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.

[22] H. B. BIGELOW, Pap. phys. Oceanogr. Met. *2*, 1 (1933).
[23] A. R. MILLER, Woods Hole oceanogr. Inst. Techn. Rept. *53*, 1 (1952).
[24] R. K. KOEHN, Science *163*, 943 (1969).
[25] H. B. MOORE, J. mar. Biol. Ass. U.K. *21*, 721 (1937).
[26] V. FRETTER and A. GRAHAM, *British Prosobranch Molluscs* (Ray Society, London 1962).
[27] We thank Mr G. COOK for technical assistance, Dr R. MAHÉO for collecting the Bailleron sample, Dr R. C. LEWONTIN for comments, and the University of Massachussetts/Boston for support (Faculty Research Grant No. C 78-71-1). This paper was presented at the Colloque sur le polymorphisme chez les mollusques, Paris, September 1971.
[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

Table I. Analysis of the cross pfp^{r-1} × wild type sensitive strain

Behaviour of the dikaryon on agar medium[a]	Analysis of basidiospore progeny						Remarks
					Segregation		
	No. of basidiospores isolated	No. of spores germinated	Germination (%)	No. of progeny tested on agar medium[a]	No. of resistant type	No. of sensitive type	
Sensitive	540	510	94.4	504	246	258	Shows 1:1 ratio

Table II. Results of testing the dikaryons arizing from crosses between the pfp^{r-1} and each of the other 4 mutants

Crosses	Morphology of the resultant dikaryons	Behaviour of the dikaryons on agar medium[a]	Fruiting (observed upto 1 month)
1. pfp^{r-1} × pfp^{r-2}	Wild type	Sensitive	Did not occur
2. pfp^{r-1} × pfp^{r-3}	Wild type	Sensitive	Did not occur
3. pfp^{r-1} × pfp^{r-4}	'Flat' (like the parents)	Resistant	Did not occur
4. pfp^{r-1} × pfp^{r-5}	Wild type	Sensitive	Did not occur

[a] Agar medium containing *p*-fluorophenylalanine (500 mg/l).

stage revealed that the hyphae initially growing on the medium became distorted and the cells became abnormal in size. These proved to be the constant phenotypic characters of all the sensitive strains.

Ethyl methane sulphonate (EMS)-treated mycelial fragments of 2 strains of *S. commune* were separately spread on complete agar medium[4] containing *p*-fluorophenylalanine at a concentration of 500 mg/l (added to the medium before sterilisation) to select resistant colony, if any, following incubation for 7 days. 5 resistant isolates were obtained and termed pfp^{r-1} (derived from one parent), pfp^{r-2}, pfp^{r-3}, pfp^{r-4}, pfp^{r-5} (derived from the other parent). Each of the resistant isolates, unlike the respective parent, lacked aerial mycelia, showed somewhat flat growth and produced bigger hyphae with blunt tips which tended to remain on the surface of agar. These characters were distinguishable in the mutant strains, even if grown on agar medium not containing the chemical.

The mutant pfp^{r-1} was crossed with a sensitive wild type strain and the resultant dikaryon was found to be sensitive. An analysis of the basidiospore progeny of this dikaryon showed a 1:1 segregation of the resistant and the sensitive types. These results indicated that the pfp^{r-1} strain carried a recessive gene conferring resistance to *p*-flurophenylalanine (Table I). This was further confirmed while some of the progeny were back-crossed with the parents and their resultant dikaryons and progeny were analyzed. It was observed that mating between any two resistant progeny arising from a cross resulted in the production of a resistant but sterile dikaryon.

Each of the remaining 4 resistant isolates was crossed with the pfp^{r-1} strain and sensitive dikaryons were obtained, except the cross $pfp^{r-1} \times pfp^{r-4}$, which produced a resistant dikaryon (Table II). The dikaryons resulting from all the crosses were, however, sterile and hence their progeny could not be analyzed. Thus, the limited test on the functional allelism in the mutants showed either that more than 1 gene locus were concerned with resistance to *p*-flurophenylalanine or that intra-allelic complementation was taking place. As all the dikaryons resulting from crosses between resistant strains were sterile, their progeny could not be analyzed and the distinction between inter- and intra-genic complementation could not be made. Isolation of many more resistant mutants and testing them for their complementation patterns or, alternatively, their recombinational patterns with different genes may throw more light on this.

A sensitive dikaryon, developed from a cross between the pfp^{r-1} strain and a sensitive one, was macerated and spread on agar medium containing *p*-flurophenylalanine at a concentration of 500 mg/l and incubated for 7 days. It was found that the resistant monokaryotic parent could be recovered roughly at a frequency of 2–4/100 colonies. This indicated that the pfp^{r-1} strain could be suitably used in a selective technique for the isolation of somatic recombinants from dikaryons of *S. commune*.

Résumé. Isolement de mutants résistants à la *p*-fluorophenylalanine et son analyse chez *Schizophyllum communis*.

M. A. Hannan[5]

Atomic Energy Centre, P.O. Box 164, Ramna, Dacca (Bangladesh), 30 August 1971.

[1] J. A. Roper and E. Käfer, J. gen. Microbiol. *16*, 660 (1957).
[2] Y. Parag, Heredity *17*, 305 (1962).
[3] M. A. Hannan, Ph. D. Thesis, London University (1968).
[4] P. J. Snider and J. R. Raper, Am. J. Bot. *45*, 538 (1958).
[5] This is a part of the Ph. D. work carried at the Department of Botany, Queen Mary College, London University, and the author wishes to express here grateful appreciation to Professor E. A. Bevan for his guidance in this study.