, Tris-HCl, pH 8.9, under the conditions detailed in Rolseth and Gooding (1978) and Sperling (1987).

For each population, 23–75 individuals were assayed for one general and 19 enzymatic proteins, as follows (EC numbers from Nomenclature Committee of the International Union of Biochemistry 1984): adenylate kinase (AK, EC 2.7.4.3), aldehyde oxidase (AO, EC 1.2.3.1), arginine phosphokinase (APK, EC 2.7.3.3), esterase (EST, EC 3.1.1.2), alpha-glycerophosphate dehydrogenase (aGPD, EC 1.1.1.8), general protein (GP), glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49), glutamate-oxaloacetate transaminase (GOT, EC 2.6.1.1), hexokinase (HK, EC 2.7.1.1), isocitrate dehydrogenase (IDH, EC 1.1.1.42), lactate dehydrogenase (LDH, EC 1.1.1.27), malic enzyme (ME, EC 1.1.1.40), malate dehydrogenase (MDH, EC 1.1.1.37), octanol dehydrogenase (ODH, EC 1.1.1.73), 6-phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44), phosphoglucose isomerase (PGI, EC 5.3.1.9), phosphoglucomutase (PGM, EC 2.7.5.1), phosphomannose isomerase (PMI, EC 5.3.1.8), sorbitol dehydrogenase (SoDH, 1.1.1.14), and xanthine oxidase (XO, EC 1.2.3.2). These 20 presumptive gene products were all that would give consistent and interpretable bands from 38 staining methods attempted. Banding patterns suggested that five loci produced monomers (AK, APK, GP, HK, and PGM), three loci produced tetramers (ME, G6PD, and SoDH), two loci produced undetermined multimers (XO, AO), and the remaining 10 loci produced dimers (see Ferguson 1980). G6PD produced a heterozygote banding pattern only in females, indicating that the locus is sex-linked.

Staining methods were standard modifications of Shaw and Prasad (1970) or Brewer (1970). It was necessary to include NADP in the homogenizing buffer (in addition to the stain) to obtain activity from the B and D alleles of G6PD, whereas the A and C alleles gave good activity irrespective of NADP in the buffer. The best cofactor for IDH was $MgCl_2$, rather than $MnCl_2$. LDH gave activity with both the LDH and the HBDH stains of Shaw and Prasad (1970). The identity of variants of APK was checked with the stain of Gooding and Rolseth (1979), though APK was assayed in most specimens with Coomassie blue, a general protein stain. PMI was resolved with the stain of Herd and Fenton (1983). Filter paper overlays were used instead of agar.

Eighteen staining methods were tried unsuccessfully, from Brewer (1970), Shaw and Prasad (1970), May *et al.* (1979), Menken (1980), and Herd and Fenton (1983). These included acid phosphatase, adenosine deaminase, alcohol dehydrogenase (using ethanol and NAD, and also isopropanol and NADP), aldolase, alkaline phosphatase, aspartate amino transferase, diaphorase, fructose dehydrogenase, fumarase, glucose oxidase, glutamate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, glycerol dehydrogenase, isocitrate dehydrogenase (using NAD), leucine napthylamidase, succinate dehydrogenase, and tetrazolium oxidase. Of these, Zera (1981*a*) obtained bands from *L. canaliculatus* in starch gels for acid phosphatase, fumarase, and leucine napthylamidase. He also recorded two GOT and IDH loci, but we only obtained occasional faint bands at a second locus of each of these. Our EST locus showed a pattern of variation similar to that found by Zera (1981*a*) for his EST-3, and may have been the same locus. Although we used a lettering system to designate alleles that was similar to that used by Zera (1981*a*), particular letters may not indicate the same alleles.

Electrophoretic data were analyzed with BIOSYS-1 (Swofford and Selander 1981) to generate allele frequency tables, tests for Hardy-Weinberg equilibrium, genetic distance measures, UPGMA phenograms, and Wagner trees. Maximum likelihood trees were obtained with the CONTML program of PHYLIP Version 3.0 (Felsenstein 1986). Tree-lengths of topologies derived from frequency data were compared using the frequency parsimony program FREQPARS Version 1.0 (Swofford and Berlocher 1987; Swofford 1988). Exhaustive searches for minimum length cladograms were performed with PAUP Version 2.4 (Swofford 1985) on datasets in which the presence or absence of each allele was considered a character.

Table 2. Allozyme frequencies detected at 20 polymorphic loci in eight *Limnoporus* populations. Alleles are listed in order of increasing electrophoretic mobility

		Limnoporus population							
			notabilis			*dissortis*			
Locus	Allele	*canaliculatus*	Coo	Mar	QC	Swan	MSM	*rufoscutellatus*	*nearcticus*
AK	(*n*)	(56)	(61)	(65)	(28)	(58)	(54)	(40)	(44)
	A	—	0.074	0.062	0.089	0.500	0.593	—	—
	B	0.009	0.910	0.938	0.804	0.500	0.398	—	—
	C	0.946	0.016	—	0.107	—	0.009	1.000	1.000
	D	0.045	—	—	—	—	—	—	—
AO	(*n*)	(38)	(61)	(64)	(23)	(68)	(54)	(35)	(44)
	A	0.013	—	—	—	0.037	—	—	0.034
	B	0.987	1.000	1.000	1.000	0.956	1.000	1.000	0.966
	C	—	—	—	—	0.007	—	—	—
APK	(*n*)	(56)	(61)	(65)	(28)	(57)	(54)	(35)	(44)
	A	—	—	—	—	0.009	—	—	—
	B	1.000	1.000	1.000	1.000	0.991	1.000	1.000	1.000
EST	(*n*)	(38)	(61)	(75)	(23)	(68)	(54)	(35)	(44)
	A	—	0.107	0.120	0.043	0.029	—	—	0.023
	B	—	0.738	0.807	0.957	0.735	0.769	0.429	0.409
	C	0.079	0.131	0.067	—	0.228	0.204	0.457	0.500
	D	0.447	0.025	0.007	—	—	0.028	0.100	0.068
	E	0.434	—	—	—	0.007	—	0.014	—
	F	0.039	—	—	—	—	—	—	—
GOT	(*n*)	(38)	(61)	(64)	(23)	(58)	(54)	(35)	(44)
	I	—	0.025	—	—	—	—	—	—
	A	0.974	0.500	0.414	0.696	0.293	0.241	0.043	0.091
	B	0.026	0.475	0.586	0.304	0.707	0.759	0.957	0.909
aGPD	(*n*)	(56)	(61)	(75)	(23)	(67)	(54)	(35)	(44)
	I	0.009	—	—	—	0.015	0.009	—	—
	A	0.063	0.861	0.760	0.978	0.582	0.583	1.000	1.000
	B	0.929	0.139	0.240	0.022	0.403	0.380	—	—
	C	—	—	—	—	—	0.028	—	—
GP	(*n*)	(56)	(61)	(64)	(28)	(58)	(54)	(40)	(44)
	I	0.616	—	—	—	—	—	—	—
	A	0.384	0.090	0.063	0.071	0.957	0.907	0.975	0.966
	B	—	0.910	0.930	0.929	0.043	0.093	0.025	0.034
	C	—	—	0.008	—	—	—	—	—

G6PD	(*n*)	(56)	(61)	(74)	(28)	(68)	(54)	(40)	(44)
	A	—	0.056	0.102	0.184	—	—	0.214	—
	B	—	—	—	—	0.990	0.937	0.036	—
	C	1.000	0.944	0.898	0.816	—	—	0.643	—
	D	—	—	—	—	0.010	0.063	0.107	1.000
HK	(*n*)	(38)	(61)	(48)	(23)	(57)	(54)	(35)	(44)
	J	0.013	—	—	—	—	—	—	—
	I	0.842	—	—	—	0.009	—	—	0.068
	A	0.145	0.115	0.208	0.022	—	0.009	0.014	0.023
	B	—	0.885	0.792	0.978	0.991	0.963	0.986	0.909
	C	—	—	—	—	—	0.028	—	—
IDH	(*n*)	(56)	(61)	(65)	(28)	(57)	(54)	(35)	(44)
	I	0.013	—	—	—	—	—	—	—
	A	0.974	—	0.008	—	0.017	—	—	—
	B	0.013	1.000	0.992	1.000	0.957	0.972	1.00	1.000
	C	—	—	—	—	0.026	0.028	—	—
LDH	(*n*)	(38)	(61)	(70)	(28)	(63)	(54)	(35)	(41)
	I	0.013	—	—	—	—	—	—	—
	A	0.974	—	0.007	0.018	0.008	0.009	—	—
	B	0.013	1.000	0.993	0.982	0.976	0.981	0.957	1.000
	C	—	—	—	—	0.016	0.009	0.043	—
MDH	(*n*)	(14)	(61)	(54)	(23)	(58)	(54)	(35)	(44)
	A	—	—	0.009	0.065	—	—	—	0.023
	B	1.000	1.000	0.991	0.935	0.991	1.000	1.000	0.977
	C	—	—	—	—	0.009	—	—	—
ME	(*n*)	(56)	(61)	(75)	(28)	(68)	(54)	(40)	(44)
	A	1.000	0.992	0.993	1.000	0.029	0.019	1.000	0.977
	B	—	0.008	0.007	—	0.404	0.380	—	0.023
	C	—	—	—	—	0.559	0.602	—	—
	D	—	—	—	—	0.007	—	—	—
ODH	(*n*)	(38)	(61)	(70)	(23)	(68)	(54)	(35)	(44)
	B	1.000	1.000	1.000	1.000	1.000	0.981	1.000	1.000
	C	—	—	—	—	—	0.019	—	—
6PGD	(*n*)	(38)	(61)	(35)	(23)	(57)	(54)	(35)	(44)
	A	—	0.008	—	—	0.053	0.120	0.071	0.034
	B	0.211	0.959	0.986	1.000	0.921	0.824	0.929	0.909
	C	0.684	0.025	0.014	—	0.018	0.056	—	0.057
	D	0.105	0.008	—	—	—	—	—	—

Table 2. (*Concluded*)

		Limnoporus population							
			notabilis			*dissortis*			
Locus	Allele	*canaliculatus*	Coo	Mar	QC	Swan	MSM	*rufoscutellatus*	*nearcticus*
PG1	(*n*)	(56)	(61)	(54)	(27)	(53)	(54)	(35)	(44)
	A	0.723	0.008	—	—	0.047	0.019	0.071	0.023
	B	0.277	0.984	1.000	1.000	0.934	0.981	0.929	0.977
	C	—	0.008	—	—	0.019	—	—	—
PGM	(*n*)	(56)	(61)	(60)	(28)	(53)	(54)	(35)	(44)
	A	—	0.008	—	—	0.028	0.019	0.014	0.034
	B	0.920	0.984	0.992	0.982	0.972	0.944	0.986	0.966
	C	0.080	0.008	0.008	0.018	—	0.037	—	—
PMI	(*n*)	(27)	(61)	(27)	(23)	(48)	(54)	(35)	(44)
	A	1.000	—	0.019	—	0.010	0.009	—	0.057
	B	—	1.000	0.981	1.00	0.979	0.981	1.000	0.943
	C	—	—	—	—	0.010	0.009	—	—
SoDH	(*n*)	(56)	(61)	(69)	(28)	(68)	(54)	(35)	(44)
	I	—	—	—	—	0.007	—	—	—
	A	0.009	0.213	0.159	0.087	0.235	0.204	0.057	0.093
	B	0.107	0.779	0.833	0.891	0.618	0.630	0.800	0.802
	C	0.268	0.008	—	0.022	0.132	0.157	0.114	0.105
	D	0.304	—	0.007	—	0.007	0.009	0.029	—
	E	0.196	—	—	—	—	—	—	—
	F	0.107	—	—	—	—	—	—	—
	G	0.009	—	—	—	—	—	—	—
XO	(*n*)	(38)	(61)	(73)	(23)	(68)	(54)	(35)	(44)
	A	1.000	—	—	—	—	—	—	—
	B	—	1.000	1.000	1.000	0.993	1.000	1.000	1.000
	C	—	—	—	—	0.007	—	—	—
Alleles/locus		2.30	2.05	1.95	1.65	2.60	2.40	1.80	1.85
5% polymor.		0.50	0.40	0.40	0.30	0.40	0.45	0.25	0.30
H $(1-\Sigma Pi^2)$		0.181	0.119	0.116	0.086	0.180	0.183	0.095	0.093

Results

A total of 76 alleles were detected in 20 loci scored across the five putative *Limnoporus* species (Table 2). Genotype frequencies within populations generally conformed to Hardy-Weinberg expectations. Only four of 108 tests performed on polymorphic loci showed significant deviations (Fisher's exact $P<0.05$, with two allele classes). These four deviations were in four different loci in three different populations, and no biological explanation is offered for their occurrence.

There were substantial shifts in allele frequency among species at most loci, although only two loci (G6PD and XO) showed complete allele substitutions among species. Six loci showed allele frequency shifts of 95% among some species, and another seven showed shifts of more than 50%. Only four loci (AO, APK, MDH, ODH) showed less than 5% variation in allele frequency among the eight sample populations. All 20 loci showed at least some polymorphism. Mean heterozygosity (H) also varied considerably among taxa (Table 2). The heterozygosity values for *L. canaliculatus* and *L. dissortis* were approximately twice as high as those for the other three species.

Phenetic comparisons between populations corresponded well with taxonomic expectations. Pairwise comparisons using Nei's (1972) standard genetic distance (D) and identity (I) are shown on Table 3. For I, intraspecific comparisons ranged from 0.992 to 0.998. The most similar pair of species was *L. rufoscutellatus* and *L. nearcticus* ($I=0.964$), while *L. canaliculatus* was the most distant from all other species ($I=0.400$ to 0.508), by a substantial margin. All other interspecific comparisons were in the range of $I=0.830$ to 0.880.

UPGMA phenograms were obtained from all similarity and distance coefficients available on BIOSYS-1 (Swofford and Selander 1981). Ten of 13 coefficients gave phenograms with the same topology (Fig. 1), showing *L. notabilis* and the *L. rufoscutellatus* – *L. nearcticus* pair as slightly more similar to each other than either was to *L. dissortis*. The remaining three phenograms grouped the *L. rufoscutellatus* – *L. nearcticus* pair with *L. dissortis* instead of with *L. notabilis*. The three coefficients that gave the latter arrangement were Nei's (1972 and 1978) minimum distances, and the modified Rogers distance (Wright 1978). Because Nei's (1972) standard distance is most commonly reported for allozyme surveys we used it for Figure 1.

Wagner trees were also obtained with the same coefficients. Here again, the only ambiguous part of the trees was the relative branching pattern of *L. notabilis*, *L. dissortis*, and the *L. rufoscutellatus* – *L. nearcticus* pair. Rogers (1986) listed three coefficients as having particularly good properties for deriving minimum length trees. One of these three, the modified Rogers distance, grouped the *L. rufoscutellatus* – *L. nearcticus* pair first with *L. dissortis* (Fig. 2), as in the UPGMA tree derived with the same genetic distance measure. The other two measures preferred by Rogers (1986) were modified Cavalli-Svorza and Edwards (1967) arc and chord coefficients, which were not available on BIOSYS-1. However, the original forms of these two coefficients grouped the *L. rufoscutellatus* – *L. nearcticus* pair first with *L. notabilis*.

Maximum likelihood solutions were obtained for 10 randomly different input orders of the eight *Limnoporus* samples (Felsenstein 1986). All of the trees had the same topology, in which *L. rufoscutellatus* was grouped with *L. nearcticus*, and *L. notabilis* was the sister group of *L. dissortis* (Fig. 3). However, the length of the branches leading to either of these pairs was not significantly different from zero, and hence a quadrichotomy cannot be statistically rejected under Felsenstein's (1981) model of divergence through genetic drift.

Two programs were used in an attempt to resolve the ambiguous relationships of the *L. rufoscutellatus* group. The FREQPARS parsimony algorithm (Swofford 1988) gave a slightly shorter length for the topology of the maximum likelihood tree than for trees in

Table 3. Genetic distance measures. Nei's (1972) genetic identity (*I*) is above diagonal, and Nei's (1972) genetic distance (*D*) is below diagonal

		notabilis			*dissortis*			
POPULATION:	*canaliculatus*	Coo	Mar	QC	Swan	MSM	*rufoscutellatus*	*nearcticus*
canaliculatus	—	0.482	0.481	0.472	0.404	0.400	0.508	0.471
notab: Coombs	0.729	—	0.998	0.992	0.840	0.839	0.880	0.834
notab: Marion	0.732	0.002	—	0.989	0.840	0.839	0.875	0.830
notab: Q.Char.	0.750	0.008	0.012	—	0.833	0.833	0.875	0.833
dissortis: Swan	0.906	0.174	0.175	0.182	—	0.998	0.856	0.842
dissortis: MSM	0.915	0.176	0.175	0.183	0.002	—	0.858	0.846
rufoscutellatus	0.678	0.127	0.134	0.134	0.156	0.153	—	0.964
nearcticus	0.753	0.181	0.186	0.183	0.172	0.167	0.037	—

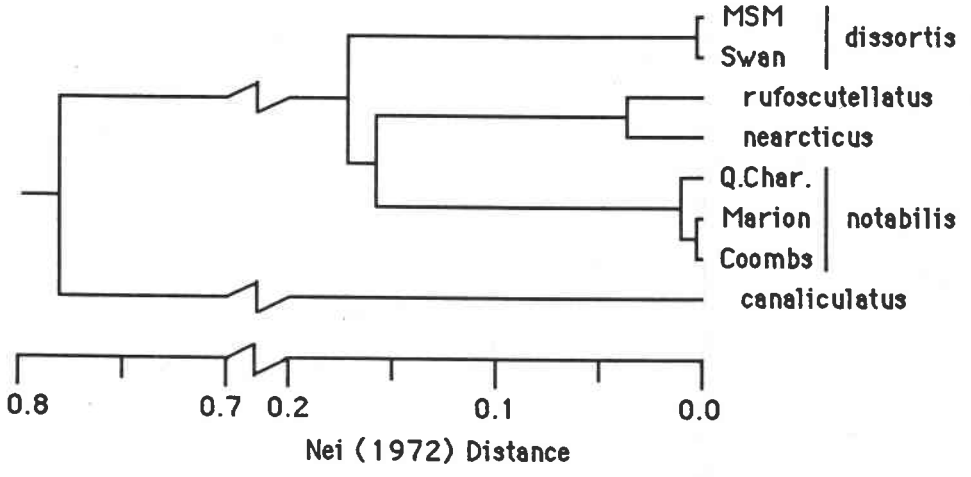

FIG. 1. UPGMA phenogram of Nei's (1972) genetic distances of *Limnoporus* populations.

which *L. notabilis* and *L. dissortis* were not sister groups. For treelength comparisons using PAUP (Swofford 1985), *L. canaliculatus* was used as outgroup and length calculations were obtained from two datasets. In one dataset the simple presence or absence of each allele was considered a character, and in the other dataset only alleles with a frequency of greater than 0.05 were considered present. A single most parsimonious tree was found in each case, but the clade comprising the three *L. notabilis* populations also included the *L. rufoscutellatus* – *L. nearcticus* pair in one case, and *L. dissortis* in the other case.

Discussion

Heterozygosity. The only other published information on electrophoretic character variation in *Limnoporus* species is that of Zera (1981*a*, 1981*b*, 1987*a*, 1987*b*). Zera examined up to 16 loci in seven populations of *L. canaliculatus* (1981*a*), and also aGPDH variation in *L. dissortis* (1981*b*). We sampled up to 11 of the 16 loci sampled by Zera (1981*a*). For

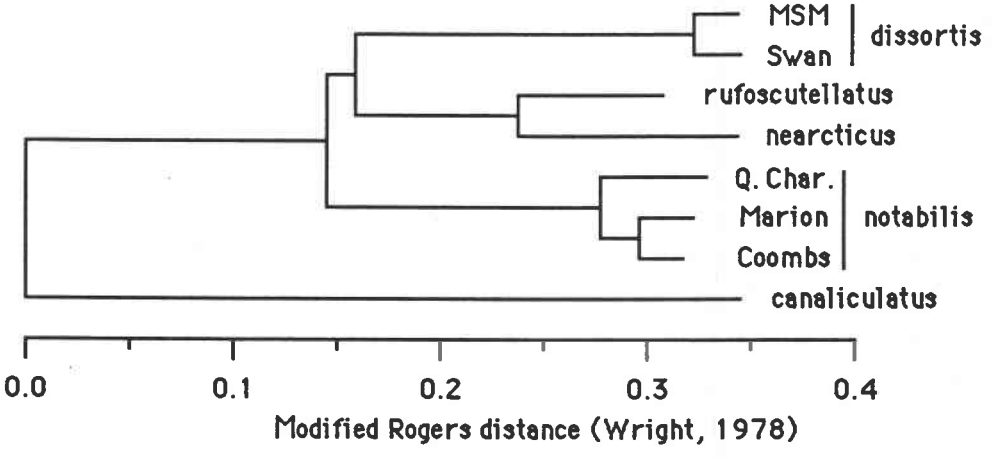

FIG. 2. Optimized Wagner tree of modified Rogers distances (Wright 1978) of *Limnoporus* populations.

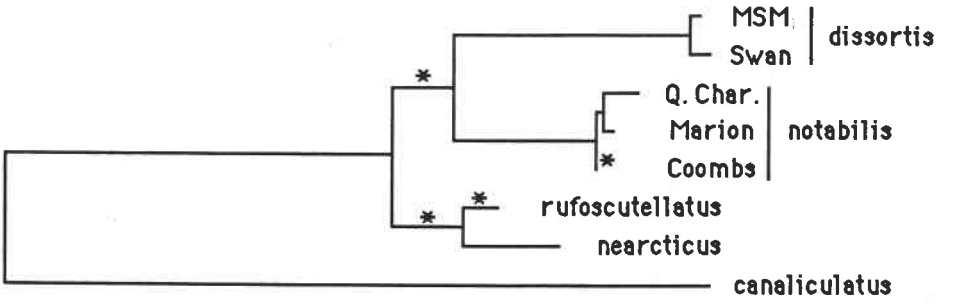

FIG. 3. Maximum likelihood tree (Felsenstein 1981) of gene frequencies of *Limnoporus* populations, with midpoint rooting between the two most distant nodes. Branches with lengths that are not significantly different from zero are indicated by an asterisk (*).

L. canaliculatus, allele frequencies were comparable among seven of the 11 loci, but four (aGPDH, 6PGD, PGI, PGM) had substantially higher frequencies of the most common allele in our sample. Zera (1981*b*) reported a heterozygosity of 0.451 for aGPDH in *L. dissortis*, which was similar to the values we obtained (0.499 and 0.515). This suggests that the differences between Zera's and our sample of *L. canaliculatus* are real, rather than an artifact of technique.

Some of the lower mean heterozygosity that we have observed within species may be related to geographic isolation of populations. For example, the Queen Charlotte Islands are 70 km off the mainland of British Columbia, and the sample from these islands had the lowest mean heterozygosity among the three *L. notabilis* populations. Also, our sample of *L. canaliculatus* was taken from the northernmost edge of the range of the species, and had a heterozygosity in the low part of the range of heterozygosities reported by Zera (1981*a*). However, Varvio-Aho *et al.* (1978) found that mean heterozygosity showed little dependence on degree of geographic isolation in *Gerris lacustris*.

Zera (1981*a*, 1981*b*) related variation in allele frequencies to degree of flightlessness in waterstriders. He found that *L. canaliculatus*, which is wing dimorphic, had low variation in allele frequencies between populations and high mean heterozygosity. In contrast, the nearly wingless species *G. remigis* Say had higher variation in allele frequencies between populations, and lower mean heterozygosity. However, for *Limnoporus*, members of the "*L. rufoscutellatus* group" are all monomorphic for long wings and seem to fly regularly (Spence 1989), and therefore we do not feel that the doubling in mean heterozygosity that we found in *L. canaliculatus* and *L. dissortis* can be attributed to differences in wing polymorphism. More general interspecific differences in population structure still need to be investigated. For example, Fairbairn (1984) found significant, heritable variation in body size and development time among geographically close populations of *L. notabilis*. In contrast, we have found relatively small differences in allele frequencies and body measurements between populations of *L. notabilis* from the same region in southwest British Columbia, and also between *L. dissortis* populations collected over an even broader geographic scale (unpublished data).

A survey of aGPDH variation across a range of waterstrider species showed uncommonly high levels of heterozygosity (Zera 1981*b*). Wing dimorphic species did not differ from wing monomorphic species in mean aGPDH heterozygosity, but Zera attributed the generally high aGPDH variability in gerrids, compared with other insects, to a group trend in reduction of the general importance of flight. Although we observed higher heterozygosity at the aGPDH locus ($H = 0.508$ vs. $H = 0.451$ in Zera's study) for *L. dissortis*, heterozygosity for *L. canaliculatus* from Quebec ($H = 0.133$) was lower than the weighted average heterozygosity of several populations from the eastern United States studied by

Zera ($H = 0.351$). Most importantly, heterozygosity at this locus was low for the other *Limnoporus* species ($H = 0.270$ for *L. notabilis* and no variation detected in either *L. nearcticus* or *L. rufoscutellatus*). Therefore, it seems that variation at the aGPDH locus is not abnormally high in *Limnoporus*. Perhaps this is because repeated flight during the reproductive period seems to be a major life-history feature for members of the *L. rufoscutellatus* group (Spence 1989; Vepsäläinen 1978).

Genetic Distances. Nei's (1972) genetic identity has frequently been used to compare species electrophoretically. Thorpe (1982) compiled many of these values and found that about 85% of *I* values between congeneric species exceed 0.35. The *I* values of 0.400–0.508 between *L. canaliculatus* and other *Limnoporus* species fall within this range, though at the low end (Table 3). Thorpe (1982) also found that 95% of *I* values between species are below 0.85, but within species 98% exceed 0.85. The values of 0.830–0.880 for comparisons among *L. notabilis, L. dissortis, L. rufoscutellatus*, and *L. nearcticus* are thus reasonably near the high end of the range for different species. However, the *I* value of 0.964 for the comparison between a Finnish population of *L. rufoscutellatus* and an Alaskan population of *L. nearcticus* is clearly within the main part of the range of values found between populations within species. This does not prove that these two taxa belong to the same species, but it does suggest that careful study of *Limnoporus* populations in Siberia, and a comparison with *L. rufoscutellatus genitalis* in Japan, would be especially informative.

The close similarity within the two samples of *L. dissortis*, and within the three samples of *L. notabilis*, is especially remarkable in light of the fact that the *L. dissortis* samples were taken 2700 km apart, and the *L. notabilis* samples were from locations separated by saltwater barriers. As well, the *L. dissortis* population from central Alberta (Swan Hills) was no closer to *L. notabilis* than was the population from Quebec (Mont Ste. Marie), suggesting that introgression has not caused large changes in the gene pool of *L. dissortis*, despite common hybridization with *L. notabilis* in western Alberta and central British Columbia. The only other genetic distance measures available for water-strider species are those of Varvio-Aho and Pamilo (1979) for 22 Finnish populations of *Gerris lacustris*. They reported Nei's (1972) genetic distances that range substantially higher than our own values, but unfortunately their values were based only on "polymorphic" loci and hence comparison with our study has little meaning.

If there is validity in the molecular clock hypothesis, as Thorpe (1982) suggests, then a value of 1.0 for Nei's standard *D* indicates a divergence time of 15–20 million years. This would mean an initial divergence for *L. canaliculatus* during the Miocene, for *L. notabilis, L. dissortis*, and the *L. rufoscutellatus – L. nearcticus* pair during the Pliocene, and for *L. rufoscutellatus* from *L. nearcticus* during the Pleistocene.

Phylogenetic Relationships. Although allozyme data are commonly used to infer phylogenetic relationships between populations or species, their analysis can prove problematical (Mickevich and Mitter 1981; Berlocher 1984; Buth 1984; Richardson *et al.* 1986). In the simplest case, when evolutionary rates are relatively homogenous in all taxa, UPGMA phenograms of Nei's (1972) distances give a good estimate of the true phylogenetic tree (Nei *et al.* 1983). Unfortunately, several rate differences are apparent in our data (Table 3). Even without such incongruences, most Nei's (1972) *I* values based on 20–25 loci will have an error range of about 0.4–0.5, which means that the hypothesis that all *Limnoporus* species except *L. canaliculatus* diverged simultaneously from one ancestor cannot be statistically refuted.

Several other methods are commonly used with frequency data to estimate minimum length trees under conditions of unequal rates of changes (Swofford and Berlocher 1987; Kim and Burgman 1988). Our results for the distance Wagner method (Swofford and

Selander 1981), Felsenstein's (1981) maximum likelihood method, and Swofford and Berlocher's (1987) frequency parsimony method all gave essentially the same topology. This topology includes an ambiguous or very close branching pattern for *L. notabilis, L. dissortis*, and the *L. rufoscutellatus – L. nearcticus* pair, and so it seems very probable that a trichotomy reflects the underlying phylogenetic relationships of these species.

Discrete-character analysis of the data with PAUP did not resolve the trichotomy. The fact that the *L. notabilis* populations were not retained as a monophyletic clade, despite their very close genetic similarity, can be explained by the large contribution of unreliably sampled rare alleles to these analyses. When *L. canaliculatus* is excluded, the remaining four species share all major alleles (frequency ≥ 0.05) except at the AK, G6PD, and ME loci. In short, the dataset contains few, if any, good cladistic characters.

Given the paucity of discrete character substitutions, we do not suggest formal taxonomic changes based solely on our electrophoretic data. Nonetheless, we are encouraged by the generally good correspondence, in other taxa, between phylogenies based on frequencies of electrophoretic characters, and those based on other characters (e.g. Green 1986; Wayne and O'Brien 1987). By coupling our information with a complementary study of morphological characters and breeding relationships of *Limnoporus* species the true phylogeny may be more fully resolved.

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