Esterase Polymorphisms in European and American Populations of the Periwinckle, *Littorina littorea* (Gastropoda)

The distribution and great abundance of *Littorina littorea* in North America, south of Nova Scotia, are due to recent dispersal. Although shells dated 600–700 years B.P. have been found near Halifax[1,2], southward colonization started only about 1840. The New England coasts were occupied in about 40 years or generations[3–7].

This rapid dispersal has no precedent in Europe. Thus *L. littorea* is suitable for comparisons of the genetical structure of two kinds of populations within the same species: recent colonists (New England), and old settlers (France). From such comparisons, the genetics of colonization[8] and the population structure of marine invertebrates[9–16] which are still poorly known, can be better understood.

In an early study BUMPUS[17] concluded that the American populations were more variable than their European counterparts. His results were criticized because the characters he used (shell morphology) are easily modified by environmental influences[18] and so he could not distinguish genetical variation from phenotypic plasticity. More recent work has shown that shell sculpture variation has a genetic component, however, and that shell morphology may be adaptive[19]. Yet we chose isozyme variation instead of shell morphology to re-analyze the differentiation within *L. littorea* because the danger of working with an unknown and complex genetical system is largely avoided[20]. We discuss here preliminary findings on esterase polymorphisms.

We collected snails at 4 US and 3 French sites between December 1970 and July 1971 (Tables I and II). Each snail (after discarding the operculum, foot, mantle edge, and columellar muscle) was ground on ice in 0.089 *M Tris*, 2.7×10^{-3} *M* Na_2 EDTA, 0.089 *M* boric acid buffer with 5% sucrose, pH 8.4. The slurry was centrifuged at 4°C at $3000 \times g$ for 15 min and the supernatant electrophoresed on polyacrylamide gel (Cyanogum-41) in a discontinuous buffer system[21] for 40 min at 200 volts and at 400 volts for another 60 min. Esterase activity was revealed by incubation for $1^1/_2$–2 h in a staining solution (filtered prior to use) of 0.024 g Fast Garnet GBC salt (Sigma) and 0.6 ml 2% α-naphthyl-acetate (in a 1:1 acetone-water solution) added to 40 ml 0.1 *M* phosphate buffer pH 6.5. Fixation was done overnight in 1% glycerol —8% acetic acid. We found 5 zones of esterase activity, numbered from 1 (fastest migrating anodally) to 5 (slowest). Cathodal bands were not detected by the apparatus. Only the activity at zones 1, 2 and 5, hereafter referred to as Est-1, Est-2 and Est-5, respectively, is described below.

Est-1 appeared in 371 of 374 individuals as a single band of identical mobility. We found a slower moving variant in 1 individual from Halibut Point, Massachusetts, and a double banded pattern (both slow and fast components) in 2 individuals from Roscoff-25, Finistère. This variation could be due to an autosomal locus with 2 alleles. Est-1 is very monomorphic throughout the US and French samples. By contrast, electrophoretic activity at Est-2 and Est-5 showed polymorphisms. Seven phenotypes were scored at Est-2: 1. a fast moving, well stained band, 2. a slow moving, well stained band, 3. a double band, fast-slow, well stained, 4. a fast moving, faintly stained band, 5. a slow moving, faintly stained band, 6. no band, and 7. a triple band, fast-intermediate-slow, relatively well stained. Activity at Est-5 was similar to that at Est-2,

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Table I. Geographical variation in phenotype frequencies at the Esterase-2 zone in *Littorina littorea*

Population sample (*n*)	Phenotype numbers (%)[a]						
	1	2	3	4	5	6	7
New England, USA							
1 Blue Hill, Maine (32)	9 (28.1)	–	2 (6.3)	4 (12.5)	3 (9.4)	14 (43.7)	–
2 Kennebunk Beach, Me (54)	21 (38.9)	1 (1.9)	1 (1.9)	12 (22.2)	–	19 (35.2)	–
3 Halibut Point, Massachusetts (74)	44 (59.5)	1 (1.4)	2 (2.7)	12 (16.2)	–	15 (20.3)	–
4 Nahant, Massachusetts (26)	10 (38.5)	2 (7.7)	–	6 (23.1)	–	8 (30.8)	–
France							
5[b] Roscoff-25, Finistère (57)	35 (61.4)	–	1 (1.8)	13 (22.8)	–	8 (14.0)	–
6[b] Roscoff-26, Finistère (61)	31 (50.8)	–	2 (3.3)	8 (13.1)	–	18 (29.5)	2 (3.3)
7 Bailleron, Morbihan (45)	36 (80.0)	–	1 (2.2)	5 (11.1)	–	3 (6.7)	–

[a] For description of phenotypes, see text. [b] Samples 5 and 6 represent two sites 1 km from each other (linear distance): Roscoff-25 in the harbor (collected 25 April 1971) and Roscoff-26 just outside the harbor (collected 26 April 1971).

Table II. Geographical variation in phenotype frequencies at the Esterase-5 zone in *Littorina littorea*

Population sample (n)	Phenotype numbers (%)[a]					
	1	2	3	4	5	6
New England, USA						
1 Blue Hill, Maine (16)	–	6 (37.5)	–	–	6 (37.5)	4 (25.0)
2 Kennebunk Beach, Me (38)	2 (5.3)	20 (52.6)	2 (5.3)	–	5 (13.1)	9 (23.7)
3 Halibut Point, Massachusetts (50)	–	32 (64.0)	2 (4.0)	–	11 (22.0)	5 (10.0)
4 Nahant, Massachusetts (15)	6 (40.0)	2 (13.3)	2 (13.3)	2 (13.3)	2 (13.3)	1 (6.7)
France						
5[b] Roscoff-25, Finistère (44)	2 (4.5)	19 (43.2)	12 (27.3)	–	6 (13.6)	5 (11.4)
6[b] Roscoff-26, Finistère (28)	3 (10.7)	11 (39.3)	6 (21.4)	1 (3.6)	6 (21.4)	1 (3.6)
7 Bailleron, Morbihan (25)	–	25 (100.0)	–	–	–	–

[a] For description of phenotypes, see text. [b] See note in Table I.

except that no 7th phenotype was observed. Such phenotypes could be explained by variation at 2 loci, each having 3 major alleles: fast, slow, and null or silent. Data on protein concentration are being gathered to test the hypothesis of a null allele.

At Est-2 (Table I) phenotype frequencies vary geographically in the USA. Either phenotype 6 (population 1) or 1 (pop. 3) is the most frequent or else both phenotypes are about equally frequent (pop. 2 and 4). In France on the other hand phenotype 1 is always the most frequent, reaching 80% at Bailleron.

At Est-5 (Table II) there is, again, greater variability among American than among French samples: the commonest phenotypes in US populations are either 1, 2 or 5, whereas phenotype 2 is always the most frequent in France, reaching 100% at Bailleron. In addition to this pattern, phenotype 6 decreases from about 25% in Maine to 7–10% in Massachusetts.

These data suggest the following conclusions and hypotheses. 1. Although the qualitative pattern of biochemical variability is similar between US and French populations, the former (recent colonists) are more variable geographically than the latter. BUMPUS' conclusion[17] is apparently substantiated.

2. The north to south cline of decreasing frequency of phenotype 6 at Est-5 in the USA could be correlated with the north to south coastal current[22,23]. Correlation between clinal variation at biochemical loci and environmental gradients has been shown by KOEHN[24] and SCHOPF and GOOCH[12].

3. The greater phenotypic uniformity (and probable greater homozygosity) of the Bailleron sample may be correlated with its isolation in the Gulf of Morbihan, which is partially cut off from the strong coastal currents of southern Brittany (personal communication of R. MAHÉO). Thus the larvae carried along such currents[25,26] might not enter the Gulf or do so in small numbers, thereby reducing gene flow and favoring inbreeding in the Gulf populations[27].

Résumé. La structure génétique de 7 populations du gastéropode marin *Littorina littorea* a été étudiée par analyse de phénotypes d'estérases révélés par électrophorèse sur gel de polyacrylamide. Les populations des USA (Maine, Massachusetts), d'origine récente, ont davantage de variation géographique que celles de France, d'origine ancienne. De plus, parmi ces dernières il y a réduction de l'hétérozygotie dans l'échantillon semi-isolé du Golfe du Morbihan.

F. VUILLEUMIER[28] and MARTHA B. MATTEO

Institut d'Ecologie animale et de Zoologie, Université de Lausanne, 19 Place du Tunnel CH-1005 Lausanne (Switzerland), and Department of Biology, University of Massachusetts, 100 Arlington Street, Boston (Massachusetts 02116, USA), 21 February 1972.

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[28] Present address: Station de biologie marine, Roscoff, Nord-Finistère, France.

Mutation in *Schizophyllum commune* for Resistance to *p*-Fluorophenylalanine

Mutants resistant to chemicals can be fruitfully used in devising techniques for the selection of vegetative segregants in fungi[1,2]. Attempts were made to isolate *p*-fluorophenylalanine resistant mutants in the basidiomycete, *Schizophyllum commune*, for the purpose of studying somatic recombination in dikaryons[3].

The minimum concentration of the chemical inhibiting the growth of any of the stock cultures was found to be 25 mg/l. It was observed that any sensitive strain, inoculated on agar medium containing the chemical, showed light background growth which ceased completely after 2–3 days of incubation. Microscopical examination at this