# Mitochondrial DNA, Allozymes, Morphology, and Hybrid Compatibility in *Limnoporus* Water Striders (Heteroptera: Gerridae): Do They All Track Species Phylogenies?

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**ABSTRACT** The six extant species of *Limnoporus* water striders provide an unusual opportunity to compare allozyme and mitochondrial DNA (mtDNA) divergences with both morphological divergences and information on interspecific hybrid inviability. Parsimony analyses of mtDNA, allozymes, and morphology did not differ greatly, and each data type provided useful information on at least some nodes in the phylogeny. Simultaneous analysis of the combined data gave a better resolved and supported tree than did any single data type. Several measures of hybrid inviability bore no relationship to genetic distances between species, thus demonstrating the poor value of hybrid compatibility as an indicator of phylogenetic relationships. When genetic distances were related to estimates of time of divergence based on fossils, standard molecular clocks for mtDNA and allozymes showed as much as a 10-fold underestimate of absolute time. The phylogeny of *Limnoporus* allows more rigorous evaluation of several prior hypotheses of ecological or developmental processes in water striders. In particular, the phylogeny supports the evolutionary lability of wing dimorphism, sexual size dimorphism, and local differentiation of ontogenetic traits.

**KEY WORDS:** *Limnoporus, Gerris, Aquarius,* hybridization, total evidence, water striders

INFORMATION MAY BE lost when phylogenies are based on a consensus of datasets that are analyzed separately. Phylogenies based on combined data sets, or "total evidence," have been advocated as a solution to this problem (Kluge 1989, Swofford 1991, Chavarria and Carpenter 1994, but see Funk et al. 1995, de Quieroz et al. 1995). Nevertheless, it remains important to evaluate carefully the contributions that fundamentally different kinds of characters make to a phylogeny based on a combined dataset. In particular, portions of the total dataset may provide misleading information, whether by lack of independence among characters or by inaccurate reflection of the true species phylogeny.

We define a species phylogeny as the modal phylogeny of the genes contained by species. Species themselves are recognized most conveniently as genetically distinct, diagnosable groups of populations. The most unambiguous test of species status when populations contact each other is that they maintain their integrity. Thus, portions of a genome may legitimately have a different phylogeny from that of the species that contain them (Pamilo and Nei 1988). Species may exchange a limited number of genes for some time after their initial divergence, and even after one or both of the original sister species have themselves undergone a division. In fact, if introgression between species occurs predominantly in only one direction, then a substantial proportion of the genome may indicate a phylogeny that is different from the history of the species.

Furthermore, lack of resolution of the relationships of species will not disappear once the species achieve complete reproductive isolation, but rather the distribution of the introgressed genes will continue to confound efforts to resolve a node even when it is deep within a much larger phylogeny. Thus, this problem is also related to a general issue concerning whether polychotomies may sometimes be the most accurate depiction of the relationships among some taxa, rather than simply an expression of ignorance (Hoelzer and Melnick 1994).

*Limnoporus* comprises a group of 6 water strider species that provide this kind of challenge to efforts to resolve a true, or at least modal, species phylogeny (Spence 1990). Four of the 6 species in the genus are placed as members of the *L. rufoscutellatus* species group: *L. notabilis* (Drake & Hottes), *L. dissortis* (Drake & Harris), *L. rufoscutellatus* (Latreille), and *L. genitalis* (Miyamoto). These species are notoriously difficult to identify and yet exhibit clear behavioral isolation, postzygotic incompatibilities, or both. For example, *L. notabilis* and *L. dissortis* differ in mating behavior and $F_1$ hybrids show marked reduc-

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**Table 1. Taxa, characters, and number of populations (p), laboratory colonies (l), and minimum number of individuals (i) sampled per population for molecular data**

| Taxa | mtDNA | Allozymes |
|---|---|---|
| *L. notabilis* | 2i: California (CA), U.S.A. & British Columbia (BC), Canada | 3p, 128–164i: SS'90 |
| *L. dissortis* | 2i: Quebec (QC), Canada & Alberta (AB), Canada | 2p, 102–122i: SS'90 |
| *L. rufoscutellatus*-FI | 1i: Hanko, Finland | 1p, 35–40i: SS'90 |
| *L. rufoscutellatus*-AK | 1i: Alaska, U.S.A. | 1p, 44i: SS'90 |
| *L. genitalis* | 1i: Hokkaido, Japan | 1l, 32–36i: Hokkaido, Japan |
| *L. canaliculatus* | 1i: Ontario, Canada | 1p, 14–56i: SS'90 |
| *L. esakii* | 1i: Honshu, Japan | 1l, 6–32i: Honshu, Japan |
| *A. remigis* | 1i: Alberta, Canada | 1p, 2–12i: Alberta, Canada |
| *G. buenoi* | 1i: Ontario, Canada | 1p, 2–4i: Alberta, Canada |
| *G. comatus* | 1i: Ontario, Canada | 1p, 2–5i: Alberta, Canada |
| *G. pingreensis* | 1i: Alberta, Canada | 1p, 2–8i: Alberta, Canada |

Source of samples is indicated unless information is published previously (Sperling and Spence 1990 [SS'90]). See Andersen and Spence (1992) for detailed information about the data base for morphological characters of *Limnoporus* and *A. remigis*; characters for the *Gerris* species were taken from 5 specimens of each species from Alberta, Canada.

tions in juvenile survival (Spence and Wilcox 1986, Spence 1990). The species are distinguished by body length and other subtle morphometric characters as well as by alleles at the G-6-PD allozyme locus (Andersen and Spence 1992, Sperling and Spence 1990). Three autosomal loci show asymmetric introgression of alleles from *L. dissortis* into *L. notabilis* (Sperling and Spence 1991), although they may not be sister species. A phylogeny of all species based on morphological characters indicated that *L. dissortis* was the sister of a species pair comprised of *L. rufoscutellatus* + *L. genitalis* (Andersen and Spence 1992), but an allozyme survey of most taxa in the genus indicated that *L. dissortis, L. notabilis,* and *L. rufoscutellatus* were genetically equidistant (Sperling and Spence 1990). The 2 remaining *Limnoporus* species, *L. canaliculatus* (Say) and *L. esakii* (Miyamoto), are distinct from the *L. rufoscutellatus* species group and are basal within *Limnoporus* (Andersen and Spence 1992), although their exact relationships remain ambiguous.

Under such circumstances it is tempting to conclude that species interactions with real potential for introgression represent the limit of our ability to resolve the genealogical relationships of species using phylogenetic systematics. However, *Limnoporus* water striders have been used extensively as experimental animals in studies of life history and habitat use (Spence 1983, 1989; Fairbairn 1984), mating behavior (Wilcox and Spence 1986), hybrid zones (Sperling and Spence 1991), wing polymorphism and sexual size dimorphism (Zera 1981a, Andersen 1993a, 1994), allometry (Klingenberg and Spence 1993), and biochemical variation (Zera 1981b). A minimum date for the divergence of the *L. rufoscutellatus* group in the genus can be estimated from 2 fossils (Andersen et al. 1993). Thus, we are compelled to provide the strongest possible phylogenetic framework for meaningful comparisons among species. Well-supported phylogenies are a prerequisite for studies of adaptation in a historical setting (Brooks and McLennan 1991, Harvey and Pagel 1991), and water striders have provided a rich opportunity for such work (Andersen 1993a, 1993b, 1994; Spence and Andersen 1994).

Therefore, the objectives of this article were to use new data from mitochondrial DNA (mtDNA) sequences, improved data on allozyme differences and a published cladistic analysis of morphological data (Andersen and Spence 1992) to reconstruct the *Limnoporus* species phylogeny and to evaluate the contribution of the different data types to this phylogeny; to determine the extent of postzygotic hybrid incompatibility between species and assess the relationship of such data to phylogeny; to use fossil ages and comparisons of molecular divergences to date and calibrate molecular data types against each other; and to reevaluate the evolution of ecological and behavioral traits in the context of *Limnoporus* phylogeny.

## Methods and Character Matrices

All currently recognized species of *Limnoporus* were included in this study (Table 1): *L. notabilis, L. dissortis, L. rufoscutellatus, L. genitalis, L. canaliculatus,* and *L. esakii* (Andersen and Spence 1992). In addition, *L. rufoscutellatus* from Alaska was included because populations from this region had been recognized previously as a separate species, *L. nearcticus* (Kelton 1961). Representatives of 2 additional genera were used as outgroups for phylogenetic analysis: *Aquarius remigis* (Say), *Gerris buenoi* Kirkaldy, *G. comatus* Drake & Hottes, and *G. pingreensis* Drake & Hottes.

**Mitochondrial DNA.** An 820-bp region of mtDNA was sequenced by F.A.H.S. from 1 or 2 specimens per taxon using the methods of Sperling and Hickey (1994). The sequenced region includes the 3′ half of the gene for cytochrome oxidase subunit 1. Sequences were obtained from fragments amplified using the polymerase chain reaction and 2 primers with sites that are conserved widely in insects (Simon et al. 1994). These 2 end primers, C1-J-2183 and TL2-N-3014, allowed amplification



**Table 2. Allozyme alleles dete**

| Taxon | AK | AO |
|---|---|---|
| | ABCDE | ABCI |
| *L. genitalis* | 00100 | 0100 |
| *L. esakii* | 00001 | 0010 |
| *A. remigis* | 00110 | 0100 |
| *G. buenoi* | 01000 | 0100 |
| *G. comatus* | 00100 | 0011 |
| *G. pingreensis* | 00100 | 0100 |
| | LDH | MD |
| | IABCD | AB |
| *L. genitalis* | 00100 | 011 |
| *L. esakii* | 00001 | 001 |
| *A. remigis* | 01000 | 100 |
| *G. buenoi* | ????? | 011 |
| *G. comatus* | ????? | 100 |
| *G. pingreensis* | ????? | 010 |

Data for 8 previously surveyed *Lim* increasing electrophoretic mobility.

of this region from all specie Using sequences generated *Gerris,* a new primer, C1-N and has a sequence of 5′ ( GAA/GTGTTCIGC 3′. This 780-bp region to be amplifie *A. remigis.* Sequence data GenBank (accession numbe U83345).

**Allozymes.** Using acrylami sis of proteins, Sperling and veyed variation in 20 loci ac *Limnoporus.* Using the same allozyme data were collec F.A.H.S. for species missing f ysis (*L. esakii* and *L. genital* taxa, including the 3 *Gerris* above and *A. remigis.* Outgr allozyme data are small (Tab *genitalis* and *L. esakii* came f had been in laboratory cultur tions. A total of 86 alleles wa

**Morphology.** The morphol posed of 46 characters descri oration (adults and nymphs), metathoracic scent apparatus, genitalia. Forty-five of these c

**Table 3. Morphological chara**

| Character no. | 01234 |
|---|---|
| *L. rufoscutellatus*-FI | 20100 |
| *L. rufoscutellatus*-AK | 20100 |
| *G. buenoi* | 01011 |
| *G. comatus* | 01011 |
| *G. pingreensis* | 00001 |

Data for character numbers 0–44 fo Each of these 6 species was coded treated as nonadditive (unordered), o

(l), and minimum number of in-

| Allozymes |
|---|
| 3p, 128–164i: SS'90 |
| 2p, 102–122i: SS'90 |
| 1p, 35–40i: SS'90 |
| 1p, 44i: SS'90 |
| 1l, 32–36i: Hokkaido, Japan |
| 1p, 14–56i: SS'90 |
| 1l, 6–32i: Honshu, Japan |
| 1p, 2–12i: Alberta, Canada |
| 1p, 2–4i: Alberta, Canada |
| 1p, 2–5i: Alberta, Canada |
| 1p, 2–8i: Alberta, Canada |

)ence 1990 [SS'90]). See Andersen and
*mnoporus* and *A. remigis*; characters for

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1994; Spence and Andersen

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ermine the extent of postzygotic
)ility between species and assess
f such data to phylogeny; to use
omparisons of molecular diver-
d calibrate molecular data types
; and to reevaluate the evolution
behavioral traits in the context of
)geny.

### and Character Matrices

ecognized species of *Limnoporus*
this study (Table 1): *L. notabilis*,
*ifoscutellatus*, *L. genitalis*, *L. can-*
*L. esakii* (Andersen and Spence
)n, *L. rufoscutellatus* from Alaska
:ause populations from this region
ıized previously as a separate spe-
*us* (Kelton 1961). Representatives
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et al. 1994). These 2 end primers,
TL2-N-3014, allowed amplification



**Table 2. Allozyme alleles detected at 20 polymorphic loci. Presence, 1; absence, 0; unscored, ?**

| Taxon | Allozyme alleles | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | AK | AO | AP | EST1 | GOT | aGPD | GP | G6PD | HK | IDH |
| | ABCDE | ABCD | AB | ABCDEF | IAB | IAaBbCD | IABC | ABCD | JIABC | IABC |
| *L. genitalis* | 00100 | 0100 | 01 | 000100 | ??? | 0100000 | 0110 | 0100 | 00010 | 0010 |
| *L. esakii* | 00001 | 0010 | 01 | 000001 | 010 | 0000011 | 0101 | 1000 | 00001 | 0001 |
| *A. remigis* | 00110 | 0100 | 01 | 000011 | 100 | 0000100 | 0100 | 1000 | 00110 | 0010 |
| *G. buenoi* | 01000 | 0100 | 01 | 000111 | 010 | 0101000 | 0010 | 0001 | 01000 | 0011 |
| *G. comatus* | 00100 | 0011 | 01 | 000101 | 100 | 0000100 | 0100 | 0010 | 11000 | 0100 |
| *G. pingreensis* | 00100 | 0100 | 01 | 001110 | 010 | 0011100 | 0100 | 0011 | 11000 | 0010 |
| | LDH | MDH | ME | OD | 6PGD | PGI | PGM | PMI | SODH | XO |
| | IABCD | ABC | IABCD | BC | ABbCD | ABCD | ABCD | ABC | IABCDEFG | ABC |
| *L. genitalis* | 00100 | 010 | 01000 | 10 | 01000 | 0100 | 0100 | 110 | 01000000 | 010 |
| *L. esakii* | 00001 | 001 | 01000 | 01 | 00100 | 1000 | 1100 | 100 | 00110000 | 100 |
| *A. remigis* | 01000 | 100 | 01000 | 10 | 00011 | 1000 | 0011 | 010 | 00011000 | 100 |
| *G. buenoi* | ????? | 011 | 00110 | 01 | 01000 | 0001 | 0001 | 010 | 11100000 | 100 |
| *G. comatus* | ????? | 100 | 01000 | 01 | 01000 | 0010 | 0010 | 001 | 10000000 | 110 |
| *G. pingreensis* | ????? | 010 | 01000 | 01 | 01010 | 0010 | 0001 | ??? | 01100000 | 011 |

Data for 8 previously surveyed *Limnoporus* populations are listed in Sperling and Spence (1990). Alleles are listed in order of increasing electrophoretic mobility.

of this region from all species except *A. remigis*. Using sequences generated for *Limnoporus* and *Gerris*, a new primer, C1-N-2968, was designed and has a sequence of 5′ GTATTTCGTTATAI-GAA/GTGTTCIGC 3′. This primer allowed a 780-bp region to be amplified and sequenced in *A. remigis*. Sequence data are available from GenBank (accession numbers U83333 through U83345).

**Allozymes.** Using acrylamide gel electrophoresis of proteins, Sperling and Spence (1990) surveyed variation in 20 loci across most species of *Limnoporus*. Using the same methods, additional allozyme data were collected by J.R.S. and F.A.H.S. for species missing from the earlier analysis (*L. esakii* and *L. genitalis*) and for outgroup taxa, including the 3 *Gerris* species mentioned above and *A. remigis*. Outgroup sample sizes for allozyme data are small (Table 1) and data for *L. genitalis* and *L. esakii* came from populations that had been in laboratory culture for several generations. A total of 86 alleles was resolved (Table 2).

**Morphology.** The morphological dataset is composed of 46 characters describing variation in coloration (adults and nymphs), external morphology, metathoracic scent apparatus, and male and female genitalia. Forty-five of these characters (numbered 0–44) were used previously in a study of the classification and phylogeny of *Limnoporus* (Andersen and Spence 1992), and this work should be consulted for definitions of characters and their states. To allow more effective comparisons with molecular characters in the current study, the same outgroup species (*A. remigis*, *G. buenoi*, *G. comatus*, and *G. pingreensis*) were examined and coded by N.M.A. for the 45 characters as described by Andersen and Spence (1992) with the following modifications: state 2 of character 10 was deleted; an extra state was added to character 41 (Gynatrial sac tubular [2]); and a new 2-state character was added (45, Sclerotization of accessory scent gland present [0]; absent [1]). Table 3 gives the revised data matrix for the 3 new outgroup species and *L. rufoscutellatus* populations from Finland and Alaska (coded as identical).

**Laboratory Hybridization.** The ability of members of different species of *Limnoporus* to interbreed and the fate of hybrid offspring were studied by J.R.S. as a practical assessment of genetic relationships among taxa. We expected that closely related species would have greater success in producing hybrid offspring than would more distant relatives. Furthermore, results of such experiments should illuminate the potential for gene flow across

**Table 3. Morphological character state matrix for 2 *Limnoporus* populations and 3 *Gerris* species**

| Character no. | 01234 | 56789 | 11111 01234 | 11111 56789 | 22222 01234 | 22222 56789 | 33333 01234 | 33333 56789 | 44444 01234 | 4 5 |
|---|---|---|---|---|---|---|---|---|---|---|
| *L. rufoscutellatus*-FI | 20100 | 00001 | 10111 | 11011 | 01010 | 11010 | 12010 | 21102 | 10101 | 0 |
| *L. rufoscutellatus*-AK | 20100 | 00001 | 10111 | 11011 | 01010 | 11010 | 12010 | 21102 | 10101 | 0 |
| *G. buenoi* | 01011 | 10010 | --120 | 00021 | 02000 | 01011 | 20101 | -2003 | 02200 | 1 |
| *G. comatus* | 01011 | 10010 | --120 | 00021 | 02000 | 00011 | 20101 | -2000 | 02200 | 1 |
| *G. pingreensis* | 00001 | 10000 | 00120 | 00001 | 02000 | 01011 | 20101 | -1000 | 02200 | 0 |

Data for character numbers 0–44 for 6 previously treated *Limnoporus* or *Aquarius* species are listed in Andersen and Spence (1992). Each of these 6 species was coded "0" for character no. 45. Characters numbers 0, 13, 18, 21, 30, 31, 36–39, 41, 42, and 45 are treated as nonadditive (unordered), other characters as additive (ordered). Unscorable character states are indicated by -.

species boundaries if populations of these species meet in nature. Because of considerable potential for hybridization among species of the *L. rufoscutellatus* group (Spence 1990, Sperling and Spence 1991), study of these species was particularly detailed.

Hybridization experiments were carried out in Edmonton, Canada, using mainly gerrids from cultures that had been selected for breeding without diapause and that had been maintained in the laboratory for a minimum of 10 generations before experiments began. The single exception was for crosses involving *L. rufoscutellatus* from Alaska, from which a direct-breeding strain could not be isolated. Only postdiapause males of this species were used for experiments, generally after they had been bred to conspecific females to maintain a laboratory culture. All cultures and experiments were held on ≈10 cm of aerated water in rectangular plastic tubs (60 by 30 cm) at 20°C and a photoperiod of 19:5 (L:D) h; gerrids were fed to satiation 3–4 times per week on fresh-frozen flesh flies, *Neobelliera bullata* (Parker).

Data on the reproductive success of crosses among various species were collected through standardized experiments to measure fertilization success, and compare egg and juvenile survivorship. Interspecific compatibility was assayed through reciprocal crosses involving all pairs of the *L. rufoscutellatus* species group. Individuals of *L. notabilis* cultured from widely separated populations (Davis, CA, and Vancouver, BC, Canada) were also mated in reciprocal crosses to provide a baseline of interpopulation data to assist in interpretation of data from interspecific crosses. The same was done for *L. dissortis* (Mont Ste. Marie, QB, Canada and George Lake, AB, Canada) although these latter experiments were done early in the study and percentage of survival measures for 2 crosses were not standardized to be strictly comparable with the values for other crosses; they are not reported here.

Breeding cultures were initiated by isolating 10 newly emerged females with 10 newly emerged males in a single tub. Small Styrofoam strips were provided as oviposition sites and cultures were checked daily for death of breeding individuals, presence of breeding pairs, and newly hatched 1st instars. Dead breeders were replaced as soon as possible if this was feasible, given the status of other cultures. Standard survival trials generally were initiated with 100 first instars collected over a 2- to 4-d period. A few survival experiments were initiated with <100 first instars when it proved difficult to obtain the desired number within the prescribed time. However, results of those started with <70 first instars are not reported here. Survival trials were checked daily to record number and sex of newly emerged adults. Once a trial had been established for a particular cross, the females remaining alive were isolated in smaller, circular containers (23 cm in diameter) and fed daily until they had laid 30–50 eggs, or for a 3-wk period. Any eggs laid were held and checked subsequently for fertility, which was determined by darkening of eggs. Hatching success was recorded as the proportion of fertile eggs that hatched. Survival was recorded as the proportion of the initial batch of 1st instar nymphs that survived to the adult stage.

Arcsine transformed data for percent fertility, percent hatch, percent survival, and percent males at emergence were analyzed by the software package SuperANOVA (Gagnon et al. 1989) on a Macintosh Power PC computer. For the first 3 measures, means for each cross were then grouped as "between species," "between populations," or "within populations" and reanalyzed to determine effects of taxonomic hierarchy. Data were further analyzed by linear contrasts to reveal any species and population effects.

**Genetic Distances.** For mtDNA, simple percent similarity of DNA sequences was used as the basic distance measure. To provide estimates that compensate for multiple mutations at single sites, distances were also calculated using Jukes and Cantor's (1969) single parameter estimate and Kimura's (1980) 2-parameter estimate. These estimates were obtained using PHYLIP version 3.5c (Felsenstein 1993). For allozymes, Nei's (1972) genetic distance was used because it theoretically is proportional to evolutionary time (Nei 1987). Not all loci were surveyed in all species, with between 17 and 20 loci common to any given pairwise comparison. Thus, 2 distance matrices were calculated: the 1st based on the 17 loci that were surveyed in all taxa, and the 2nd based on the maximum number of loci common in any single pairwise comparison.

**Phylogenetic Analysis.** Parsimony analysis of morphology, allozymes, and mtDNA was performed using PAUP version 3.1.1 (Swofford 1993). Each dataset was analyzed separately as well as in combination with other datasets. All default parameters of PAUP were used unless indicated otherwise, including equal weightings for all characters. Bootstrap analysis, using 500 replications, was used to obtain a measure of the relative support for different nodes within a tree.

There has been much recent discussion about the merits of consensus trees (summary trees that show points of agreement between separately derived trees) versus trees obtained from a single combined dataset (de Quieroz et al. 1995). We employ a conservative strategy in which we perform both kinds of analyses and compare the results. Furthermore, we leave all characters as unweighted to minimize the number of ad hoc assumptions that might bias our phylogenetic analyses.

For allozyme data, various coding schemes were applied in recognition of possible biases introduced by small sample sizes for some taxa, including simple presence or absence of alleles in a species, and presence versus absence with presence defined by occurrence of an allele at a frequency of >5%. These coding methods are equivalent to



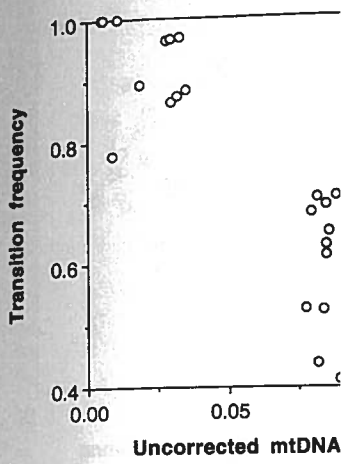


**Fig. 1.** Correspondence betwe DNA sequence divergence and freq

the "independent allele" metho Mitter (1981, 1983). Using this an inferred ancestor may be ass a given locus during cladogram complicating interpretation. To as the basic unit of homology, t ditional coding methods were locus was treated as a multist states being different allelic c Carpenter et al. 1993); and ( turnover" method was implem coding (Mickevich and Weller

**Voucher Specimens.** The ab legs of specimens used for mtD placed in gelatin capsules. The pinned and curated the same a specimens. Additional whole sp as vouchers for allozyme po Vouchers have been deposited Museum of the University of mark.

## Results

**Character Variation.** Of the in the mtDNA of *Limnoporus*

**Table 4. Genetic distances**

| | *notabilis* | *dis* |
|---|---|---|
| *L. notablilis* | 0.5/0.6 | 0 |
| *L. dissortis* | 1.8 | 0. |
| *L. rufoscutellatus*-FI | 2.8 | |
| *L. rufoscutellatus*-AK | 3.3 | |
| *L. genitalis* | 3.0 | |
| *L. canaliculatus* | 8.6 | |
| *L. esakii* | 8.2 | |
| *A. remigis* | 12.2 | |
| *G. buenoi* | 11.9 | |
| *G. comatus* | 10.8 | |
| *G. pingreensis* | 9.4 | |

Uncorrected mtDNA percent sequenc
Values on diagonal are intraspecific dist

were held and checked sub-
which was determined by dark-
ng success was recorded as the
ggs that hatched. Survival was
ortion of the initial batch of 1st
rvived to the adult stage.
ed data for percent fertility,
nt survival, and percent males
nalyzed by the software pack-
Gagnon et al. 1989) on a Mac-
mputer. For the first 3 mea-
h cross were then grouped as
"between populations," or
" and reanalyzed to determine
hierarchy. Data were further
contrasts to reveal any species
cts.

**es.** For mtDNA, simple per-
NA sequences was used as the
sure. To provide estimates that
ltiple mutations at single sites,
o calculated using Jukes and
gle parameter estimate and Ki-
arameter estimate. These esti-
ed using PHYLIP version 3.5c
. For allozymes, Nei's (1972) ge-
used because it theoretically is
olutionary time (Nei 1987). Not
yed in all species, with between
mon to any given pairwise com-
istance matrices were calculated:
he 17 loci that were surveyed in
nd based on the maximum num-
on in any single pairwise com-

**Analysis.** Parsimony analysis of
zymes, and mtDNA was per-
JP version 3.1.1 (Swofford 1993).
analyzed separately as well as in
1 other datasets. All default pa-
P were used unless indicated oth-
equal weightings for all charac-
nalysis, using 500 replications, was
measure of the relative support
es within a tree.
en much recent discussion about
nsensus trees (summary trees that
greement between separately de-
sus trees obtained from a single
t (de Quieroz et al. 1995). We em-
ive strategy in which we perform
nalyses and compare the results.
e leave all characters as unweight-
the number of ad hoc assumptions
our phylogenetic analyses.
data, various coding schemes were
gnition of possible biases intro-
sample sizes for some taxa, includ-
ence or absence of alleles in a spe-
nce versus absence with presence
urrence of an allele at a frequency
coding methods are equivalent to

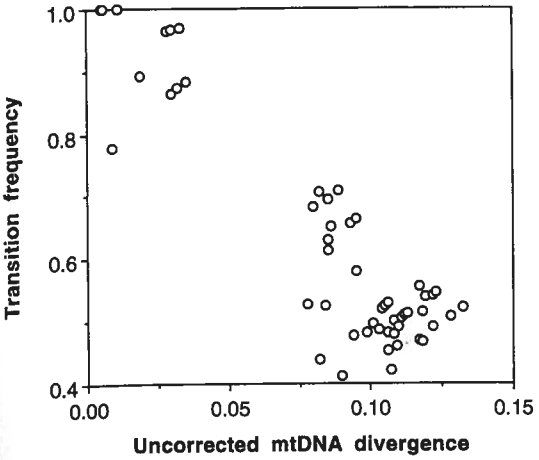


**Fig. 1.** Correspondence between uncorrected mtDNA sequence divergence and frequency of transitions.

the "independent allele" method of Mickevich and Mitter (1981, 1983). Using this method, however, an inferred ancestor may be assigned no alleles at a given locus during cladogram construction, thus complicating interpretation. To regard each locus as the basic unit of homology, the following 2 additional coding methods were explored: (1) each locus was treated as a multistate character, the states being different allelic combinations (e.g., Carpenter et al. 1993); and (2) the "minimum turnover" method was implemented with ordinal coding (Mickevich and Weller 1990).

**Voucher Specimens.** The abdomens, head, and legs of specimens used for mtDNA analyses were placed in gelatin capsules. The capsules were then pinned and curated the same as for normal insect specimens. Additional whole specimens were used as vouchers for allozyme population samples. Vouchers have been deposited in the Zoological Museum of the University of Copenhagen, Denmark.

## Results

**Character Variation.** Of the 820 bp sequenced in the mtDNA of *Limnoporus* and outgroups, 204 (25%) varied in comparisons across all taxa, 125 (15%) varied within *Limnoporus*, and 39 (5%) varied within the *rufoscutellatus* species group. The sequences were A+T rich, the A+T content ranging from 70.8 to 73.9%. Such frequencies are typical for mitochondrial protein coding genes in hemimetabolous insects (Simon et al. 1994). Nucleotide frequencies for A ranged from 34.4 to 36.6%, for T from 34.9 to 38.5%, for C from 13.2 to 15.7%, and for G from 12.9 to 13.5%. In addition, strong bias toward transitions was evident in pairwise comparisons at low divergences (Fig. 1). For sequence divergences of up to 3.4%, the average ratio of transitions observed was 93%. For divergences of >10%, the frequency of transversions had declined to ≈50%. Such strong initial transition biases have been documented widely for insect mtDNA (Simon et al. 1994). When protein sequences were inferred using an mtDNA code with 8 codons for serine, 14 out of 273 residues varied across all taxa, only 5 showed variation within *Limnoporus*, and there was a single replacement within the *rufoscutellatus* group. No insertions or deletions were found in any of the sequences.

Allozyme variation in *Limnoporus* has been described by Sperling and Spence (1990) and patterns of character variation are similar in the current study (Table 2). However, for the additional species surveyed here, laboratory colonies and much smaller sample sizes were used, and therefore these sample allele frequencies are more likely to contain deviations from true population values. Morphological character variation is discussed in Andersen and Spence (1992), and only 3 additional species were scored for the current study (Table 3).

**Genetic Distances.** Uncorrected mtDNA percent sequence differences (Table 4) were 1.1% or less within *L. notabilis* and *L. dissortis*, as well as among both populations of *L. rufoscutellatus* and *L. genitalis*. Although the 2 samples within each species represent opposite ends of large geographic ranges, these divergences are well within the interspecific divergences found in this region of mtDNA in most other insects (see Simon et al. 1994 for a list of sequences available for this region

**Table 4. Genetic distances**

| | *notabilis* | *dissortis* | *rufo*-FI | *rufo*-AK | *genit.* | *canal.* | *esakii* | *remig.* | *buenoi* | *coma.* | *pingr.* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *L. notabliis* | 0.5/0.6 | 0.204 | 0.148 | 0.201 | 0.204 | 0.636 | 1.351 | 0.894 | 0.809 | 1.230 | 0.678 |
| *L. dissortis* | 1.8 | 0.9/0.2 | 0.182 | 0.201 | 0.186 | 0.777 | 1.372 | 0.960 | 0.883 | 1.518 | 0.806 |
| *L. rufoscutellatus*-FI | 2.8 | 3.0 | — | 0.043 | 0.094 | 0.504 | 1.142 | 0.714 | 1.080 | 0.944 | 0.488 |
| *L. rufoscutellatus*-AK | 3.3 | 3.2 | 0.6 | — | 0.117 | 0.597 | 1.221 | 0.770 | 0.917 | 1.071 | 0.473 |
| *L. genitalis* | 3.0 | 3.4 | 0.5 | 1.1 | — | 0.599 | 1.309 | 0.826 | 1.006 | 1.027 | 0.601 |
| *L. canaliculatus* | 8.6 | 8.5 | 9.3 | 9.5 | 9.3 | — | 0.865 | 0.591 | 0.924 | 0.749 | 0.617 |
| *L. esakii* | 8.2 | 8.0 | 8.5 | 8.9 | 8.5 | 9.5 | — | 0.831 | 1.232 | 1.131 | 1.222 |
| *A. remigis* | 12.2 | 11.8 | 13.2 | 12.8 | 13.2 | 10.6 | 10.3 | — | 1.070 | 0.801 | 0.787 |
| *G. buenoi* | 11.9 | 11.8 | 12.3 | 12.2 | 12.3 | 10.9 | 11.7 | 10.6 | — | 1.037 | 0.546 |
| *G. comatus* | 10.8 | 10.8 | 11.1 | 11.2 | 11.3 | 11.7 | 11.0 | 10.1 | 8.4 | — | 0.547 |
| *G. pingreensis* | 9.4 | 9.8 | 10.4 | 10.5 | 10.6 | 10.7 | 8.2 | 9.0 | 8.5 | 7.8 | — |

Uncorrected mtDNA percent sequence divergences are below diagonal. Nei's (1972) distances, based on 17 loci, are above diagonal. Values on diagonal are intraspecific distances (mtDNA/Nei's D). Column headings are abbreviations of row headings.

of mtDNA). After that, the most similar pair of species was *L. notabilis* and *L. dissortis*, with 1.8% divergence; these 2 species showed 2.8–3.4% divergence from *L. rufoscutellatus*-FI and -AK and *L. genitalis*. In contrast, *L. esakii* had divergences of 8.0–9.5% from the *rufoscutellatus* species group, and *L. canaliculatus* had divergences of 8.5–9.4%, and these 2 species showed a divergence of 9.5% from each other. Higher divergences were obtained by applying formulae designed as a correction for multiple substitutions at single sites. Low divergences within the *rufoscutellatus* group remained essentially the same when corrected for multiple hits, whereas estimates for deeper divergences were 8% higher for the Jukes–Cantor correction (Jukes and Cantor 1969), and 16% higher using the Kimura 2-parameter correction (Kimura 1980).

Allozyme variation expressed as Nei's (1972) distances showed negligible divergences among populations within *L. notabilis* and *L. dissortis* (Table 4). Divergences were slightly larger between *L. rufoscutellatus* (Finland and Alaska) and *L. genitalis*, although the *L. genitalis* sample was taken from a laboratory colony and some genetic drift may have occurred. *L. notabilis*, *L. dissortis*, and *L. rufoscutellatus* were essentially equidistant in allozyme distances. Among other species of *Limnoporus*, *L. canaliculatus* showed substantially lower divergences from the *rufoscutellatus* group species than did *L. esakii*. Because it was not possible to score all loci in all species, divergences are expressed based on the 17 loci common to all species. Previously published values for Nei's distances based on a survey of 20 loci in *Limnoporus* (Sperling and Spence 1990) were on average 4% higher than those based on 17 loci.

As would be expected if divergences accumulate over time, mtDNA differences were correlated with allozyme distances when analyzed over all taxa (Fig. 2; $r = 0.73$, $P < 0.001$). The values fell in 2 clusters. One, on the lower left, reflects the relatively small divergences within the *L. rufoscutellatus* group and shows a higher correlation ($r = 0.84$, $P < 0.001$) between the 2 distance measures. The other, larger cluster is composed of comparisons involving the *L. rufoscutellatus* group and other species included in the analysis together with those involving only species outside the *L. rufoscutellatus* group. This cluster is centered on higher overall divergences, and there is no significant correlation between allozyme and mtDNA measures within the cluster ($r = -0.11$, $P = 0.46$). This probably reflects both the less accurate allozyme data and saturation of mtDNA divergence. The gap between these 2 groups of values can be related to phylogenetic history. The fossil record for *Limnoporus* (Andersen et al. 1993, N.M.A., unpublished data) indicates that divergence between the *L. rufoscutellatus* group and the other taxa must be at least 50 million years in age. We expect

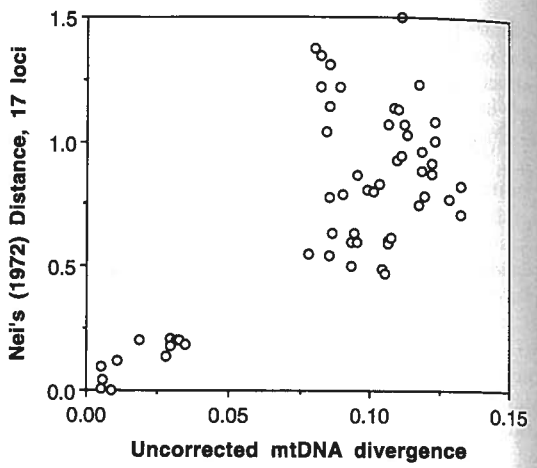


**Fig. 2.** Correspondence between uncorrected mtDNA sequence divergence and allozyme divergence.

that divergences within the extant species of the *L. rufoscutellatus* group are much younger.

**Phylogeny.** Parsimony analysis of mtDNA gave a well-supported and almost fully resolved tree (Fig. 3). The 2 individuals of *L. notabilis* clearly formed a clade, as did the 2 *L. dissortis*, and these 2 species were weakly paired with each other (65% bootstrap support). The relationship between the 2 populations of *L. rufoscutellatus* and *L. genitalis* remained unresolved. *L. canaliculatus* branched off below the *L. rufoscutellatus* species group (86% bootstrap), and *L. esakii* was the 1st species to diverge within *Limnoporus*. The monophyly of the *rufoscutellatus* group and of *Limnoporus* was strongly supported (100 and 97% bootstrap, respectively).

Parsimony analysis of allozyme data did not resolve relationships satisfactorily, as in a previous analysis of four *Limnoporus* species (Sperling and Spence 1990). When alleles were coded on the basis of simple presence and absence, only the 2 populations of *L. dissortis* were grouped together with a level of bootstrap support >50%. In the strict consensus of 8 equally most parsimonious trees, *L. dissortis* emerged as the sister group of *L. notabilis*, the Alaskan population of *L. rufoscutellatus* diverging below them. Levels of bootstrap support were higher for some nodes when alleles were considered to be present only if they occurred with a frequency of >5%, but the structure of the tree remained as ambiguous as for the simple presence/absence data. All other coding schemes gave a large number of most parsimonious trees and a completely unresolved strict consensus tree.

The phylogeny obtained in the current analysis of morphological data was topologically similar to that of Andersen and Spence (1992). The new tree (Fig. 3) grouped together the 2 populations of *L. rufoscutellatus* with *L. genitalis* at a high level of bootstrap support (96%). In addition, *L. dissortis* branched off below these 3 taxa (92% bootstrap)



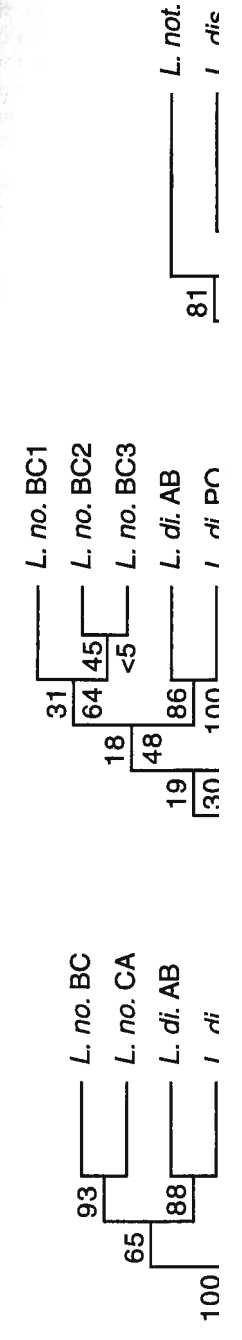

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**Fig. 3.** Most-parsimonious trees obtained from analysis of single equally weighted data types. Each tree represents a strict consensus. Percent support obtained from 500 replicates of a separate bootstrap analysis is indicated above branches. For allozymes, characters are coded by presence/absence of alleles (bootstrap percentage above branches), and also by analysis when alleles were considered present only if frequencies were >5% (bootstrap percentage below branches). tl, Tree length; ci(-u), consistency index excluding uninformative characters; ri, retention index.

and *L. notabilis* is included as the sister taxon of all other *L. rufosuctellatus* group species (81% bootstrap). Relationships among *L. esakii, L. canaliculatus,* and the *L. rufoscutellatus* species group remained unresolved. Andersen and Spence (1992, figure 30), in contrast, arrived at a single preferred arrangement that linked *L. canaliculatus* and *L. esakii* as sister species.

Combined analyses using pairs of data sets (Fig. 4) demonstrated that the resolution of *L. dissortis* as most closely related to *L. rufoscutellatus* rather than *L. notabilis* was caused primarily by the morphology data. In fact, when allozyme and mtDNA data were combined, *L. dissortis* was weakly paired with *L. notabilis* (53% bootstrap). However, support for the sister group relationship between *L. canaliculatus* and the *L. rufoscutellatus* species group came from both mtDNA and allozymes, and all 3 pairwise data combinations gave the same topology at this node.

The tree based on simultaneous parsimony analysis of morphology, allozymes, and mtDNA gave a completely resolved topology (Fig. 4). *L. rufoscutellatus* from Finland was weakly paired with *L. genitalis* (66% bootstrap) and there was 75% bootstrap support for a sister group relationship between *L. dissortis* and *L. rufoscutellatus* + *genitalis*. *L. esakii* was supported strongly as the 1st species to diverge within *Limnoporus* (93% bootstrap).

**Laboratory Hybridization.** Because of large differences in body size between members of the *L. rufoscutellatus* group and either *L. canaliculatus* or *L. esakii,* there are simple mechanical barriers to mating that are unlikely to be overcome in nature. Nonetheless, under no-choice situations in the laboratory, males of the 2 smaller species occasionally couple with small-bodied, virgin females of *L. rufoscutellatus* group species. Such pairings have been observed between *L. esakii* males and *L. dissortis* females and between *L. canaliculatus* males and females of *L. dissortis* and *L. rufoscutellatus*. Two *L. dissortis* females were dissected immediately after the termination of long (>30 min) pairings and both appeared to have sperm in the gynatrial sac. However, no fertile eggs have been laid by any virgin *L. rufoscutellatus* group female held in culture with males of either *L. canaliculatus* or *L. esakii*. Data on crosses between *L. canaliculatus* and *L. esakii* are not available.

All species in the *L. rufoscutellatus* group bred with each other under laboratory conditions, and hybrid offspring have been reared to the adult stage from all crosses attempted to date, except for *L. genitalis* males × *L. dissortis* females (Tables 5 and 6). However, the pattern of hybrid compatibility does not correspond obviously to the phylogeny presented above. In general, the main variation in measures of hybrid compatibility for interspecific crosses is between crosses involving *L. dissortis* and other species.

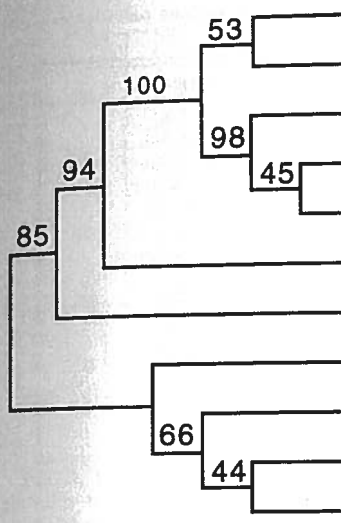
Analysis of variance (ANOVA) of arcsine-transformed data for each compatibility measure, however, did show significant effects of taxonomic status and geographical isolation (Fig. 5; Table 7). Percent fertility in interspecific crosses, though substantial on average (>75%), was significantly less than in crosses involving members of the same species. Furthermore, linear contrast revealed that fertility was lower in crosses between populations than for those made within populations. Percent hatch was reduced dramatically in interspecific crosses on average, but there was no significant difference between inter- and intrapopulation crosses. Overall differences in percent survival were only of marginal significance, but the linear contrasts suggest that survivorship was significantly higher in intraspecific crosses than in those made between species. Although other interspecific crosses show reductions in compatibility, we point out that most of the overall effect comes from crosses involving *L. dissortis*. Note, for example, the high hatch rate and survivorship in the *L. notabilis* × *L. genitalis* crosses (Tables 5 and 6), despite the fact that these species are well separated in the reconstructed phylogeny. Although the above analysis suggests that the overall picture of hybrid compatibility reflects simple expectations based on the hierarchical relationships of populations sampled, the detailed pattern does not map well on the phylogeny.

Most importantly, we emphasize that the pattern of relative success of species pairs in hybridization bears little discernible relationship to the two molecular measures of genetic similarity (Fig. 6). Neither mtDNA distances nor Nei's distances are significantly correlated with variation in percent survival ($r = 0.16$, $P = 0.96$; $r = -0.19$, $P = 0.64$), percent hatch ($r = 0.48$, $P = 0.21$; $r = 0.43$, $P = 0.26$), and percent fertility ($r = -0.48$, $P = 0.20$; $r = -0.44$, $P = 0.25$).

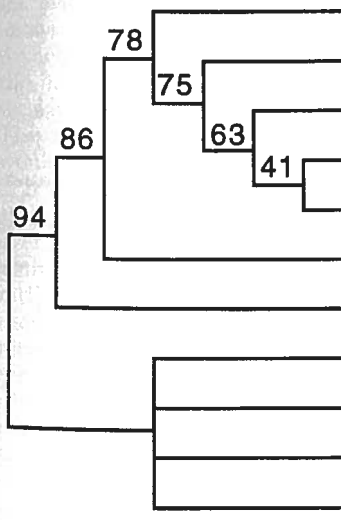
## Discussion

***Limnoporus* Phylogeny.** Despite the potential for introgression between at least 2 species that are not a sister pair, there is reassuring agreement in the phylogenetic information provided by each of the 3 different types of data. The new best-estimate of the phylogeny of *Limnoporus* is shown by the single most parsimonious tree obtained from the combined analysis of mtDNA, allozymes, and morphology (Fig. 4). Genetic distances also clearly grouped together separate samples within each of *L. notabilis, L. dissortis,* and *L. rufoscutellatus* + *L. genitalis,* and demonstrated the cohesiveness of the *rufoscutellatus* species group. Parsimony analysis of mtDNA and genetic distances for allozymes supported a topology in which *L. esakii* branched off before *L. canaliculatus* at the base of the *Limnoporus* phylogeny. Thus, use of data about mtDNA and allozymes altered the phylogeny ob-






**mtDNA + allozymes:**
tl =525, ci (- u.) = .513,




**alloz. + morph.:** 3 mp
tl = 270, ci (- u.) = .477,

**Fig. 4.** Most-parsimonious tree represents a strict consensus. Alloz abbreviations are as for Fig. 3.

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## Discussion

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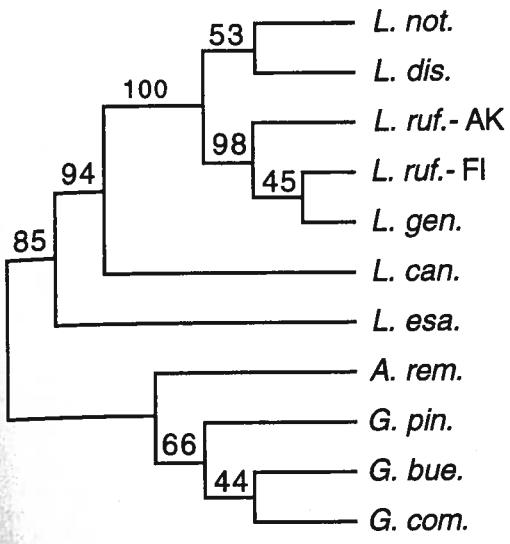
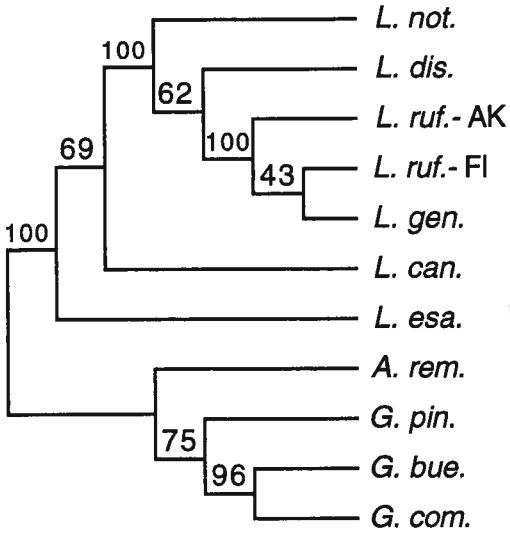
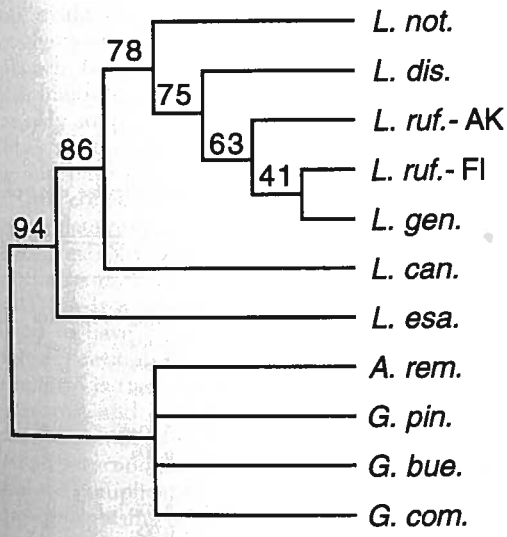


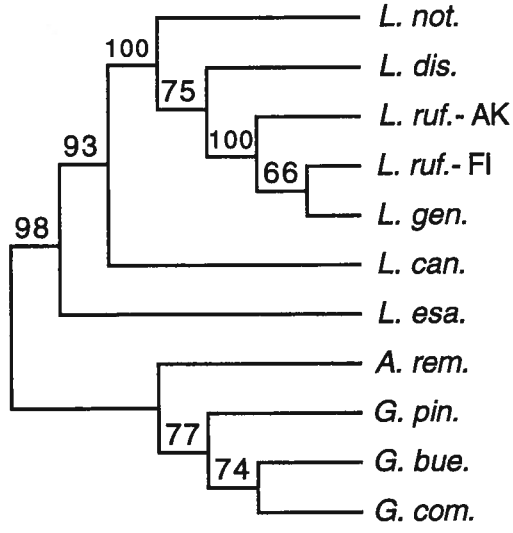

**mtDNA + allozymes:** 1 mp tree,
tl =525, ci (- u.) = .513, ri = .546




**mtDNA + morphology:** 1 mp tree,
tl =415, ci (- u.) = .625, ri = .694




**alloz. + morph.:** 3 mp trees,
tl = 270, ci (- u.) = .477, ri = .534




**mtDNA + alloz. + morph.:** 1 mp tree,
tl =607, ci (- u.) = .540, ri = .593

**Fig. 4.** Most-parsimonious trees obtained from analysis of combinations of unweighted data types. Each tree represents a strict consensus. Allozyme data is treated by simple presence/absence of alleles. Bootstrap percent and abbreviations are as for Fig. 3.

tained from previous analysis of morphology data alone (Andersen and Spence 1992).

Although there were some disagreements in the topologies of the most parsimonious trees from single data types, these were not supported strongly by bootstrap analyses (Fig. 3). For example, both mtDNA and allozymes gave trees in which *L. notabilis* and *L. dissortis* were sister species, but did not strongly support this grouping (65 and 18% bootstrap, respectively). In contrast, the morpho-

**Table 5. Measures of reproductive performance for *L. rufoscutellatus*-group species in various pairwise crosses: Fertility and egg hatch**

| Males | Females | % fertility | *n* | % hatch | *n* |
|---|---|---|---|---|---|
| | | Intraspecific crosses | | | |
| *L. dissortis* (AB) | *L. dissortis* (AB) | 98.2 ± 3.62ab | 10 | 98.1 ± 2.78ab | 10 |
| *L. dissortis* (AB) | *L. dissortis* (QC) | 98.5 ± 5.12ab | 5 | 94.7 ± 3.93ab | 5 |
| *L. dissortis* (QC) | *L. dissortis* (AB) | 99.4 ± 5.12a | 5 | 98.1 ± 3.93abc | 5 |
| *L. notabilis* (BC) | *L. notabilis* (BC) | 97.5 ± 3.62ab | 10 | 98.5 ± 2.78a | 10 |
| *L. notabilis* (CA) | *L. notabilis* (BC) | 87.2 ± 4.68bcde | 6 | 93.0 ± 3.59abc | 6 |
| *L. notabilis* (BC) | *L. notabilis* (CA) | 91.6 ± 4.33abc | 7 | 95.5 ± 3.32ab | 7 |
| | | Interspecific crosses | | | |
| *L. notabilis* | *L. dissortis* | 92.4 ± 5.12abc | 5 | 71.7 ± 3.92d | 5 |
| *L. dissortis* | *L. notabilis* | 68.9 ± 3.62cde | 10 | 53.7 ± 2.78e | 10 |
| *L. notabilis* | *L. genitalis* | 67.5 ± 6.62de | 3 | 91.0 ± 5.07bcd | 3 |
| *L. notabilis* | *L. rufoscutellatus* (FI) | 90.1 ± 4.68abcd | 6 | 77.7 ± 3.59d | 6 |
| *L. rufoscutellatus* (FI) | *L. notabilis* | 87.3 ± 5.12abcd | 5 | 74.5 ± 3.93d | 5 |
| *L. rufoscutellatus* (AK) | *L. notabilis* | 81.8 ± 5.73cde | 4 | 83.6 ± 4.39cd | 4 |
| *L. dissortis* | *L. genitalis* | 59.6 ± 3.62c | 10 | 49.0 ± 2.78e | 10 |
| *L. genitalis* | *L. dissortis* | 23.0 ± 8.10f | 2 | 48.2 ± 6.21e | 2 |
| *L. dissortis* | *L. rufoscutellatus* (FI) | 88.4 ± 5.12abcd | 5 | 55.1 ± 3.93e | 5 |
| *L. rufoscutellatus* (FI) | *L. dissortis* | 82.5 ± 4.05cde | 8 | 53.0 ± 3.11e | 8 |
| *L. rufoscutellatus* (AK) | *L. dissortis* | 86.5 ± 6.62bcde | 3 | 50.0 ± 5.07e | 3 |
| *L. genitalis* | *L. rufoscutellatus* (FI) | 86.3 ± 3.82bcde | 9 | 79.6 ± 2.93d | 9 |
| *L. rufoscutellatus* (FI) | *L. genitalis* | 71.8 ± 5.12cde | 5 | 75.7 ± 3.93d | 5 |
| *L. rufoscutellatus* (AK) | *L. genitalis* | 75.3 ± 8.10cde | 2 | 84.2 ± 6.21cd | 2 |

All entries are given as least square mean ± SE (*n*). Letters following each entry designate groups that did not differ significantly based on Student–Newman–Keuls a posteriori tests on arcsine transformed data ($P \leq 0.05$). Numbers in brackets indicate sample sizes. Two-letter abbreviations indicate geographical origin of cultures used to assess geographical differentiation within species as follows: AB, Alberta; AK, Alaska; BC, British Columbia; CA, California; FI, Finland; QC, Quebec.

**Table 6. Measures of reproductive performance for *L. rufoscutellatus*-group species in various pairwise crosses: Survival and sex ratio of progeny**

| Males | Females | % fertility | *n* | % hatch | *n* |
|---|---|---|---|---|---|
| | | Intraspecific crosses | | | |
| *L. dissortis* (AB) | *L. dissortis* (AB) | 21.3 ± 4.21ab | 4 | 48.1 ± 1.40a | 10 |
| *L. dissortis* (AB) | *L. dissortis* (QC) | — | | 47.0 ± 3.50a | 3 |
| *L. dissortis* (QC) | *L. dissortis* (AB) | — | | 45.5 ± 1.50a | 2 |
| *L. notabilis* (BC) | *L. notabilis* (BC) | 28.2 ± 3.76a | 5 | 49.2 ± 1.80a | 10 |
| *L. notabilis* (CA) | *L. notabilis* (CA) | 28.8 ± 4.21a | 4 | | |
| *L. notabilis* (CA) | *L. notabilis* (BC) | 28.8 ± 3.76a | 5 | 43.6 ± 5.50a | 5 |
| *L. notabilis* (BC) | *L. notabilis* (CA) | 33.4 ± 3.76a | 5 | 40.0 ± 6.00a | 5 |
| | | Interspecific crosses | | | |
| *L. notabilis* | *L. dissortis* | 14.2 ± 3.76ab | 5 | 89.5 ± 3.70b | 9 |
| *L. dissortis* | *L. notabilis* | 13.5 ± 2.53ab | 11 | 100 ± 0.00c | 8 |
| *L. notabilis* | *L. genitalis* | 32.8 ± 3.76a | 5 | 43.4 ± 5.60a | 5 |
| *L. notabilis* | *L. rufoscutellatus* (FI) | 20.4 ± 2.81ab | 9 | 48.2 ± 3.40a | 10 |
| *L. rufoscutellatus* (FI) | *L. notabilis* | 20.8 ± 3.76ab | 5 | 51.7 ± 3.00a | 7 |
| *L. rufoscutellatus* (AK) | *L. notabilis* | 27.2 ± 4.21ab | 4 | 55.8 ± 6.30a | 4 |
| *L. dissortis* | *L. genitalis* | 22.1 ± 3.44ab | 6 | 100 ± 0.00c | 6 |
| *L. genitalis* | *L. dissortis* | 0[a] | | — | |
| *L. dissortis* | *L. rufoscutellatus* (FI) | 16.8 ± 3.76ab | 5 | 100 ± 0.00c | 9 |
| *L. rufoscutellatus* (FI) | *L. dissortis* | 21.8 ± 3.18ab | 7 | 97.2 ± 1.80c | 6 |
| *L. rufoscutellatus* (AK) | *L. dissortis* | 18.0 ± 4.86ab | 3 | 100 ± 0.00c | 3 |
| *L. genitalis* | *L. rufoscutellatus* (FI) | 12.0 ± 3.76ab | 5 | 58.3 ± 3.50a | 6 |
| *L. rufoscutellatus* (FI) | *L. genitalis* | 17.6 ± 4.21ab | 4 | 51.5 ± 4.70a | 4 |
| *L. rufoscutellatus* (AK) | *L. genitalis* | 2.0 | 1 | 100[b] | |

Symbols and arrangement of entries are the same as in Table 5.
[a] No bugs survived to adult stage in either of 2 survivorship trials.
[b] Two males only reared in 1 standard survival cross; not included in ANOVA.



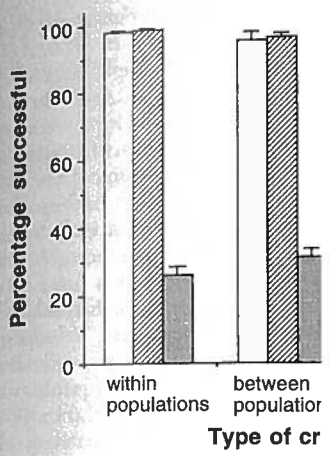



**Fig. 5.** Correspondence of 3 mea patibility to type of cross, with stan above histogram.

logical data strongly supported ( alternative topology. In combin phological characters overrode t to give a sister group relationshi *sortis* and *L. rufoscutellatus* + *L* This example illustrates the adv tal evidence rather than cons analyses of different data types. proach, *L. notabilis* + *L. dissorti* the majority arrangement even weakly supported by both mtD The strong contribution from would have been discounted.

It is conceivable that some am zyme data may be the result of ir ever, mtDNA gave a similarly a at the base of the *L. rufoscutella* mtDNA is a single locus that is ered to have clonal inheritance nity for reticulation (Avise 1991) scenerio is that the divergences *dissortis*, and *L. rufoscutellatus* curred simultaneously or nearly small internodal genetic distanc tial for complications because of polymorphisms in allozyme loci.

Interplay of the 3 data sets a teresting hypothesis about relat *foscutellatus* and *L. genitalis*. Th cific genetic distances for ei

**Table 7. Results of ANOVA for species and population effects**

| Variable | Overall |
|---|---|
| % fertility | $F = 6.6$; df = 2 |
| % hatch | $F = 17.2$; df = 2 |
| % survival | $F = 3.1$; df = 2 |

cies in various pairwise crosses:

| % hatch | n |
|---|---|
| 98.1 ± 2.78ab | 10 |
| 94.7 ± 3.93ab | 5 |
| 98.1 ± 3.93abc | 5 |
| 98.5 ± 2.78a | 10 |
| 93.0 ± 3.59abc | 6 |
| 95.5 ± 3.32ab | 7 |
| 71.7 ± 3.92d | 5 |
| 53.7 ± 2.78e | 10 |
| 91.0 ± 5.07bcd | 3 |
| 77.7 ± 3.59d | 6 |
| 74.5 ± 3.93d | 5 |
| 83.6 ± 4.39cd | 4 |
| 49.0 ± 2.78e | 10 |
| 48.2 ± 6.21e | 2 |
| 55.1 ± 3.93e | 5 |
| 53.0 ± 3.11e | 8 |
| 50.0 ± 5.07e | 3 |
| 79.6 ± 2.93d | 9 |
| 75.7 ± 3.93d | 5 |
| 84.2 ± 6.21cd | 2 |

e groups that did not differ significantly ). Numbers in brackets indicate sample aphical differentiation within species as ebec.

species in various pairwise crosses:

| | % hatch | n |
|---|---|---|
| | 48.1 ± 1.40a | 10 |
| | 47.0 ± 3.50a | 3 |
| | 45.5 ± 1.50a | 2 |
| 5 | 49.2 ± 1.80a | 10 |
| 4 | | |
| 5 | 43.6 ± 5.50a | 5 |
| 5 | 40.0 ± 6.00a | 5 |
| 5 | 89.5 ± 3.70b | 9 |
| 1 | 100 ± 0.00c | 8 |
| 5 | 43.4 ± 5.60a | 5 |
| 9 | 48.2 ± 3.40a | 10 |
| 5 | 51.7 ± 3.00a | 7 |
| 4 | 55.8 ± 6.30a | 4 |
| 6 | 100 ± 0.00c | 6 |
| 5 | 100 ± 0.00c | 9 |
| 7 | 97.2 ± 1.80c | 6 |
| 3 | 100 ± 0.00c | 3 |
| 5 | 58.3 ± 3.50a | 6 |
| 4 | 51.5 ± 4.70a | 4 |
| 1 | 100[b] | |



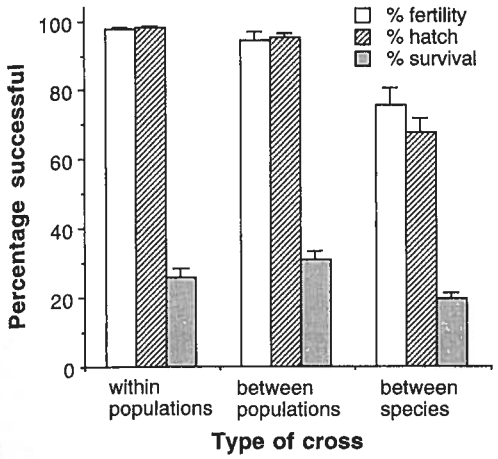
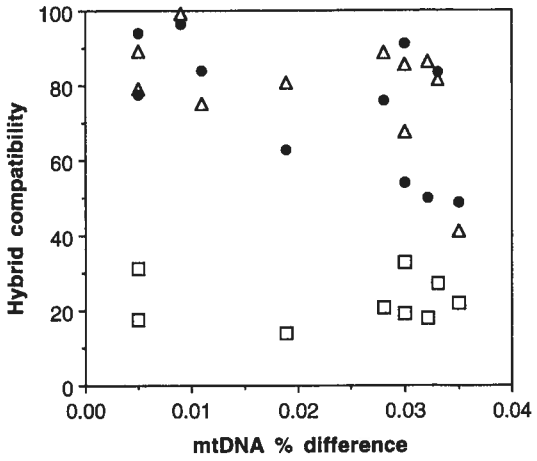



**Fig. 5.** Correspondence of 3 measures of hybrid compatibility to type of cross, with standard error indicated above histogram.

logical data strongly supported (92% bootstrap) an alternative topology. In combined analyses, morphological characters overrode the molecular data to give a sister group relationship between *L. dissortis* and *L. rufoscutellatus* + *L. genitalis* (Fig. 4). This example illustrates the advantage of using total evidence rather than consensus of separate analyses of different data types. In a consensus approach, *L. notabilis* + *L. dissortis* would have been the majority arrangement even though it was only weakly supported by both mtDNA and allozymes. The strong contribution from morphology data would have been discounted.

It is conceivable that some ambiguity in the allozyme data may be the result of introgression. However, mtDNA gave a similarly ambiguous pattern at the base of the *L. rufoscutellatus* group, and yet mtDNA is a single locus that is generally considered to have clonal inheritance with no opportunity for reticulation (Avise 1991). A more plausible scenerio is that the divergences of *L. notabilis*, *L. dissortis*, and *L. rufoscutellatus* + *L. genitalis* occurred simultaneously or nearly so, leading to very small internodal genetic distances and the potential for complications because of retained ancestral polymorphisms in allozyme loci.

Interplay of the 3 data sets also leads to an interesting hypothesis about relationships of *L. rufoscutellatus* and *L. genitalis*. The lowest interspecific genetic distances for either mtDNA or



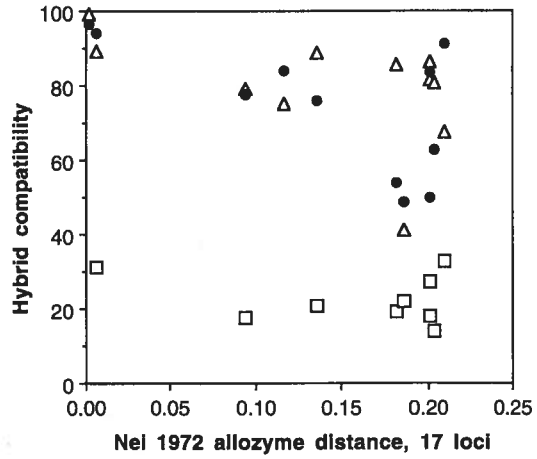




**Fig. 6.** Correspondence between hybrid compatibility and mtDNA divergences or allozyme divergences. Triangles, percent fertility; dots, percent hatch; squares, percent survival.

allozyme data occur among the 2 *L. rufoscutellatus* populations and *L. genitalis* (Table 4). Our parsimony analyses for these single data sets generated trees compatible with these distances for mtDNA but not for allozymes. In contrast, the morphological data clearly set *L. genitalis* apart from the 2 *L. rufoscutellatus* populations by virtue of 3 autapomorphies (Andersen and Spence 1992; Table 3). In addition, percent survival of hybrids between *L. genitalis* and *L. rufoscutellatus* was low. Perhaps

**Table 7. Results of ANOVA for 3 measures of hybrid compatibility and the linear contrasts designed to reveal species and population effects**

| Variable | Overall analysis | Linear contrasts | |
|---|---|---|---|
| | | Species vs Population | Within vs Between populations |
| % fertility | $F = 6.6$; df = 2, 17; $P = 0.008$ | $F = 12.9$; $P = 0.002$ | $F = 8.2$; $P = 0.01$ |
| % hatch | $F = 17.2$; df = 2, 17; $P < 0.001$ | $F = 33.4$; $P < 0.001$ | $F = 0.5$; $P = 0.50$ |
| % survival | $F = 3.1$; df = 2, 15; $P = 0.08$ | $F = 5.4$; $P = 0.03$ | $F = 0.02$; $P = 0.89$ |

isolation of *L. genitalis* on Hokkaido, the Kurile Islands, and the southern part of Sakhalin (Andersen and Spence 1992, figure 31) has been associated with rapid morphological and behavioral divergence of these populations, although divergence is not reflected in the molecular data considered in our analysis. Such peripheral speciation of *L. genitalis* may have left *L. rufoscutellatus* as a paraphyletic species. However, all 3 character sets agree that the extreme eastern and western populations of *L. rufoscutellatus* have diverged very little from each other. Further resolution will require genetic analysis of more samples of *L. rufoscutellatus* from Asia.

**Calibration of Genetic Distances with Evolutionary Time.** There are few insect taxa for which data are available about both allozyme and DNA divergences (e.g., Futuyma and McCafferty 1990, Sperling 1993, Sperling and Hickey 1994, Funk et al. 1995, Harrison and Bogdanowicz 1995, Sperling et al. 1995), and few that can also be coupled with direct information from insect fossils to calibrate genetic divergences with real time (Brower 1994). Fortunately, 2 fossils are known for *Limnoporus* from the middle Eocene (Andersen et al. 1993), and these indicate that the divergence of both *L. canaliculatus* and *L. esakii* from the remainder of *Limnoporus* occurred at least 50 million years ago. At least the mtDNA data remained phylogenetically informative at this level, as evidenced by high bootstrap values at the base of the *Limnoporus* phylogeny (Figs. 3 and 4).

Using a commonly employed molecular clock (Thorpe 1982, Nei 1987, but see Thorpe and Solé-Cava 1994), a value of 1.0 for Nei's standard D indicates a divergence time of 15–20 million years. Thus, the value of 0.50–0.78 for the divergence between *L. canaliculatus* and the *rufoscutellatus* group estimates a divergence time of 7.5–15.6 million years. The same logic has been applied to mtDNA sequences, where a 1% sequence divergence per lineage has been suggested to correspond to a divergence time of 1–2 million years (Avise 1991, Brower 1994). *L. canaliculatus* and *L. esakii* have uncorrected divergences of 8.2–9.5% from the *rufoscutellatus* group, suggesting a divergence time of ≈4–9 million years. When the divergence is estimated using the Kimura 2-parameter correction, a divergence time of ≈5–11 million years is suggested. These values are a fraction of the minimal time established by fossils. Thus, at least for *Limnoporus,* evolution in both proteins and mtDNA appears to be 5–10 times slower than generally expected for arthropods.

**Ecological and Developmental Implications of Phylogeny.** The morphology-based phylogeny of Andersen and Spence (1992) was used to interpret ecological and developmental patterns in *Limnoporus* (Andersen 1993a, 1994; Klingenberg and Spence 1993). Because the phylogeny presented here differs from the one based only on morphology, some reinterpretation of previously proposed ecological and developmental scenarios is appropriate.

Andersen (1993a) examined the evolution of wing polymorphism in *Limnoporus, Aquarius,* and *Gerris* and concluded that several traits were synapomorphies of *L. esakii* and the *rufoscutellatus* group. These traits included monomorphic long-winged adults (both diapausing and nondiapausing generations) and nymphs. Andersen and Spence (1992) illustrated the relationship among *L. canaliculatus, L. esakii,* and the *L. rufscutellatus* group as a trichotomy, leaving open the possibility that the common ancestor of *Limnoporus* (including *L. canaliculatus*) had dimorphic nymphs and short-winged or dimorphic adults. The unambiguous placement of *L. esakii* at the base of *Limnoporus* in the current analysis suggests alternatively that dimorphism was derived in *L. canaliculatus* independently of its occurrence in *Aquarius* and *Gerris.* Both hypotheses require 2 transitions between dimorphism and obligatory long-wingedness in *Limnoporus.* However, we favor the hypothesis that suggests independent loss of the short-winged adult form in *L. esakii* and the *L. rufoscutellatus* group species as paralleled in some *Aquarius* and *Gerris* species (Andersen 1993a, b). We hold in accordance with Dollo's rule about evolution of complex structures that the short-winged adult form is more likely to be lost than to reappear using the same developmental pathway.

Andersen (1994) also examined the evolution of sexual size dimorphism in *Limnoporus, Aquarius,* and *Gerris.* He concluded that this was a complex trait associated with a range of mating systems, and it is not simply a nonadaptive outcome of allometric growth. *L. esakii* and *L. canaliculatus* were shown as a group that shared an unusually high ratio of female to male size. In general, however, Andersen (1994) found that size dimorphism did not show phylogenetic effects. This conclusion is supported in the phylogeny obtained using combined evidence, so the separation of *L. esakii* and *L. canaliculatus* as separate branches off the main stem leading to the *rufoscutellatus* group decreases still further the correspondence between monophyletic groups and shared possession of unusual size ratios.

Finally, Klingenberg and Spence (1993) studied heterochrony and allometry in *Limnoporus* by examining rates of increase of various size measures in immatures and adults. They found a remarkable variety of heterochronic changes among the 6 species, indicating that current models could be related to the observed patterns only if combinations of several processes were assumed. Furthermore, local trends within species groups were at least as important as global trends in large clades. The phylogeny they used to interpret their results linked *L. esakii* and *L. canaliculatus* as a species group, as it was the topology preferred by Andersen and Spence (1992). However, in the arrangement supported by combined evidence, any traits that



linked *L. esakii* and *L. canali* guished them from the *L. rufosc* more confidently interpreted This gives more information ab evolutionary change of traits fo *rufoscutellatus* group. For exam eral transposition of ontogeneti in *L. esakii* and *L. canaliculatus* ancestral characteristic, and it that value in the *rufoscutellatus* sents an evolutionary change.

**Hybrid Incompatibilities.** I reasonable to expect hybrid inc crease as species diverge. Gene tion is supported in *Drosophil* 1989). Hybrid incompatibility l to reconstruct the phylogeny of Ae 1979). However, although in viously usually greater betwee species than in intrapopulation less prior reason to expect hyb between any particular species a gradual manner with interm tances. Indeed, if a few genes fects on incompatibility, then i patibility could be relatively dis

In the *L. rufoscutellatus* gro measures of hybrid incompatibi ity, hatch, and survival) were sig ed with either molecular meas tance between species. Althou significant, the overall pattern gests that any subtle effects ar early developmental traits of fe development than for the post juvenile survival. Once a young successfully, chances of furthe opment seem to vary with little ship to parentage. Hybrid in even be the result of relatively f major gene causing death befc male hybrids that are the result *dissortis.* Because no other crc *rufoscutellatus* group show this hypothesize that this trait is a r within *L. dissortis.*

Hybrid compatibility is there dicator of genealogical relation *rus,* because it is correlated so distance. This result is not par in a phylogenetic context; there son for an autapomorphy relatec to arise if species evolve in iso velopment of new incompatibi previously evolved incompatibil

**Are Some Characters Be Markers Than Others?** The as viability of crosses involving *L.* that variation in mitochondrial a markers should have a better species boundaries than shoulc This is because there is dispro

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lysis suggests alternatively that
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/ever, we favor the hypothesis
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sed to interpret their results linked
*L. canaliculatus* as a species group,
topology preferred by Andersen and
). However, in the arrangement sup-
ombined evidence, any traits that

linked *L. esakii* and *L. canaliculatus* and distinguished them from the *L. rufoscutellatus* group are more confidently interpreted as ancestral traits. This gives more information about the direction of evolutionary change of traits found only in the *L. rufoscutellatus* group. For example, the higher lateral transposition of ontogenetic trajectories found in *L. esakii* and *L. canaliculatus* is more clearly an ancestral characteristic, and it is the reduction of that value in the *rufoscutellatus* group that represents an evolutionary change.

**Hybrid Incompatibilities.** It seems intuitively reasonable to expect hybrid incompatibility to increase as species diverge. Generally, this expectation is supported in *Drosophila* (Coyne and Orr 1989). Hybrid incompatibility has also been used to reconstruct the phylogeny of some insects (e.g., Ae 1979). However, although incompatibility is obviously usually greater between distantly related species than in intrapopulation matings, there is less prior reason to expect hybrid incompatibility between any particular species pair to increase in a gradual manner with intermediate genetic distances. Indeed, if a few genes can have large effects on incompatibility, then increases in incompatibility could be relatively discontinuous.

In the *L. rufoscutellatus* group, none of the 3 measures of hybrid incompatibility (percent fertility, hatch, and survival) were significantly correlated with either molecular measure of genetic distance between species. Although not statistically significant, the overall pattern of correlation suggests that any subtle effects are stronger for the early developmental traits of fertilization and egg development than for the postembryonic trait of juvenile survival. Once a young gerrid has hatched successfully, chances of further successful development seem to vary with little consistent relationship to parentage. Hybrid incompatibility may even be the result of relatively few genes, with one major gene causing death before hatching of female hybrids that are the result of crosses with *L. dissortis.* Because no other crosses within the *L. rufoscutellatus* group show this dramatic effect, we hypothesize that this trait is a new autapomorphy within *L. dissortis.*

Hybrid compatibility is therefore not a good indicator of genealogical relationships in *Limnoporus,* because it is correlated so poorly with genetic distance. This result is not particularly surprising in a phylogenetic context; there may be little reason for an autapomorphy related to incompatibility to arise if species evolve in isolation, and the development of new incompatibilities may obscure previously evolved incompatibilities.

**Are Some Characters Better Phylogenetic Markers Than Others?** The asymetric hybrid inviability of crosses involving *L. dissortis* suggests that variation in mitochondrial and X chromosome markers should have a better correspondence to species boundaries than should autosomal genes. This is because there is disproportionate elimination of females among $F_1$ hybrids. Females carry 2 copies of the X chromosomes, whereas males carry only 1 copy plus a Y chromosome (Spence and Maddison 1986). Assuming an equal sex ratio, females are the repository of ⅔ of the X chromosomes in the total gene pool, whereas they contain only ½ of the autosomal genes. Thus, elimination of females will disproportionately remove X chromosomes from hybrid interactions.

An allozyme survey using both X-linked and autosomal loci already has been performed in a hybrid zone between *L. notabilis* and *L. dissortis* (Sperling and Spence 1991). One of four loci surveyed was X-linked. It had fewer heterozygotes and a better correspondence to a diagnostic morphological character than did the 3 autosomal genes surveyed. Thus, empirical evidence indicates that X-linked loci in *L. dissortis* and related species do indeed make better markers of species boundaries than do autosomal genes.

However, judging from the pattern found for alleles of G-6-pdh across *Limnoporus,* this X-linked marker may not provide a particularly good indicator of species phylogenies in deeper divergences. The primary alleles that distinguish *L. notabilis* from *L. dissortis* are both present in *L. rufoscutellatus,* and there is a mixed pattern of possession of these allelic electromorphs in related species. For example, the allele that is predominant in *L. notabilis* is apparently the only allele present in the Alaskan population of *L. rufoscutellatus* and in *L. canaliculatus* (Sperling and Spence 1990). Also, the allele that is predominant in *L. dissortis* is also shared by *L. genitalis.* The opportunity now exists to survey sequence data directly in G-6-pdh (Soto-Adames et al. 1994), and this could give more detailed information on the homologies of alleles at this locus.

As females are the carriers of mtDNA, this marker also should be eliminated disproportionately in hybrid interactions involving *L. dissortis.* We have not extensively surveyed mtDNA variation in the hybrid zones between *L. notabilis* and *L. dissortis.* However, the fact that nearly identical mtDNA sequences were sampled from individuals collected at opposite ends of the range of these 2 species suggests that mtDNA sequence variation within these species may generally be very small relative to differences between the species. The correspondence between mtDNA and nuclear genes remains to be investigated.

In total, mtDNA, allozymes and morphology gave complementary information on phylogenetic relationships within *Limnoporus.* In particular, mtDNA sequences gave abundant, easily resolved characters that largely support previous studies of morphology. Furthermore, as discussed above, mtDNA should provide a particularly useful marker for species boundaries involving *L. dissortis.* However, mtDNA comprises only a single locus that has an unusual mode of inheritance, and hence might have a different phylogeny from the

modal gene phylogeny of the species. In contrast, allozymes represent numerous loci and therefore should give a better estimate of the species phylogeny. However, they provided more useful information as genetic distances than they did as character data. One major reason is that there was a substantial amount of shared polymorphism among allozyme loci and it was difficult to code this effectively for phylogenetic analysis.

Finally, this study clearly illustrates advantages of basing phylogenetic reconstruction on simultaneous analysis of several kinds of data. Morphological data are the traditional basis of phylogenetic reconstruction and are already available for many groups. However, there have been concerns that they could be swamped in combined analyses with large molecular data sets. In our combined analyses, morphological characters dominated the more ambiguous patterns in mtDNA and allozymes, despite the smaller number of characters in the morphological data. This has been noted in other studies (e.g., Chavarria and Carpenter 1994, de Quieroz et al. 1995), and this pattern of empirical results should reduce a priori resistance to combined analyses. For *Limnoporus*, each of the 3 data types contributed in substantive ways to the resolution at different nodes and their combined analysis gave the best resolved phylogeny.

## Acknowledgments

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## References Cited

**Ae, S. A. 1979.** The phylogeny of some *Papilio* species based on interspecific hybridization data. Syst. Entomol. 4: 1–16.

**Andersen, N. M. 1993a.** The evolution of wing polymorphism in water striders (Gerridae): A phylogenetic approach. Oikos 67: 433–443.

**1993b.** Classification, phylogeny, and zoogeography of the pond skater genus *Gerris* Fabricius (Hemiptera: Gerridae). Can. J. Zool. 71: 2473–2508.

**1994.** The evolution of sexual size dimorphism and mating systems in water striders (Hemiptera: Gerridae): a phylogenetic approach. Ecoscience 1: 208–214.

**Andersen, N. M., and J. R. Spence. 1992.** Classification and phylogeny of the Holarctic water strider genus *Limnoporus* Stål (Hemiptera: Gerridae). Can. J. Zool. 70: 753–785.

**Andersen, N. M., J. R. Spence, and M.V.H. Wilson. 1993.** 50 million years of structural stasis in water striders (Hemiptera: Gerridae). Am. Entomol. 39: 174–176.

**Avise, J. C. 1991.** Ten unorthodox perspectives on evolution prompted by comparative population genetic findings on mitochondrial DNA. Annu. Rev. Genet. 25: 45–69.

**Brooks, D. R., and D. A. McLennan. 1991.** Phylogeny, ecology, and behavior. A research program in comparative biology. University of Chicago Press, Chicago.

**Brower, A.V.Z. 1994.** Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. Proc. Natl. Acad. Sci. U.S.A. 91: 6491–6495.

**Carpenter, J. M., J. E. Strassmann, S. Turillazzi, C. R. Hughes, C. R. Solis, and R. Cervo. 1993.** Phylogenetic relationships among paper wasp social parasites and their hosts (Hymenoptera: Vespidae; Polistinae). Cladistics 9: 129–146.

**Chavarria, G., and J. M. Carpenter. 1994.** "Total evidence" and the evolution of highly social bees. Cladistics 10: 229–258.

**Coyne, J. A., and H. A. Orr. 1989.** Patterns of speciation in *Drosophila*. Evolution 43: 362–381.

**Fairbairn, D. J. 1984.** Microgeographic variation in body size and development time in the waterstrider, *Limnoporus notabilis*. Oecologia (Berl.) 61: 126–133.

**Felsenstein, J. 1993.** PHYLIP (phylogeny inference package) version 3.5c. Department of Genetics, University of Washington, Seattle.

**Funk, D. J., D. J. Futuyma, G. Ortí, and A. Meyer. 1995.** Mitochondrial DNA sequences and multiple data sets: a phylogenetic study of phytophagous beetles (Chrysomelidae: *Ophraella*). Mol. Biol. Evol. 12: 627–640.

**Futuyma, D. J., and S. McCafferty. 1990.** Phylogeny and the evolution of host plant associations in the leaf beetle genus *Ophraella* (Coleoptera: Chrysomelidae). Evolution 44: 1885–1913.

**Gagnon, J., J. M. Roth, W. F. Finzer, K. A. Haycock, D. S. Feldman, Jr., R. Hoffman, and J. Simpson. 1989.** SuperANOVA, accessible general linear modeling. Abacus Concepts, Berkeley, CA.

**Harrison, R. G., and S. M. Bogdanowicz. 1995.** Mitochondrial DNA phylogeny of North American field crickets: perspectives on the evolution of life cycles, songs, and habitat associations. J. Evol. Biol. 8: 209–232.

**Harvey, P. H., and M. D. Pagel. 1991.** The comparative method in evolutionary biology. Oxford University Press, Oxford.

**Hoelzer, G. A., and D. J. Melnick. 1994.** Patterns of speciation and limits to phylogenetic resolution. Trends Ecol. Evol. 9: 104–107.

**Jukes, T. H., and C. R. Cantor. 1969.** Evolution of protein molecules, pp. 21–132. *In* H. N. Munro [ed.], Mammalian protein metabolism. Academic, New York.

**Kelton, L. A. 1961.** A new species of *Gerris* F. from Yukon and Alaska (Hemiptera: Gerridae). Can. Entomol. 93: 663–665.

**Kimura, M. 1980.** A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16: 111–120.

**Klingenberg, C. P., and J. R. Spence. 1993.** Heterochrony and allometry: lessons from the water strider genus *Limnoporus*. Evolution 47: 1834–1853.

**Kluge, A. G. 1989.** A concern for evidence and a phylogenetic hypothesis of relationships among *Epicrates* (Boidae: Serpentes). Syst. Zool. 38: 7–25.



**Mickevich, M. F., and C. Mitter.** morphic characters in systema treatment of electrophoretic dat Funk and D. R. Brooks [eds.], A vol. 1. New York Botanical Garc

**1983.** Evolutionary patterns in tematic approach, pp. 169–176. V. A. Funk [eds.], Advances in lumbia University Press, New Yo

**Mickevich, M. F., and S. Weller.** character analysis: tracing cha cladogram. Cladistics 6: 137–171

**Nei, M. 1972.** Genetic distance Am. Nat. 106: 283–292.

**1987.** Molecular evolutionary ger versity Press, New York.

**Pamilo, P., and M. Nei. 1988.** gene trees and species trees. 568–583.

**de Quieroz, A., M. J. Donoghue,** Separate versus combined analys idence. Annu. Rev. Ecol. Syst. 2

**Simon, C., F. Frati, A. Beckenb Liu, and P. Flook. 1994.** Evol phylogenetic utility of mitochon and a compilation of conserved Entomol. Soc. Am. 87: 1–51.

**Soto-Adames, F. N., H. M. Robert locher. 1994.** Phylogenetic u sequences of *C6pdh* at differen Hexapoda with emphasis on Di Soc. Am. 87: 723–736.

**Spence, J. R. 1983.** Pattern an tence of water-striders (Heteropt imal Ecol. 52: 497–512.

**1989.** The habitat templet and lif pond skaters (Heteroptera: Ge potential, phenology, and wing Zool. 67: 2432–2447.

**1990.** Introgressive hybridization example of *Limnoporus* Stål ( western Canada. Can. J. Zool. 6

**Spence, J. R., and N. M. Anderse** water striders: interactions betw ecology. Annu. Rev. Entomol. 3

**Spence, J. R., and D. R. Maddis** somes of two hybridizing species eroptera: Gerridae). Proc. Ento 502–508.

ondrial DNA. Annu. Rev. Genet.

**D. A. McLennan. 1991.** Phylog-
behavior. A research program in
y. University of Chicago Press, Chi-

**)4.** Rapid morphological radiation
among races of the butterfly *Heli-*
red from patterns of mitochondrial
Proc. Natl. Acad. Sci. U.S.A. 91:

**E. Strassmann, S. Turillazzi, C. R.**
**)lis, and R. Cervo. 1993.** Phyloge-
among paper wasp social parasites and
:noptera: Vespidae; Polistinae). Cladis-

**J. M. Carpenter. 1994.** "Total ev-
evolution of highly social bees. Cla-
58.

**H. A. Orr. 1989.** Patterns of spe-
*ohila*. Evolution 43: 362–381.

**1984.** Microgeographic variation in
evelopment time in the waterstrider,
*abilis*. Oecologia (Berl.) 61: 126–133.

**)93.** PHYLIP (phylogeny inference
n 3.5c. Department of Genetics, Uni-
ington, Seattle.

**. Futuyma, G. Ortí, and A. Meyer.**
ondrial DNA sequences and multiple
/logenetic study of phytophagous bee-
lidae: *Ophraella*). Mol. Biol. Evol. 12:

**and S. McCafferty. 1990.** Phylogeny
on of host plant associations in the leaf
*)phraella* (Coleoptera: Chrysomelidae).
1885–1913.

**. Roth, W. F. Finzer, K. A. Haycock,**
**ın, Jr., R. Hoffman, and J. Simpson.**
ANOVA, accessible general linear mod-
Concepts, Berkeley, CA.

**, and S. M. Bogdanowicz. 1995.** Mi-
)NA phylogeny of North American field
pectives on the evolution of life cycles,
ıbitat associations. J. Evol. Biol. 8: 209–

**and M. D. Pagel. 1991.** The compar-
in evolutionary biology. Oxford Univer-
xford.

**, and D. J. Melnick. 1994.** Patterns of
ınd limits to phylogenetic resolution.
. Evol. 9: 104–107.

**and C. R. Cantor. 1969.** Evolution of
ecules, pp. 21–132. *In* H. N. Munro [ed.],
protein metabolism. Academic, New

**1961.** A new species of *Gerris* F. from
Alaska (Hemiptera: Gerridae). Can. En-
363–665.

**980.** A simple method for estimating evo-
ıtes of base substitutions through compar-
es of nucleotide sequences. J. Mol. Evol
0.

**C. P., and J. R. Spence. 1993.** Heter-
ıd allometry: lessons from the water strider
*noporus*. Evolution 47: 1834–1853.

**1989.** A concern for evidence and a phy-
ıypothesis of relationships among *Epicrates*
ierpentes). Syst. Zool. 38: 7–25.



**Mickevich, M. F., and C. Mitter. 1981.** Treating polymorphic characters in systematics: a phylogenetic treatment of electrophoretic data, pp. 45–60. *In* V. A. Funk and D. R. Brooks [eds.], Advances in cladistics, vol. 1. New York Botanical Garden, New York.

**1983.** Evolutionary patterns in allozyme data: a systematic approach, pp. 169–176. *In* N. I. Platnick and V. A. Funk [eds.], Advances in cladistics, vol. 2. Columbia University Press, New York.

**Mickevich, M. F., and S. Weller. 1990.** Evolutionary character analysis: tracing character change on a cladogram. Cladistics 6: 137–171.

**Nei, M. 1972.** Genetic distance between populations. Am. Nat. 106: 283–292.

**1987.** Molecular evolutionary genetics. Columbia University Press, New York.

**Pamilo, P., and M. Nei. 1988.** Relationships between gene trees and species trees. Mol. Biol. Evol. 5: 568–583.

**de Quieroz, A., M. J. Donoghue, and J. Kim. 1995.** Separate versus combined analysis of phylogenetic evidence. Annu. Rev. Ecol. Syst. 26: 657–681.

**Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu, and P. Flook. 1994.** Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved PCR primers. Ann. Entomol. Soc. Am. 87: 1–51.

**Soto-Adames, F. N., H. M. Robertson, and S. H. Berlocher. 1994.** Phylogenetic utility of partial DNA sequences of *G6pdh* at different taxonomic levels in Hexapoda with emphasis on Diptera. Ann. Entomol. Soc. Am. 87: 723–736.

**Spence, J. R. 1983.** Pattern and process in co-existence of water-striders (Heteroptera: Gerridae). J. Animal Ecol. 52: 497–512.

**1989.** The habitat templet and life history strategies of pond skaters (Heteroptera: Gerridae): reproductive potential, phenology, and wing dimorphism. Can. J. Zool. 67: 2432–2447.

**1990.** Introgressive hybridization in Heteroptera: the example of *Limnoporus* Stål (Gerridae) species in western Canada. Can. J. Zool. 68: 1770–1782.

**Spence, J. R., and N. M. Andersen. 1994.** Biology of water striders: interactions between systematics and ecology. Annu. Rev. Entomol. 39: 97–124.

**Spence, J. R., and D. R. Maddison. 1986.** Chromosomes of two hybridizing species of *Limnoporus* (Heteroptera: Gerridae). Proc. Entomol. Soc. Wash. 88: 502–508.

**Spence, J. R., and R. S. Wilcox. 1986.** The mating system of two hybridizing species of water striders (Gerridae). II. Alternative tactics of males and females. Behav. Ecol. Sociobiol. 19: 87–95.

**Sperling, F.A.H. 1993.** Mitochondrial DNA variation and Haldane's rule in the *Papilio glaucus* and *P. troilus* species groups. Heredity 70: 227–233.

**Sperling, F.A.H., and D. A. Hickey. 1994.** Mitochondrial DNA sequence variation in the spruce budworm species complex (*Choristoneura*: Lepidoptera). Mol. Biol. Evol. 11: 656–665.

**Sperling, F.A.H., and J. R. Spence. 1990.** Allozyme survey and relationships of *Limnoporus* Stål species (Heteroptera: Gerridae). Can. Entomol. 122: 29–42.

**1991.** Structure of an asymmetric hybrid zone between two water strider species (Hemiptera: Gerridae: *Limnoporus*). Evolution 45: 1370–1383.

**Sperling, F.A.H., J.-F. Landry, and D. A. Hickey. 1995.** DNA-based identification of introduced ermine moth species in North America (Lepidoptera: Yponomeutidae). Ann. Entomol. Soc. Am. 88: 155–162.

**Swofford, D. L. 1991.** When are phylogeny estimates from molecular and morphological data incongruent?, pp. 295–333. *In* M. M. Miyamoto and J. Cracraft [eds.], Phylogenetic analysis of DNA sequences. Oxford University Press, Oxford.

**1993.** PAUP: phylogenetic analysis using parsimony, version 3.1.1. Illinois Natural History Survey, Champaign.

**Thorpe, J. P. 1982.** The molecular clock hypothesis: biochemical evolution, genetic differentiation and systematics. Annu. Rev. Ecol. Syst. 13: 139–168.

**Thorpe, J. P., and A. M. Solé-Cava. 1994.** The use of allozyme electrophoresis in invertebrate systematics. Zool. Scr. 23: 3–18.

**Wilcox, R. S., and J. R. Spence. 1986.** The mating system of two hybridizing species of water striders (Gerridae). I. Ripple signal functions. Behav. Ecol. Sociobiol. 19: 79–85.

**Zera, A. J. 1981a.** Genetic structure of two species of waterstriders (Gerridae: Hemiptera) with differing degrees of winglessness. Evolution 35: 218–225.

**1981b.** Extensive variation at the a-glycerophosphate dehydrogenase locus in species of waterstriders (Gerridae: Hemiptera). Biochem. Genet. 19: 797–812.

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