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Geographically Widespread, Non-hybridizing, Sympatric Strains of the Hermaphroditic, Brooding Clam *Lasaea* in the Northeastern Pacific Ocean

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Abstract. We have studied phenotypic variation in six enzymes of *Lasaea,* a taxonomically complex genus of small brooding clams, from nine northeastern Pacific sites. Each of the individuals examined produced one of five combinations of electromorph patterns. *Lasaea* phenotypes could be differentiated into two main types, one containing two and the other three phenotype combinations. Samples from each population contained from one to three phenotype combinations and there was no evidence for crossbreeding among phenotypes. These results are strongly at variance with random mating expectations and indicate that the phenotype combinations represent reproductively isolated strains. This is substantiated by a more detailed study of the McNeill Bay, British Columbia, population where both main strains coexist. Electrophoretic characterization of *Lasaea* from individual 100 cm^2 samples of barnacle cover revealed that strains are not spatially segregated. Progeny of (1) pair mating experiments, (2) brooding field individuals, and (3) specimens that reproduced in isolation, all perpetuated the maternal electromorphs. Data from previous studies of reproduction in northeastern Pacific *Lasaea* suggest that the formation of non-hybridizing strains has resulted either from the predominance of self-fertilization or as a result of pseudogamy combined with meiotic parthenogenesis. We currently favor the former hypothesis. The two main strains are largely conserved between geographically distant sites, despite the lack of a planktonic larval stage. Separation of the main strains in Victoria, British Columbia, populations on the basis of shell color phenotype is to some degree possible, but is often equivocal. Electrophoretic analysis (especially of reddish specimens) is necessary for their reliable identification.

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Introduction

Members of the taxonomically complex bivalve genus, *Lasaea,* are reproductively specialized, minute, intertidal, crevice dwellers (Keen, 1938; Oldfield, 1964; Glynn, 1965; Ponder, 1971) with a near-cosmopolitan distribution (Chavan, 1969). A prominent reproductive/developmental dichotomy exists within the genus. *Lasaea australis* (Lamarck, 1818) engages in random mating and broods its young to a straight-hinged, planktotrophic veliger stage of development (Ó Foighil, 1988). All congeners studied to date, release their young as crawl-away juveniles (Pelseneer, 1903; Oldfield, 1964; Glynn, 1965; Rosewater, 1975; Booth, 1979; Kay, 1979). There is as yet no evidence for cross-fertilization in *Lasaea* that have crawl-away juveniles (Crisp *et al.,* 1983; Ó Foighil, 1986a, Crisp and Standen, 1988), which together form a complex assembledge of nominal species and subspecies of unknown phylogenetic affinity.

Molluscan systematists have conventionally relied heavily on shell morphology to distinguish between species. There is great individual variation in *Lasaea* valves (Dall, 1899; Ponder, 1971; Roberts, 1984; Ó Foighil, 1986a), even among those collected from any particular site, and this poses a difficult taxonomic dilemma. Keen (1938) lists >40 species of *Lasaea,* distinguished from each other on the basis of slight differences in shell morphology and color. However, subsequent workers, have been unable to separate many of these nominal species

(Soot-Ryen, 1960; Barnard, 1964; Dell, 1964; Ponder, 1971; Haderlie and Abbott, 1980; Beauchamp, 1985). In the northeastern Pacific, Keen recognized two species, *L. subviridis* Dall, 1899 and *L. cistula* Keen, 1938. In a more recent worldwide review of *Lasaea,* Ponder (1971) concluded that most of the nominal species, including *L. subviridis* and *L. cistula,* are merely regional subspecies or ecotypes of the type species *L. rubra* (Montagu, 1803).

Population genetic studies of European *Lasaea* have revealed the existence of a variety of non-hybridizing, sympatric, genetic strains capable of reproducing in isolation (Crisp *et al.,* 1983, Crisp and Standen, 1988). These results have important implications for understanding morphological variation and systematic relationships within the genus. Crisp *et al.* (1983) concluded that the populations studied were composed of female, apomictic (*i.e.,* ameiotic) clones. However, Oldfield (1961) described European *Lasaea* as simultaneous hermaphrodites with minute sperm production, recently confirmed by McGrath and Ó Foighil (1986) and by D. J. Crisp and associates (pers. comm.). Crisp and Standen (1988) proposed that European *Lasaea* reproduce by a combination of pseudogamy and apomixis. As yet there is no available data on spawning and gamete interaction in European *Lasaea.* Thiriot-Quiévreux *et al.* (1988) found an unusually large chromosome complement ($100 \leq 2n \geq 120$) in Kerguelen *Lasaea* and proposed that this resulted from an apomictic mode of reproduction. However, re-examination of their histological material has revealed that Kerguelen *Lasaea* are simultaneous hermaphrodites with disproportionately tiny testicular *versus* ovarian tissue (Ó Foighil, unpub.). The presence of sperm in European and Kerguelen *Lasaea* leaves open the possibility of reproductive modes besides apomixis, including self-fertilization.

Considerable data are available on the reproduction of northeastern Pacific *Lasaea.* They brood their young to a crawl-away juvenile developmental stage (Keen, 1938; Glynn, 1965; Beauchamp, 1986; Ó Foighil, 1986b), are simultaneous hermaphrodites (Glynn, 1965; Ó Foighil, 1985a; Beauchamp, 1986), and can reproduce in isolation (Ó Foighil, 1986b, 1987). Isolated individuals simultaneously spawn sperm and eggs into the suprabranchial chamber, sperm attach to eggs by an acrosomal reaction, the male pronucleus is incorporated into the egg cytoplasm and the oocyte produces two polar bodies before first cleavage (Ó Foighil, 1987). A relatively tiny amount of testis is produced in the ovotestis, sperm occupy approximately 5% of gonadal volume, the rest being devoted to oogenesis (Ó Foighil, 1985a). This pattern of reduced sperm production is theoretically consistent with the hypothesis that self-fertilization is common (Heath, 1979; Fischer, 1981; Charlesworth and Charlesworth, 1981). Indeed, sperm production in the randomly mating *L. australis* is an order of magnitude greater (approximately 50% of gonadal volume) than in northeastern Pacific congeners (Ó Foighil, 1988). Several observations imply that cross-fertilization might be a relatively rare phenomenon in northeastern Pacific *Lasaea* populations. Sperm are present only in small numbers and have reduced motility (Ó Foighil, 1985a), and there is an apparent absence of specialized sperm transfer mechanisms typically found in cross-fertilizing brooding bivalves, such as spermatophores/spermatozeugmata (Coe, 1931; Ó Foighil, 1985b), dwarf/complemental males (Turner and Yakovlev, 1983; Ó Foighil, 1985c), and pseudocopulation (Townsley *et al.,* 1965).

In this study, we examined isozyme variation of four British Columbia (B. C.), Canada, and five California, U. S. A. populations of *Lasaea.* The F_1 progeny of adults from a B. C. site, either resulting from pairs or single individuals that reproduced when placed in isolation, or from broods collected from field individuals, were also characterized electrophoretically. We used isozyme data from adults and juveniles (1) to help identify the breeding system of *Lasaea* by comparing observed phenotype frequencies to random mating expectations and (2) to assess the systematic status of *Lasaea* populations using shell and protein phenotypes.

Materials and Methods

After collecting, adult nonbrooding animals were either analyzed electrophoretically within 2 days following storage in seawater tables or placed at −70°C until processed. Electrophoresis was performed at Friday Harbor Laboratories using 13% starch (Sigma hydrolyzed potato starch) gels, standard power supplies, and horizontal electrophoretic apparatus. Whole animals were individually homogenized with glass rods in an approximately equal volume of gel buffer and gels were run, not exceeding 200 volts, until the front had reached a preset "destiny" 80 mm from the origin. A single discontinuous Tris-citrate buffer system (electrode: 18.55 g boric acid/l and 2.4 g sodium hydroxide/l, pH 8.2; gel: 9.21 g tris/l and 1.05 g monohydrate citric acid/l, pH 8.7) was used for the following enzymes: esterase with α-naphtyl acetate substrate (EST; nonspecific), (leucine) aminopeptidase (LAP; E.C. 3.4.11.1), peptidase with glycyl-leucine substrate (PEP-GL), peptidase with leucyl-glycyl-glycine substrate (PEP-LGG), and peptidase with leucyl-valine and leucyl-tyrosine substrate (PEP-LVLT). In addition, phosphoglucomutase (PGM; E.C. 2.7.5.1) and glucose-phosphate isomerase (GPI; E.C. 5.3.1.9) were investigated using Crisp *et al.*'s (1983) discontinuous Tris-citrate buffer system. Most individuals showed no activity for PGM and the results for this enzyme are not presented. Enzyme staining assays for EST, LAP, and PGM

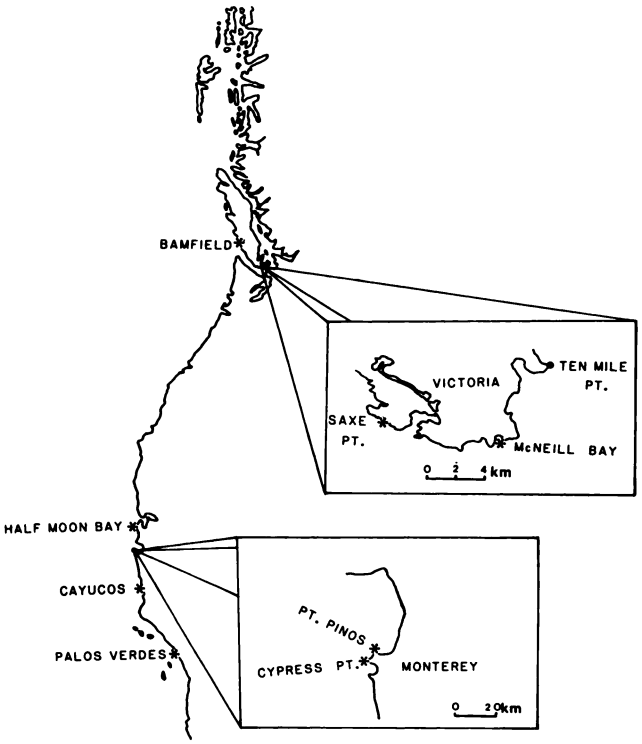

Figure 1. Sampling sites for northeastern Pacific *Lasaea* used in the electrophoretic study.

were as described by Ayala *et al.* (1972) and for GPI by Tracey *et al.* (1975). The PEP-GL, PEP-PP and PEP-LVLT staining assays consisted of 30 ml of a 2% agar solution (60°C) added to 20 mg of peptide substrate, 2000 units of peroxidase, 10 mg of O-Dianisidine, 5 mg of *Corotalus adamanteus* toxin, 0.5 ml of 0.1 *M* $MnCl_2$ and 20 ml of 0.1 *M* Na_2HPO_4 buffer.

At least one McNeill Bay individual of known protein phenotype was included per gel to provide a standard electromorph, and specimens from all nine populations were repeatedly run together on the same gels to verify electromorph scoring. The right valves of 73 McNeill Bay specimens were retained and analyzed for possible correlations of shell morphology and color to protein phenotype.

Subsequent to the initial electrophoretic survey of *Lasaea* from the nine study sites (Fig. 1), a more detailed investigation of the McNeill Bay population was performed. This involved electrophoretic characterization of the progeny of (1) pair mating experiments, (2) specimens that reproduced in isolation, (3) individuals that reproduced in the field. The degree of spatial overlap between strains in the McNeill Bay study site was also electrophoretically assessed.

In February 1987, non-brooding *Lasaea* adults were sampled from McNeill Bay and specimens were sorted according to shell color phenotype. Thirty pairs, each containing one white and one red shelled individual, were placed in separate culture vials containing 20 mls of 1 μm filtered seawater. *Lasaea* are positively thigmotactic (Morton, 1960) and each pair was positioned in close physical contact to ensure mutual attachment with byssal threads to facilitate potential cross-fertilization. The culture vials were maintained at 18°C and the clams were fed on cultured *Thalassiosira pseudonana* (strain 3H) and had salt water changes twice weekly. All individuals were checked weekly for brooding activity using a dissecting microscope (broods are visable through the translucent shells). Once a brooding individual was detected, the non-brooding partner was preserved at −70°C, as was the brooding parent when all the F_1 juveniles had been released from the brood chamber. The F_1 juveniles were cultured under the same conditions until September 1987, when parents, non-brooding partners, and the F_1 progenies of pairs containing adults of different enzyme phenotypes were electrophoretically analyzed. Originally, it was hoped to type the offspring (0.7–1.2 mm in valve lengths) using both LAP and PEP-GL enzyme assays, however, results were obtained only for the latter.

Ten red-shelled non-brooding adults collected from McNeill Bay in February 1987 were placed individually in culture vials and maintained in the laboratory, as previously described, until they released juveniles. In May 1987, 30 *Lasaea* adults brooding early embryos were selected from pooled McNeill Bay samples. They were also individually raised in laboratory conditions until juvenile release. Both sets of parents were then frozen at −70°C and electrophoretically characterized together with their offspring in September 1987 for the PEP-GL enzyme phenotypes.

Twenty five random 100 cm^2 samples of barnacle cover containing *Lasaea* were removed from the McNeill Bay site in October 1987 to investigate the degree of spatial overlap between strains. Samples were collected from the mid to high intertidal along an 80 m stretch of shore and were representative of the various crevice habitats available to *Lasaea* at the study site. All individuals in each sample were characterized for the PEP-GL phenotype after embryos had been dissected from brooding individuals.

Results

The electromorphs obtained from the survey of nine populations for EST, LAP, PEP-GL, and PEP-LGG are presented diagramatically in Figure 2. GPI was monomorphic in all individuals, PEP-LVLT phenotypes for each specimen were identical to the combined PEP-GL and PEP-LGG electromorphs for that individual and only one esterase presumed locus (EST) was consistently

Table I

Composite electromorph composition of the five Lasaea *strains observed in B.C. and California populations. Refer to Figure 2 for diagrammatic representation of electromorph patterns*

		Composite electromorph pattern			
	Strains	PEP-GL	PEP-LGG	LAP	EST
"A"	1	1	1	1	1
	2	2	1	2	1
	3	3	2	3	2
"B"	4	3	2	4	2
	5	3	3	4	2

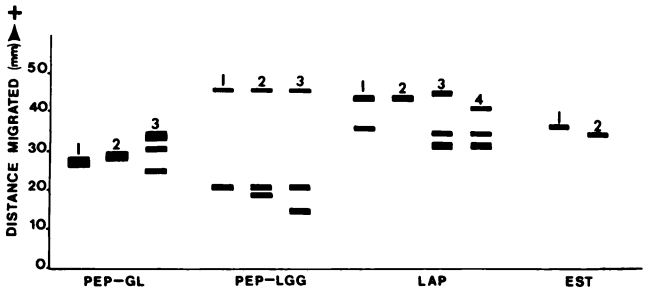

Figure 2. Diagrammatic representation of the total electromorph variation observed over four polymorphic gene-enzyme systems for northeastern Pacific *Lasaea*. Each electromorph pattern is individually numbered. For instance, the three PEP-GL protein phenotypes are labelled PEP-GL1, PEP-GL2, and PEP-GL3, respectively.

resolved in this study. Each *Lasaea* individual examined produced one of only five different composite electromorph patterns observed in this study (Table I). These patterns could be differentiated into two main sets ("A," "B") on the basis of similarity of the protein phenotypes. Patterns 1, 2 are of the "A" set and share identical PEP-LGG and EST phenotypes; "B" patterns 3, 4, 5 produce identical PEP-GL and EST phenotypes. Table II shows the distribution of the five composite electromorph patterns in the nine populations studied. The Bamfield, Pt. Pinos, and Palos Verdes samples were fixed for patterns 1, 4, and 2 respectively, while the remaining samples were polymorphic. The two main electromorph patterns ("A," "B") were respectively represented by patterns (1, 4) in both B. C. and California populations.

Genetic interpretation of these complex protein phenotypes is affected by the absence of expected heterozygous or homozygous phenotypes from polymorphic populations. For example, populations containing both "A" and "B" EST phenotypes (Table II) lacked obvious heterozygotes. Because all specimens had a single GPI band of identical mobility, we assume that all were homozygous at a single GPI coding locus. Genetic interpretation was more difficult for other, multiple-banded patterns and are thus provisional in nature. The three-banded PEP-GL 3 pattern (Fig. 2) probably does not represent a heterozygous phenotype for a dimer protein because the middle band is neither equidistant from, nor twice as prominent as, the two outlying bands (see Fig. 6). PEP-GL 1, 2 electromorphs share identical staining characteristics with the fastest of the three PEP-GL 3 bands and they may represent three alleles of a single locus (Fig. 2). The two slower PEP-GL 3 bands may represent a heterozygous monomer or two homozygous loci. PEP-LGG patterns 1, 2, and 3 all share an identical fast band which may represent a distinct locus (Fig. 2). It is unclear whether the slower PEP-LGG bands are products of one or more loci. Similarly, LAP 1 may represent a heterozygous monomer condition or else these individuals may be homozygous at two LAP loci (Fig. 2). The other LAP patterns 2, 3, and 4 do not appear to be consistent with a uniform heterozygous condition. Three potential loci may be represented by LAP 3 and 4; the three-banded patterns do not have the characteristics of a heterozygous dimer, the two slower bands are composed of a relatively weak faster band and a more pronounced slower one and probably do not represent a heterozygous monomer condition (Fig. 2).

None of the five composite electromorphs appeared to be the result of matings among electromorphs in any of the individuals characterized from the six populations that contained >1 strain. Assuming that these electromorph patterns have a similar genetic basis to that observed in other organisms, this is markedly at variance with random mating expectations. The observed and

Table II

Distribution of the five composite electromorph patterns (strains) in the nine Lasaea *populations studied. n = number of individuals analyzed per population*

		Frequency of *Lasaea* with each composite electromorph pattern per population				
		"A"		"B"		
Population	n	1	2	3	4	5
Bamfield	84	1.0	—	—	—	—
Saxe Point	78	0.15	—	0.71	0.14	—
McNeill Bay	140	0.29	—	0.63	0.08	—
Ten Mile Point	79	0.56	—	0.42	0.03	—
Half Moon Bay	19	—	—	—	0.63	0.37
Point Pinos	50	—	—	—	1.0	—
Cypress Point	25	—	—	—	0.64	0.36
Cayucos	54	0.04	—	—	0.72	0.24
Palos Verdes	14	—	1.0	—	—	—

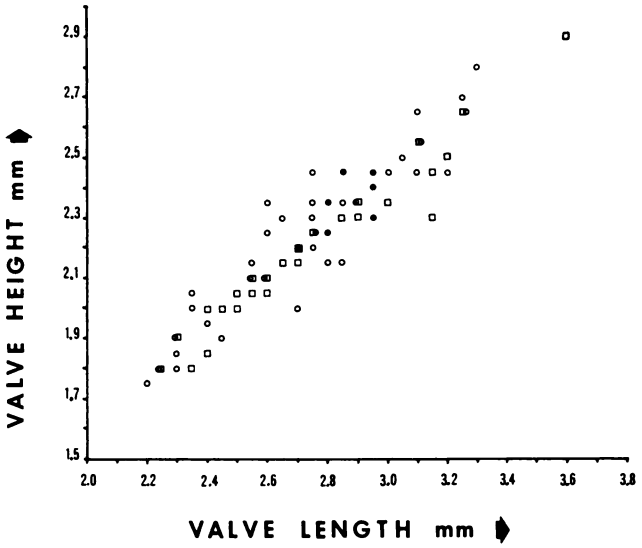

Figure 3. Plot of valve height against valve length for 73 McNeill Bay *Lasaea* characterized according to protein phenotype. Squares represent strain 1 animals (n = 27), solid squares are double scores, correlation coefficient (r) = 0.976. Circles represent strain 3 animals (n = 46), solid circles are double scores, r = 0.921 ($0.01 < P < 0.02$). When the large strain 1 individual (3.6 mm in length) is excluded, strain 1 r = 0.967 is not significantly different from 0.921 ($0.05 < P < 0.10$).

Hardy-Weinberg-Castle expected genotype frequencies for the EST locus of 140 McNeill Bay individuals characterized during the initial electrophoretic survey (Table II) are very different ($P < 0.001$). In addition, the apparent gametic disequilibrium between sympatric strains indicates that they are reproductively isolated.

The height and length of the right valves were positively correlated for 73 individuals from McNeill Bay, characterized according to protein phenotype and shell coloration. Although the height/length dimensions of strains 1, 3 overlap substantially (Fig. 3), their respective correlation coefficients (0.976 and 0.921) are significantly different ($0.01 < P < 0.02$) due to the presence of an unusually large (3.7 mm in length) strain 1 specimen. When this specimen is removed from the calculation, a correlation coefficient of 0.967 is obtained for strain 1, which is not significantly different from that of strain 3 ($0.05 < P < 0.10$). Individuals varied in shell coloration from totally white to totally red. Intermediate forms commonly occurred which were dorso-laterally red with white ventral patches. Some individuals appeared to have switched abruptly from forming a red shell to secreting a lighter zone of white growth. The height/length correlation coefficients do not differ significantly ($P > 0.50$) among white and predominantly red-shelled individuals (0.942 and 0.953 respectively) (Fig. 4). Anteriodorsal shell margins varied considerably in shape within each strain and shell color group, *e.g.*, see specimens 2, 4, 5 and 31, 37, 38 (Fig. 5). There was no consistent difference in the maximum sizes attained by either protein phenotype or shell color groupings.

The relationship between shell color and protein phenotype was also investigated for *Lasaea* from Victoria's McNeill Bay population. Figures 5 and 6 show the right valves and the respective PEP-GL electromorphs for forty specimens (20 white; 20 predominantly red). The 20 white-shelled individuals, sorted before electrophoretic analysis, all exhibited the PEP-GL 3 electromorph. Four hundred and forty-one (441) adult specimens from McNeill Bay were typed for the PEP-GL 1 enzyme during the initial electrophoretic survey, and the subsequent investigation of spatial overlap between the two main strains. Of these, 272 and 169, respectively, expressed PEP-GL 3 and PEP-GL 1 phenotypes. If shell color is independent of PEP-GL protein phenotype, a ratio of 12.34 PEP-GL 3 to 7.66 PEP-GL 1 for the 20 white specimens in Figure 5 is expected (when analyzed by Chi square test: $P < 0.001$). The 19 predominantly red-shelled animals for whom electromorphs were obtained, contained both PEP-GL 1 (11 specimens) and PEP-GL 3 (8 specimens) patterns (expected ratios: 11.72 PEP-GL 3, 7.27 PEP-GL 1; Chi square test: $0.25 < P < 0.50$). Of these 19 predominently red animals, 11 were uniform in color and 8 had some white patches. Both of these two color subgroupings, however, were heterogenous in protein phenotype expression. Eight of the totally red specimens had the PEP-GL 1 pattern and four were type PEP-GL 3. Four of the eight mixed color individuals were PEP-GL 1 and the rest PEP-GL 3. Similar results were obtained when shell coloration and protein phenotypes of the Ten Mile Pt. population were investigated; white

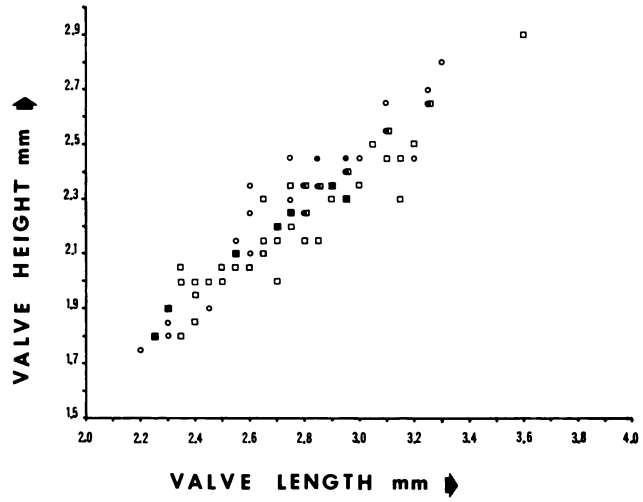

Figure 4. Plot of valve height against valve length for 73 McNeill Bay *Lasaea* characterized according to shell color. Squares represent predominently red individuals (n = 48), solid squares are double scores, correlation coefficient (r) = 0.953. Circles represent white shells (n = 25), solid circles are double scores, r = 0.942, ($P > 0.50$).

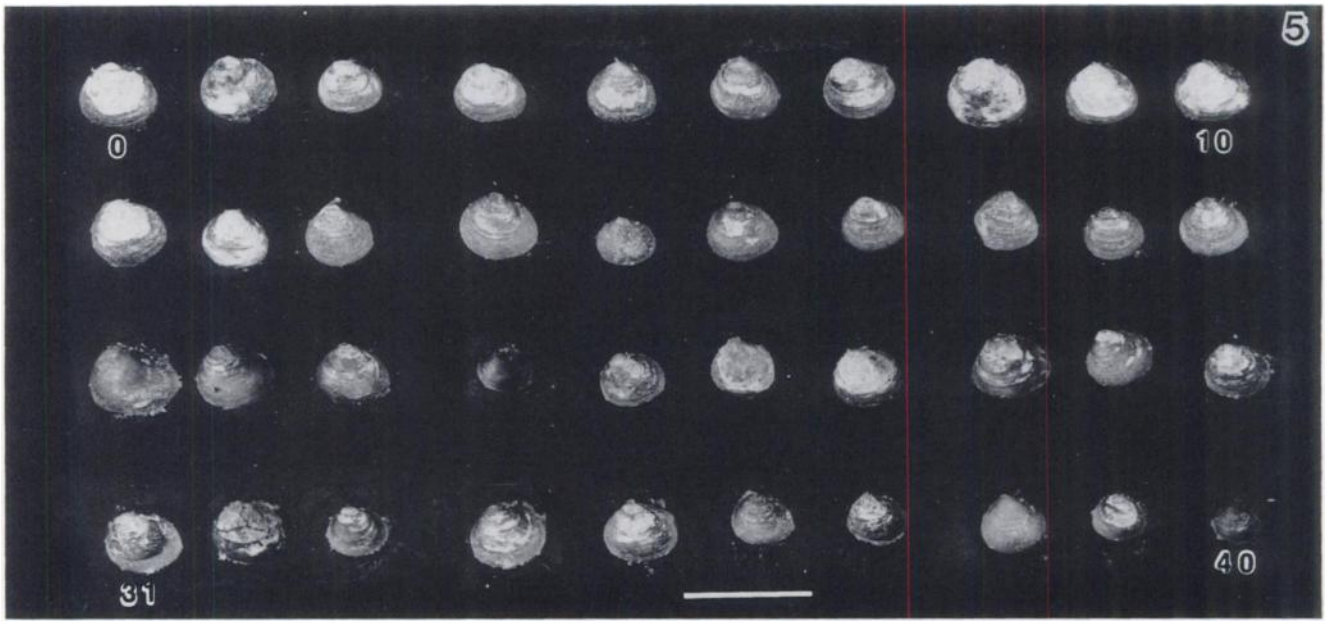

Figure 5. Right valves of 40 *Lasaea* individuals from McNeill Bay, Victoria. Specimens 1–20 (top two rows) have white shells and specimens 21–40 (bottom two rows) are predominantly or totally red. Scale = 5 mm.

individuals all expressed the PEP-GL 3 phenotype (15 specimens) and totally red or mixed color specimens produced either the PEP-GL 1 or PEP-GL 3 phenotypes (64 specimens).

No mortality of adult McNeill Bay *Lasaea* occurred during laboratory culture. Less than 5% of F_1 progeny died in culture, mainly as a result of premature release from the brood chamber before the development of valve opposition. Four of the 30 *Lasaea* pairs used in the pair mating experiments did not spawn while in the laboratory. One member of all remaining pairs released F_1 progeny, however, in eight of these pairs both adult specimens expressed the PEP-GL 3 electromorph. The remaining 18 pairs were composed of individuals exhibiting different PEP-GL phenotypes and in 17 of these cases the individual that initiated brooding expressed a PEP-GL 1 electromorph. When analyzed by Chi square test, this result is significantly different ($0.005 < P < 0.01$) from an expected ratio of 9 PEP-GL 1 to 9 PEP-GL 3, if precedence in the onset of brooding were independent of PEP-GL phenotype. Mean brood size was 17.8 ± 6.4 S.E. and a total of 330 F_1 progeny were typed for the PEP-GL enzyme. In all cases, the F_1 PEP-GL phenotypes were identical to those of the confirmed parents (brooding individuals) and did not reveal any evidence of cross-fertilization by the potential sperm donors (non-brooding partners) (see Fig. 7).

The 10 adult McNeill Bay *Lasaea* maintained in isolation during laboratory culture reproduced successfully. Eight and 2 individuals, respectively, expressed the PEP-GL 1 and PEP-GL 3 electromorphs. All 146 F_1 progeny (mean brood size of 14.6 ± 5.0 S.E.) perpetuated the parental PEP-GL phenotypes, including the 26 progeny of the 2 PEP-GL 3 parents. Assuming that the isolated PEP-GL 3 phenotype parents reproduced by self-fertilization, as previous cytological evidence would suggest (Ó Foighil, 1987), the result for the 26 F_1 progeny is significantly different from the 1:2:1 phenotype ratios expected if the PEP-GL 3 electromorph represented a heterozygous dimer protein, or if the 2 slow bands represented a heterozygous monomer protein (Chi square test, $P < 0.001$).

Eleven of the 30 brooding adults sampled in McNeill Bay in May 1987 expressed a PEP-GL 3 phenotype and the remainder exhibited the PEP-GL 1 electromorph. Altogether, 435 F_1 progeny (mean brood size = 14.5 ± 8.4 S.E.) were typed and all perpetuated the parental phenotypes, with a single exception. This exceptional individual occurred in a brood of 21 juveniles, 20 of which expressed the maternal PEP-GL 3 electromorph, the other produced the PEP-GL 1 phenotype. It is more likely that this individual resulted from inadvertent transfer between cultures, rather than from cross-fertilization between the strains, because it totally lacked the maternal phenotype for this brood.

Three hundred and one (301) individuals were characterized for the PEP-GL enzyme from the 25 samples of barnacle cover (100^2 cm^2) taken from McNeill Bay in October 1987. Both main *Lasaea* strains co-occurred in 18 of these samples (Figs. 8, 9) and there was no signifi-

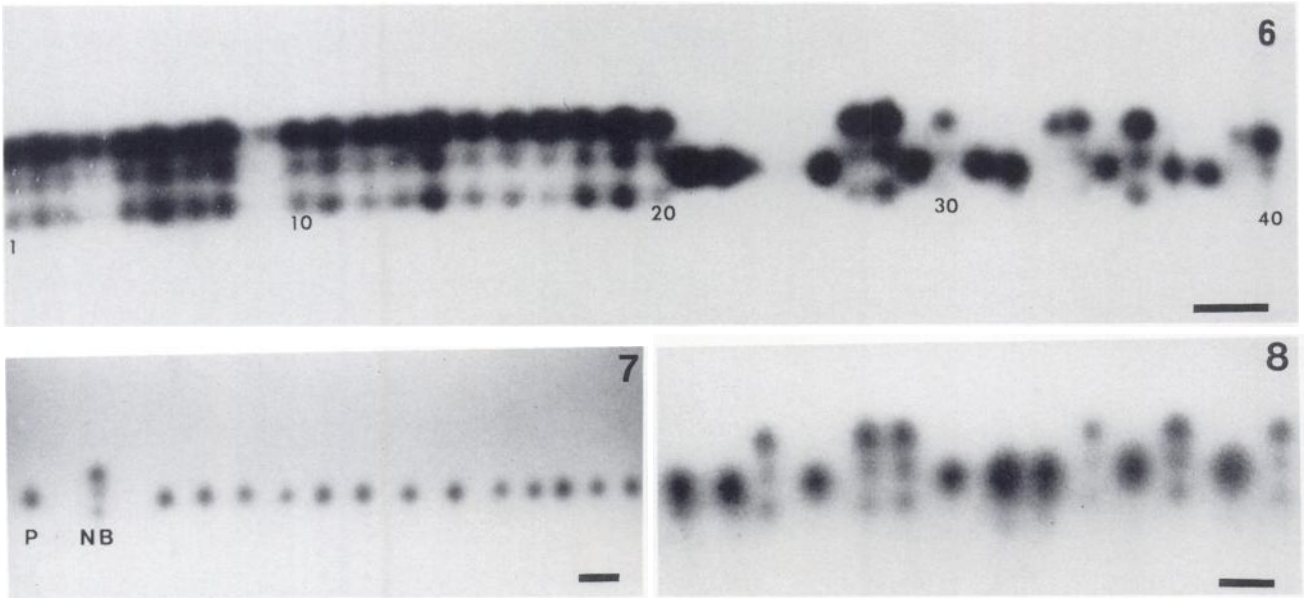

Figure 6. PEP-GL electromorphs of the same 40 *Lasaea* individuals in Figure 5. Specimens 1–20, 27, 28, 30, 33, 34, 36, 39, and 40 produced a PEP-GL 3 electromorph pattern. Individuals 21–23, 25, 26, 29, 31, 32, 35, 37, and 38 gave a PEP-GL 1 electromorph pattern. Specimen 24 gave no detectable result and specimen 25 was too faint to photograph well.

Figure 7. PEP-GL phenotypes of McNeill Bay *Lasaea* pair mating experiment showing parent (P), non-brooding partner (NB) and the 13 F1 progeny. Note that all offspring perpetuate the electromorph of the confirmed parent.

Figure 8. PEP-GL phenotypes of 14 McNeill Bay *Lasaea* recovered in a 100 cm^2 sample of barnacle cover. Both PEP-GL 1 (8) and PEP-GL 3 (6) phenotypes are expressed.

cant correlation between the distributions of the main strains ($r = 0.0399$, $P > 0.5$). It appears that the 2 main *Lasaea* strains are not segregated in McNeill Bay on this spatial scale and show a high degree of overlap.

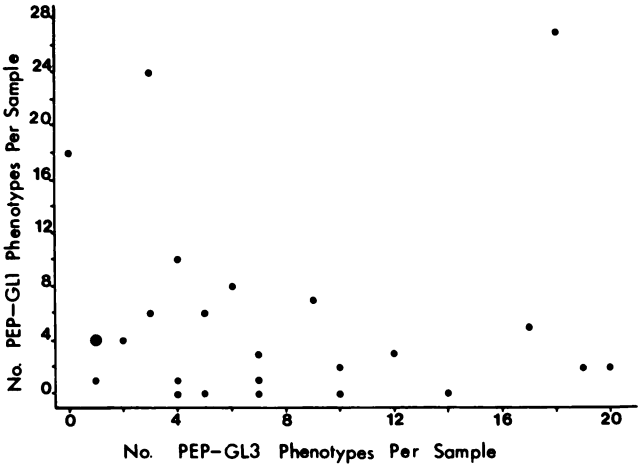

Figure 9. Relative numbers of PEP-GL 1 and PEP-GL 3 phenotypes in 25 100 cm^2 samples of barnacle cover from McNeill Bay, Victoria, B. C. Large circle = double score. Correlation coefficient (r) = −0.0399, $P > 0.5$.

Discussion

The absence of putative intermediate protein phenotypes and the consequent deviations from random mating expectations in spatially overlapping, sympatric field populations and in the progeny of pair mating experiments suggest that (1) several to many strains of *Lasaea* coexist and are widespread along the west coast of North America, and (2) if mating occurs between the various strains, it must be very rare. Population genetic structure of our study populations resemble those described by Crisp *et al.* (1983) and Crisp and Standen (1988) for European *Lasaea* but are markedly different from the randomly mating *L. australis* which has a planktotrophic larval development (Ó Foighil, 1988).

Formation of non-hybridizing, genetic strains can result from a number of reproductive modes, including apomixis, autosegregation, pseudogamy, and self-fertilization (Bell, 1982). Identifying the reproductive mode employed by *Lasaea* that lack dispersive larvae is proving to be problematical, due in part to the difficulty of identifying sperm in the ovotestis. In populations examined, a minute quantity of testis (averaging approximately 5% of gonadal volume) is produced in the posterio-ventral lobe of the ovotestis; the sperm heads vary

in shape and exhibit incomplete nuclear condensation (Pelseneer, 1903; Oldfield, 1961; Ó Foighil, 1985a, 1987; McGrath and Ó Foighil, 1986; Beauchamp, 1986). It is now apparent that both British and Kerguelen *Lasaea*, respectively, described by Crisp *et al.* (1983) and Thiriot-Quiévreux *et al.* (1988) as female, apomictic clones, are actually simultaneous hermaphrodites which appear identical to northeastern Pacific *Lasaea* in their gonadal and sperm morphologies (Oldfield, 1961; Ó Foighil, 1985a; McGrath and Ó Foighil, 1986; Ó Foighil, unpub.). Despite their unusual morphology, these sperm appear to be functionally mature and each is capable of swimming and undergoing an acrosomal reaction, binding to, and penetrating an egg (Ó Foighil, 1987).

Important data on post-spawning cytological events in isolated northeastern Pacific individuals are available (Ó Foighil, 1987), as reviewed in the introduction. These data, especially the evidence for production of two polar bodies before first cleavage, indicate that individuals from populations we studied reproduce not by apomixis but by automixis. Extrapolation to all northeastern Pacific strains assumes that results from these isolated individuals of unknown electrophoretic phenotype represent the norm. Additionally, since sperm were seen to penetrate eggs (Ó Foighil, 1987), these data suggest that the particular automitic mode must be either self-fertilization or meiotic parthenogenesis with some form of pseudogamy. In practice, self-fertilizing organisms and meiotic parthenogens are distinguished because the former produce both sperm and eggs, giving differentiation of gender at the gametic level (Bell, 1982).

Ideally, electrophoretic examination of the progeny of isolated heterozygous (Aa) adults could provide strong evidence for self-fertilization, or for some types of automictic parthenogenesis that have similar consequences to selfing (White, 1973; Suomalainen *et al.*, 1987), if the progeny do not deviate significantly from an expected 1AA:2Aa:1aa ratio. However, lack of segregation of the protein phenotypes in sampled populations makes positive identification of heterozygous loci difficult, because multiple bands on a gel that resemble typical heterozygous monomers or dimers could also be explained as the results of multiple loci. Similarly, if all progeny resemble their parent at a particular locus, as they did in our study and as reported by Crisp *et al.* (1983), this is not conclusive evidence for apomixis. Only a detailed study of male and female pronuclear interaction is likely to resolve the reproductive mode in these cases.

Crisp and Standen (1988) used a different buffer system from the one used by Crisp *et al.* (1983) and found non-segregating protein phenotypes they interpreted as suggestive evidence for fixed-heterozygosity at PGM and GPI loci of European *Lasaea* (*L. rubra*). They then concluded that *L. rubra* reproduces by a combination of pseudogamy and apomixis, citing Ó Foighil's (1987) description for sperm penetration in *L. subviridis* in support of this view. Although this remains a possibility, a number of important points need to be clarified before Crisp and Standen's (1988) interpretation can be accepted. Ó Foighil (1987) rejected apomixis in *L. subviridis*, not because of sperm penetration, but because the egg apparently undergoes a meiotic division before first cleavage, producing two polar bodies. It is quite possible that European *L. rubra* eggs are likewise meiotic. However, this remains to be established by observation of cytological events that occur before first cleavage. An alternate interpretation of the protein phenotype patterns observed by Crisp and Standen (1988) is that they represent multiple homozygous loci. C. Thiriot-Quiévreux (pers. comm.) found very high chromosome numbers in *L. rubra*, and these represent potential sources of such loci. Finally, pseudogamy is employed by all-female clones (Moore *et al.*, 1956; Kallman, 1962; Uzzell, 1964; Schultz, 1971, 1977; Kiester *et al.*, 1981; Stenseth *et al.*, 1985) or hermaphrodites (*e.g.*, the enchytraeid oligochaete *Lumricillus*, see Christensen and O'Connor, 1958; Christensen, 1980) that are sexual parasites of closely related cross-fertilizing species and are incapable of reproducing in isolation. *L. rubra* can reproduce in isolation to at least an F2 generation (Crisp *et al.*, 1983). The combination of pseudogamy with apomixis proposed by Crisp and Standen (1988) would indeed be noteworthy, if further substantiated, because we are presently unaware of any species where individuals are known to use their *own* sperm to initiate parthenogenic development.

Thiriot-Quiévreux *et al.* (1988) concluded that Kerguelen *Lasaea* were apomictic based on the absence of sperm and meiotic stages. It is now known that Kerguelen *Lasaea* are simultaneous hermaphrodites with greatly reduced sperm production (Ó Foighil, unpub.). Because of the relatively tiny testis, it is possible that sperm were overlooked by Thiriot-Quiévreux *et al.* (1988) in their chromosome preparations. We consider that a self-fertilizing mode of reproduction has yet to be ruled out for European and Kerguelen *Lasaea* populations. As is the case for northeastern Pacific congeners, a detailed cytological study is needed to firmly establish the reproductive mode of these populations.

Our present working hypothesis is that northeastern Pacific *Lasaea* reproduce by automixis, probably by self-fertilization. This interpretation is more parsimonious than that of pseudogamy combined with automictic parthenogenesis and, as discussed above, pseudogamy is only known to be employed by sexual parasites incapable of reproducing in isolation. If self-fertilization is in-

deed the predominate norm in northeastern Pacific *Lasaea* populations, heterozygous loci should be extremely rare within each strain, and each band in the complex multi-banded PEP-GL, PEP-LGG and LAP electromorph patterns recorded in this study should represent a separate homozygous locus. Each "B" strain typically yielded a greater number of protein phenotypes (*i.e.*, bands) than did each "A" strain in our study. One interesting possibility is that the strains differ in ploidy and perhaps one or more ploidy duplication events has been responsible, in part, for the reproductive isolation(s) of *Lasaea* strains (C. Moritz, pers. comm.). The only published work to date on *Lasaea* karyology is that of Thiriot-Quiévreux *et al.* (1988) who found a record (though variable) number of chromosomes for the class Bivalvia in Kerguelen *Lasaea* ($100 \leq 2n \leq 120$). A similarly high chromosome complement may be present in northeastern Pacific *Lasaea* (especially the "B" strains) and may represent a source of multiple homozygous loci.

Species that reproduce by self-fertilization still, in theory, retain the potential for cross-fertilization, and exclusively self-fertilizing populations [*e.g., Rivulus marmoratus* (Kallman and Harrington 1964; Vrijenhoek, 1985)] are thought to be extremely rare in nature (Bell, 1982). A mixed self- and cross-fertilizing mating pattern has the potential of creating a wide variety of new genotypes, as occasional outcrossing between inbred lineages will result in highly heterozygous progeny (Antonovics, 1968; Bucklin *et al.*, 1984). Cross-fertilizations in northeastern Pacific *Lasaea* populations might be exceptionally rare or absent, judging from the lack of intermediate protein phenotypes encountered in this study and the apparent absence of sperm transfer and sperm storage mechanisms that enable other suprabranchial brooding bivalves to cross-fertilize (Coe, 1931; Townsley *et al.*, 1965; Turner and Yakovlev, 1983; Ó Foighil, 1985b, c). Still, even rare cross-fertilization could provide an alternative mechanism, besides ploidy duplication, for the creation of new strains.

The majority of marine invertebrates including bivalves cross-fertilize, so it is interesting to consider how predominate self-fertilization might arise. Various genetic models of the evolution of self-fertilization predict that a history of inbreeding predisposes originally cross-fertilizing populations to the development of automixis (self-fertilization) by removing recessive deleterious alleles (Charlesworth and Charlesworth, 1981; Lande and Schemske, 1985; Uyenoyama, 1986). This forms the premise for Strathmann *et al.*'s (1984) hypothesis to account for the association of simultaneous hermaphroditism and brooding of young to a crawl-away juvenile stage in many marine invertebrate taxa. Strathmann *et al.* suggest that reduced dispersal promotes inbreeding which lowers heterozygosity, exposing deleterious homozygous combinations to selection and eventual elimination. Thus prolonged inbreeding dilutes the genetic penalty of inbreeding depression caused by self-fertilization. If a self-fertile individual happens to arise in an already inbred population, and it additionally produces relatively few sperm, it would then be at a reproductive advantage, because of the lowered cost of spermatogenesis and at a genetic advantage due to the reduced "cost of meiosis" (Williams, 1975; Maynard Smith, 1978; Bell, 1982). Indeed, the genetic advantage of a reduced "cost of meiosis" implies that once developed, the evolution of completely self-fertilizing lineages may be an irreversible step (Bull and Charnov, 1985). Strathmann *et al.* (1984) were concerned primarily with explaining hermaphroditism in groups that are normally gonochoric (*e.g.*, echinoderms, anemones, chitons, sipunculans, etc.), but they also point out that the basic model should apply to explaining exceptional self-fertilizers in hermaphroditic taxa that normally have effective blocks to self-fertilization (*e.g.*, tunicates, nudibranchs, certain bivalve taxa including *Lasaea*). The lack of a planktonic dispersive stage, simultaneous hermaphroditism, and apparent high level of self-fertilization of northeastern Pacific *Lasaea* are consistent with the proposal (Strathmann *et al.*, 1984; Eernisse, 1988) that having crawl-away offspring can lead to departure from cross-fertilization.

Cross-fertilizing marine invertebrate species that are typically sedentary as adults and lack a planktonic larval stage show significant interpopulational genetic divergence on a relatively small geographic scale (Berger, 1973, 1977; Snyder and Gooch, 1973; Campbell, 1978; Ward and Warwick, 1980; Bulnheim and Scholl, 1981; Burton, 1983; Janson and Ward, 1984; Palmer, 1984; Grant and Utter, 1988). Interpopulational genetic drift in these cases may originate from a founder effect (Holgate, 1966; Nei *et al.*, 1975) during an initial colonization, or a later genetic bottleneck event, that may be maintained and enhanced by infrequent genetic exchange with other populations over time. In contrast, our results for northeastern Pacific *Lasaea* show that some strains (1, 4) are present in geographically distant sites. A number of potential factors may have contributed to this, including alternative dispersal techniques such as byssus drifting (Lane *et al.*, 1985) and rafting (Highsmith, 1985) (short and long distances respectively), low rates of mutation, and a predominantly self-compatible reproductive mode. Self-fertilization is genetically conservative because new alleles formed by mutation are rapidly expressed in homozygous combinations and are thus directly exposed to selection (Bell, 1982). Newly formed populations will not experience a founder effect if the initial colonizers previously existed as reproductively isolated strains at the source site.

Taxonomic interpretations based on a small number

of loci can lead to a potentially serious bias (Nei, 1972). Even though we examined relatively few loci, it is clear that northeastern Pacific *Lasaea* populations are composed of a variety of reproductively isolated strains as Crisp *et al.* (1983) found for British populations. These strains are readily distinguishable by electrophoretic analysis.

Diagnostic separation of strains from Victoria, B. C. populations on the basis of shell phenotype appears unreliable, except for some apparent color differences between the strains. We can predict with a high degree of confidence that white-shelled *Lasaea* from Victoria will express particular protein electromorphs. However, specimens with the same protein phenotypes may also have mixed shell coloration (red/white) or totally red shells. Shell pigmentation is known to be light-induced in juvenile mussels (Trevelyan and Chang, 1987), and, in *Lasaea rubra hinemoa,* the extent of red coloration in the valves is thought to be related to the degree of exposure to sunlight (Ponder, 1971). This may also be the case for at least strain 3 of northeastern Pacific *Lasaea* because specimens recovered from deep crevices had the whitest shells. There may be a partial habitat difference between the two main strains in Victoria, with strain 3 occurring in both deep and shallow crevices and strain 1 found only in shallow crevices. Data from the laboratory pair mating experiments hint at physiological or spawning differences between the two main strains: 17 of the first individuals to spawn from the 18 heterogeneous pairs expressed a PEP-GL 1 phenotype.

Criteria used by Keen (1938) to separate two nominal *Lasaea* species in Californian populations are inadequate when applied to Victoria *Lasaea* of known protein phenotype. Keen (1938) distinguished *L. cistula* from *L. subviridis* by its smaller size, less oblique outline, darker color, higher umbones and by its more abrupt slope from the umbone to the anterior margin. However, Ponder (1971) and Beauchamp (1985) could not distinguish on morphological grounds two different forms of *Lasaea* in Californian populations. As in this present study, Ponder (1971) found Keen's (1938) distinguishing morphological characteristics to be highly variable among individual shells. For the present, it would appear that electrophoretic analysis is necessary for the reliable identification of northeastern Pacific *Lasaea* strains. Whether the two main strains detected in this study correspond to *L. subviridis* and/or *L. cistula* will require careful comparisons of any diagnostic morphological distinctions, should they be found, with existing type material for northeastern Pacific *Lasaea.*

There is as yet no evidence for cross-fertilization between the strains found in British (Crisp *et al.,* 1983) and northeastern Pacific *Lasaea* populations. Therefore the biological species concept (Mayr, 1957, 1963), which applies best to randomly mating organisms, would seem inappropriate for these populations. Ponder (1971) concluded that most nominal species of *Lasaea* are merely regional subspecies, or ecotypes of the type species *L. rubra.* Alternatively, one could conclude that every distinct electrophoretic strain is a distinct historical entity or, perhaps, species. Efforts to determine the level at which the category "species" best applies would best wait for reproductive investigations of other *Lasaea* populations and a better understanding of *Lasaea* historical relationships, which are undoubtedly complex among *Lasaea* that lack a dispersive larval stage (Ponder, 1971; Crisp *et al.,* 1983; Ó Foighil, 1986a; Thiriot-Quiévreux *et al.,* 1988). Resolution of these relationships will require a multidisciplinary approach applied to a variety of populations of this near-cosmopolitan genus.

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