G6PD phenotypes in larval progenies from crosses of *Drosophila melanogaster* populations: number of determinations per cross. Assignment to mobility classes was confirmed through control runs of mixed samples

Electromorph mobility	Crosses vg × PMvg		PMvg × vg		vg × PFvg		PFvg × vg		vg × PKvg		PKvg × vg	
	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
1.10	4	...	11	...	6	...	8	...	6	...	9	...
1.00	...	4	...	3	...	3	...	5	...	3	...	5

bution to a multiband pattern, because codominance seems to be the rule in isozyme variation. A Mendelian hypothesis also fails to apply in our further observations of electrophoretic phenotype. In the progeny from vg strain × PMvg, PFvg, PKvg population crosses, neither F_1s nor F_2s exhibit multiband patterns.

Within each generation we did not observe mobility differences among populations, but it is worth pointing out that F_1s show single fast bands of the same mobility as parental vg strain and F_2s single slow bands, the same as parental PMvg, PFvg, PKvg (table). We could interpret this phenomenon as a maternal effect if we could find any difference between F_1 direct and reciprocal crosses: figure 2

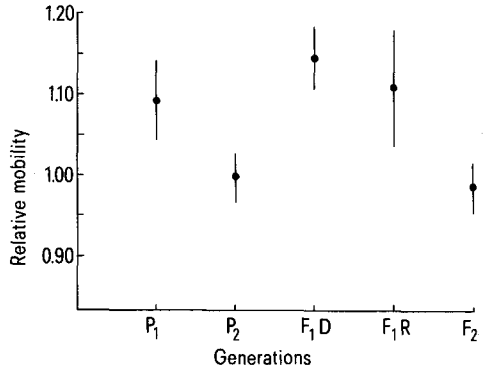

Fig. 2. Comparison between parents and progeny of the cross between electrophoretic variants of larval stage. From left to right, vg population (P_1), averaged PMvg, PFvg, PKvg (P_2), averaged mobilities of 1st generation progeny in direct and reciprocal crosses (F_1D and F_1R), averaged mobilities of 2nd generation progeny (F_2), obtained by random matings within each F_1.

shows that this hypothesis is not verified in our populations. On the basis of these results, we might suggest the existence of regulatory factors affecting the electrophoretic expression of G6PD. They could be contributed by PMvg, PFvg, PKvg populations and favour the appearance of the slow electromorphs; if this regulatory mechanism were unable to operate early on in development, it would not affect F_1 larvae but would do so in F_2 ones; alternatively, vg strain could carry a factor masking the presence of the slow electromorphs in F_1 larvae that is silenced later on.

In any case, the G6PD gene-enzyme system seems to be rich in non-structural variation[2,5,7]; we intend to continue the analysis of the biochemical and genetic relationships of electrophoretic variants observed in larval populations and in their corresponding pupae and imagoes.

Similar phenomena are reported for other gene-enzyme systems in the recent literature[8]: this kind of information can prove most useful to estimate the relative importance of structural and regulatory variations and evaluate their evolutionary rôle[9].

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A genetic study of intergradation between *Mytilus edulis* and *Mytilus galloprovincialis*[1]

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Summary. A study of geographic variation at 2 diagnostic allozyme loci in the mussels *Mytilus edulis* and *M. galloprovincialis* reveals considerable spatial variation, both in allele frequencies and in the extent of intergradation.

Electrophoretic techniques have been used extensively for the characterisation of variation at protein loci in natural populations[2,3], yet considerable problems have arisen in attempts to identify the forces that maintain and modify this variation[4]. Electrophoretic investigation has certain advantages in systematic studies[5] and has proved powerful and informative in analyses of the genetic structure of hybrid zones[6–9]. Here we report the results of an electrophoretic survey of populations of the mussels *Mytilus edulis* and *M. galloprovincialis* in the British Isles which provides evidence for a zone of intergradation of unusual complexity.

M. galloprovincialis is confined largely to the Mediterranean, Adriatic, and Black Seas, where it may have evolved[10], but morphological and electrophoretic evidence suggests that it occurs sympatrically and interbreeds with *M. edulis* in S.W. England and Ireland[11–15]. Its systematic status is uncertain; it has been regarded as a subspecies of *edulis*[16], though in S.W. England good morphological separation and different spawning times provides initial evidence for 2 biological species[11].

Samples of adult mussels were collected from the sites given in figure 1 and assayed electrophoretically at 2 diagnostic loci *Est-D* and *Lap-1*. Technical details and

typical allele frequencies for *edulis* and *galloprovincialis* have been described previously[14,17]. To simplify analysis, alleles at high frequency in *edulis* have been combined as have alleles at high frequency in *galloprovincialis;* the result is 2 'compound' alleles, *E* (for *edulis*) and *G* (for *galloprovincialis*), at each locus. Frequencies of *G* for *Est-D* and $1-G$ (to avoid overlap) for *Lap-1* are given in figure 2. There is striking covariation between loci and large changes in frequency between sites, often occurring over small geographic distances. Frequencies of *G* are highest in *galloprovincialis* from site 8 and southern Europe, and in morphologically typical *galloprovincialis* from site 7. Little geographic variation occurs at both loci among *edulis* samples from N.W. England to S. Wales, the east coast of Ireland, in S.E. England from the Wash southwards and in N. America, Denmark and Holland. In S.W. England, the south and west of Ireland, N.E. England and Scotland *G* rises to intermediate frequencies at some sites. The pattern of variation is consistent with morphological evidence that *galloprovincialis* occurs sympatrically with *edulis* in S.W. England and Ireland[11,12].

Of great interest for studies of *Mytilus* biology is the suggestion of *galloprovincialis* in N.E. England and Scotland. *Galloprovincialis* anatomical characters have been reported in these regions but regarded as ecotypic variation in *edulis*[18,19]. In the absence of genetic evidence, this interpretation is justified as it is well known that such characters may arise as a developmental response to certain environmental conditions[20].

The extent of intergradation resulting from interbreeding in a mixed sample of 2 species having diagnostic loci can be assessed by measuring deviations from Hardy-Weinberg equilibrium and genotypic correlations between loci. A measure of H-W deviation (F) and 2 measures of interlocus correlation (R1 and R2) are given in figure 3 for samples of intermediate frequency (*G* between 0.2 and 0.8). R1 which measures the strength of association of homozygote genotypes, and R2 which measures the deviation of the double heterozygote frequency from expected are defined in the footnote. Mixtures of pure *edulis* and pure *galloprovincialis* having intermediate compound allele frequencies will have F-values of 0.6–0.8, R1-values of 0.95–1.0, and R2-values close to zero for the observed frequencies of *G* between 0.9 (0.1) and 0.95 (0.05) in *galloprovincialis (edulis).* On hybridisation F will decrease, R2 increase, and R1 remain unaltered. If intergradation proceeds, all 3 measures will decrease at different rates to zero values, when the process is complete.

Many samples have positive and significant R1 and F-values below the expected for a 'pure' mixture. This suggests widespread though incomplete intergradation. F, R1 and R2 show considerable variation among samples, which probably reflects real local differences in population genetic structure. Consider the following:

a) High F, high R1, high R2 (e.g. sites 6 and 7) suggests hybridisation but little if any intergradation.

b) Low F, high R1, high R2 (e.g. sites 3 and 4) – again little intergradation but greater hybridisation than for a.

c) Low F, high R1, low R2 (e.g. site 41) – greater intergradation than for b.

d) Low F *(Est-D),* high F *(Lap-1),* low R1, high R2 (e.g. site 37) – considerable intergradation (with differences between loci), and possible selective superiority of double heterozygote.

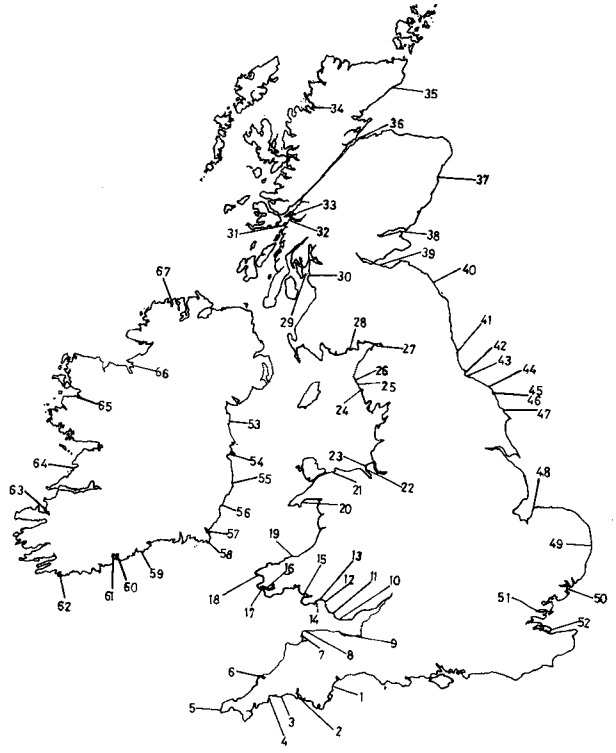

Fig. 1. Location of sample sites. Numbers of individuals scored electrophoretically at each site are as follows: site 1 (168), 2 (238), 3 (120), 4 (120), 5 (45), 6 (411), 7 (832), 8 (34), 9 (62), 10 (68), 11 (100), 12 (102), 13 (205), 14 (1286), 15 (79), 16 (68), 17 (201), 18 (365), 19 (277), 20 (66), 21 (100), 22 (64), 23 (34), 24 (100), 25 (120), 26 (180), 27 (60), 28 (80), 29 (80), 30 (66), 31 (100), 32 (80), 33 (40), 34 (133), 35 (80), 36 (100), 37 (80), 38 (38), 39 (100), 40 (100), 41 (100), 42 (91), 43 (100), 44 (34), 45 (119), 46 (292), 47 (34), 48 (76), 49 (67), 50 (79), 51 (55), 52 (68), 53 (40), 54 (40), 55 (40), 56 (40), 57 (80), 58 (78), 59 (79), 60 (88), 61 (137), 62 (37), 63 (121), 64 (79), 65 (75), 66 (40), 67 (80). Additional samples were analyzed from Ronas Voe in the Shetlands, 68 (118); Long Island (N.Y., USA), 69 (80); New Bristol (Maine, USA), 70 (50); Gibraltar, 71 (90); Venice, 72 (103); Kilyos, Black Sea, 73 (50); Zeeland, Holland, 74 (70); Søttrup, Denmark, 75 (50).

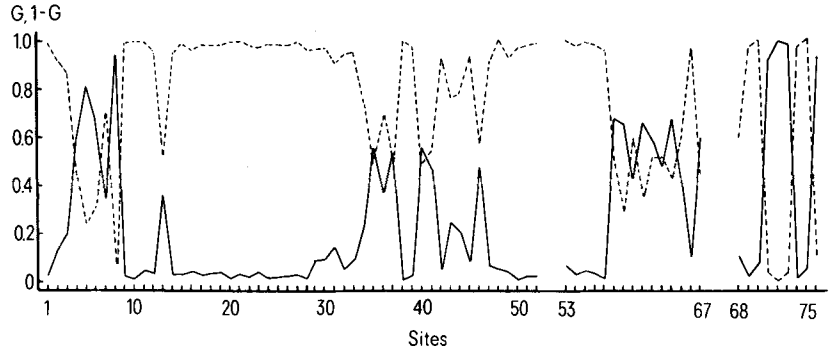

Fig. 2. Frequencies of *G* for *Est-D* (——) and $1-G$ for *Lap-1* (---). Values to the right of site 75 are for morphologically typical *galloprovincialis* picked out of the sample from site 7.

e) Low F, low R1, low R2 (e.g. sites 13, 59 and 61) - complete or a very high level of intergradation. A variety of intermediate situations occur and on the whole intergradation is greater in N.E. England, Ireland and Scotland than S.W. England.

Parallel variation in allele frequencies and in F *(Est-D)* and F *(Lap-1)* ($r=0.54$, $p<0.01$) indicates broad similarity overall in the extent of intergradation between loci. There are exceptions; see d) above and also site 68 in the Shetlands where *G* has much lower frequency at *Est-D* than *Lap-1*.

The pattern of variation can be interpreted in several ways. Hybridisation and secondary intergradation might be occurring between *edulis* and *galloprovincialis* gene pools which have differentiated allopatrically. Large scale migration of *galloprovincialis* to particular regions could explain the macrogeographic differences in allele frequencies in the British Isles, but could not alone explain the sharp local differentiations in Ireland, N.E. England and Scotland. Selection resulting from environmental gradients or ecotones, which differ from locality to locality, is probably involved in these regions. At many sites intergradation, though not complete, is considerable which suggests that some genotypes of mixed ancestry have a selective advantage over *edulis*. This observation provides little support for the traditional view that secondary contact will lead ultimately either to speciation as a result of hybrid inferiority or to complete intergradation[21-23]. Instead it favours the idea that the apparent stability of many zones of intergradation results from superior fitness of hybrids and intergrades[24,25].

Experimental and theoretical results suggest that primary intergradation, that is the evolution of spatial differentiation or sharp clines within a single continuous population, is possible even with extensive gene flow[26]. Primary intergradation resulting from selection on an *edulis*-like base population therefore provides an alternative explanation of the observed macro- and microgeographic variation. Unfortunately the problem of distinguishing between primary and secondary intergradation is considerable without knowledge of the dynamics of population genetic structure. Finally, a decision as to the systematic status of *M. galloprovincialis* is not facilitated by the observed variety of genetic structure among sites, and will depend critically on the geographic location under consideration.

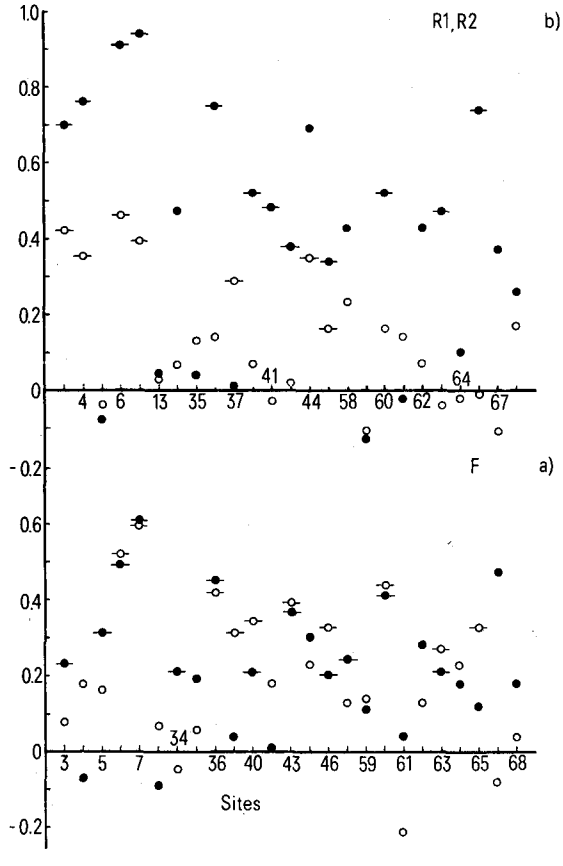

Fig. 3. Values of F, R1 and R2 for sites having intermediate allele frequencies. *a* —●—, ●, F for *Est-D* (significant, non-significant); –○–, ○, F for *Lap-1* (significant, non-significant). *b* —●—, ●, R1 (significant, non-significant); –○–, ○, R2 (significant, non-significant). Significance level is $p<0.05$ or smaller. If single locus genotype frequencies are *EE*(a), *EG*(b) and *GG*(c), $F=(4ac-b^2)/(2a+b)(2c+b)$; value is significant if $|F|>1.96\sqrt{(a+b+c)}$. If 2 locus genotype frequencies are *EE*(*Est-D*) *EE*(*Lap-1*) (r), *EEEG*(s), *EEGG*(t), *EGEE*(u), *EGEG*(v), *EGGG*(w), *GGEE*(x), *GGEG*(y), *GGGG*(z),

$R1=(rz-xt)/((r+t)(x+z)(r+x)(t+z))^{1/2}$ and

$R2=(v(r+t+x+z)-(s+y)(u+w))/((r+t+x+z+s+y)(u+w+v)(r+t+x+z+u+w)(s+y+v))^{1/2}$.

R1 and R2 are product moment correlation coefficients, significance levels determined by a 2×2 contingency test. There is some dependance of F, R1 and R2 on allele frequencies; the effect is relatively small at intermediate frequencies. In localities having low *G* frequencies F, R1 and R2 are uniformly close to zero.

1 This work was supported in part by grant GR3/2452 from the NERC. We thank M. Hunt, A. Dunn and M. Newton for technical assistance and the many people who gave advice or helped in the collection of specimens.

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