Genetic studies of laboratory reared *Mytilus edulis*.

II. Selection at the leucine amino peptidase (*Lap*) locus

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Offspring from several families and a mass mating of *Mytilus edulis* were cultured in the laboratory up to the juvenile stage under different temperature regimes. Mussels were genotyped at the leucine aminopeptidase (*Lap*) locus at the spat stage (post-metamorphosis) using cellulose acetate electrophoresis and at the juvenile stage using starch gel electrophoresis. Significant deviations from expected genotype frequencies were found in many cultures at both stages and certain genotypes were generally found to be favoured irrespective of temperature regime during culture. Estimates of relative fitnesses of the six common *Lap* genotypes suggest overdominance for the alleles *Lap*⁹⁴ and *Lap*⁹⁶ and semi-dominance for *Lap*⁹⁸, but calculations based on this array of estimated fitnesses indicate that *Lap*⁹⁸ would eventually become fixed in a panmictic population. Since a balanced *Lap* polymorphism exists in the wild we conclude that selection differentials at the *Lap* locus in laboratory cultures may be very different from those in nature, and suggest that nutritional constraints may account for some of these differences in selection. Also, in contrast to wild populations, the laboratory cultures showed a significant excess of *Lap* heterozygotes.

INTRODUCTION

The leucine aminopeptidase (*Lap*) locus which codes for an α -aminoacylpeptide hydrolase (Young *et al.*, 1979) in *Mytilus edulis* has been extensively studied in recent years (for reviews see Levinton and Koehn, 1976; Koehn, 1985; Hilbish and Koehn, 1985). Five *Lap* alleles have been detected, although only three are common in any one area (Levinton and Koehn, 1976). In UK waters and on the eastern coast of America, south of Cape Cod, *Lap*⁹⁴, *Lap*⁹⁶, and *Lap*⁹⁸ are the commonest alleles (Koehn *et al.*, 1976; Skibinski *et al.*, 1983; Ahmad and Hedrick, 1985). In Long Island Sound, New York, there is a cline in the frequency of *Lap*⁹⁴ that is associated with changes in salinity along the Sound. Under varying salinities, biochemical differences in *Lap* allozymes produce genotype specific rates of change in the free amino acid pool which is an important element in cell volume regulation (Hilbish *et al.*, 1982). In a detailed study of this cline, Hilbish and Koehn (1985) concluded that a high degree of dominance existed for the biochemical and physiological phenotypic expression of *Lap*⁹⁴ genotypes

and that a selection model incorporating dominance gave a close fit to observed *Lap* genotype frequencies among natural populations in the cline.

This paper reports on genotype specific mortalities at the *Lap* locus in offspring from a number of families and a mass mating of *M. edulis* which were reared in the laboratory at different temperatures but at constant salinity. Evidence is provided for selection at the *Lap* locus, and estimates are made of the relative fitnesses of *Lap* genotypes.

MATERIALS AND METHODS

Ripe *M. edulis* were collected in the Menai Strait, North Wales, UK, during 1984 and 1985 and cultures of larvae were reared as described by Beaumont *et al.* (1988). Larvae and juveniles from several families (M1, M2, M3 and M4) and a mass mating (MM) were cultured under a number of temperature regimes. The temperature regime used for each culture is indicated in tables 1–4. Samples for electrophoresis were taken at the spat stage (500–1000 μ m) in all cultures, and again at the

juvenile stage in some of the cultures. Techniques for cellulose acetate microelectrophoresis of spat, starch gel electrophoresis of juveniles and staining for *Lap* are detailed in Beaumont *et al.* (1988).

Expected *Lap* genotype and allele frequencies in the offspring from single families were calculated from the known genotypes of the parents. Expected genotype and allele frequencies for the mass mating were calculated on the assumption of equal and random mixing of all gametes from the 15 mussels (nine males, six females) spawned. Evidence for equal gametic contribution of all progenitors in this mass mating are presented in a previous paper (Beaumont *et al.*, 1988). Deviations of observed from expected genotype and allele frequencies were tested for significance using the χ^2 goodness of fit test.

RESULTS

Lap genotype and allele frequencies are shown for family M2 in table 1. In almost every sample taken from cultures in this family, regardless of temperature and age, heterozygous *Lap*^{94/96} individuals were significantly more frequent than expected. The frequency of the *Lap*⁹⁴ allele was therefore significantly higher than expected in most samples. Four *Lap* genotypes, each expected at equal frequency, were present in family M3 and their observed frequencies in all M3 cultures are

given in table 2. Irrespective of temperature or age at sampling, the *Lap*^{94/96} heterozygote was again significantly more frequent than expected, and in most cases this was also true of the *Lap*^{94/98} genotype. Too few *Lap*^{96/96} homozygotes and *Lap*^{96/98} heterozygotes were observed. Allele frequencies reflect the high abundance of *Lap*^{94/96} and *Lap*^{94/98} individuals with the *Lap*⁹⁴ allele generally being significantly more frequent than expected.

Lap genotype and allele frequencies in samples of spat from cultures of families M1 and M4 were not in disagreement with expected frequencies (table 3). Data from samples taken principally at the spat stage in the mass mating (MM) cultures are presented in table 4. In most samples, observed genotype and allele frequencies differ significantly from those predicted. The genotypes *Lap*^{94/96}, *Lap*^{94/98} and *Lap*^{98/98} are generally more common than expected while the *Lap*^{96/96} genotype is deficient in every culture sampled. No clear trends were evident with regard to the temperature at which the cultures were held. Allele frequency data reveal that the *Lap*⁹⁴ and *Lap*⁹⁸ alleles are generally more common, and the *Lap*⁹⁶ allele always less common than predicted.

Table 5 details the numbers of samples over all families and the mass mating in which the observed frequencies of the various *Lap* genotypes were either greater than, or less than the expected frequencies. If mortalities are random with respect

Table 1 Genotype and allele frequencies at the *Lap* locus in Family M2 of *Mytilus edulis* sampled at the spat and juvenile stages and reared at different temperatures

Expected frequencies		Genotype frequencies			Allele frequencies		
		94/96	96/96	χ^2	94	96	χ^2
		0.500	0.500		0.250	0.750	
A [15, 15]	Spat	0.793 (46)	0.207 (12)	***	0.397	0.603	***
	Juvenile	0.628 (64)	0.373 (38)	*	0.314	0.686	*
B [15, 22]	Spat	0.750 (45)	0.250 (15)	***	0.375	0.625	**
	Juvenile	0.610 (61)	0.390 (39)	*	0.305	0.695	ns
C [22, 15]	Spat	0.660 (33)	0.340 (17)	*	0.330	0.670	ns
	Juvenile	0.500 (51)	0.500 (51)	ns	0.250	0.750	ns
D [22, 22]	Spat	0.667 (38)	0.333 (19)	*	0.333	0.667	*
	Juvenile	0.696 (71)	0.304 (31)	***	0.348	0.652	**

Numbers in round brackets are sample sizes. Temperature regimes for each culture are given in square brackets; the first figure represents the temperature (°C) before sampling at the spat stage, the second, the temperature after spat were sampled.

* = $P < 0.05$.

** = $P < 0.01$.

*** = $P < 0.001$.

ns = not significant.

Table 2 Genotype and allele frequencies at the *Lap* locus in Family M3 of *Mytilus edulis*, sampled at the spat and juvenile stages and reared at different temperatures. For full details see table 1

Expected frequencies		Genotype frequencies					Allele frequencies			
		94/96	94/98	96/96	96/98	χ^2	94	96	98	χ^2
		0.250	0.250	0.250	0.250		0.250	0.500	0.250	
B [15, 22]	Spat	0.343 (34)	0.202 (20)	0.313 (31)	0.141 (14)	*	0.273	0.555	0.172	*
	Juveniles	0.451 (46)	0.333 (34)	0.137 (14)	0.078 (8)	***	0.392	0.402	0.206	***
C [22, 15]	Spat	0.372 (35)	0.266 (25)	0.202 (19)	0.160 (15)	*	0.319	0.468	0.213	ns
	Juveniles	0.549 (56)	0.343 (35)	0.088 (9)	0.020 (2)	***	0.446	0.373	0.181	***
D [22, 22]	Spat	0.365 (35)	0.354 (34)	0.167 (16)	0.115 (11)	***	0.359	0.406	0.235	**
	Juveniles	0.500 (51)	0.373 (38)	0.059 (6)	0.069 (7)	***	0.436	0.343	0.221	***

Table 3 Genotype and allele frequencies at the *Lap* locus in families M1 and M4 of *Mytilus edulis*, sampled at the spat and juvenile stages and reared at different temperatures. For full details see table 1

		Genotype frequencies					Allele frequencies				
Expected frequencies	M1	94/96	94/98	96/96	96/98	χ^2	94	96	98	χ^2	
		0.250	0.250	0.250	0.250		0.250	0.500	0.250		
A [15, 15]	Spat	0.253 (23)	0.274 (25)	0.154 (14)	0.319 (29)	ns	0.264	0.440	0.296	ns	
	Juveniles	0.235 (24)	0.216 (22)	0.333 (34)	0.216 (22)	ns	0.226	0.559	0.215	ns	
B[15]	Spat	0.327 (33)	0.297 (30)	0.218 (22)	0.158 (16)	ns	0.312	0.460	0.228	ns	
		Genotype frequencies					Allele frequencies				
Expected frequencies	M4	92/94	92/96	94/98	96/98	χ^2	92	94	96	98	χ^2
		0.250	0.250	0.250	0.250		0.250	0.250	0.250	0.250	
A [15]	Spat	0.324 (33)	0.294 (30)	0.206 (21)	0.177 (18)	ns	0.309	0.265	0.235	0.191	ns
B [8]	Spat	0.202 (22)	0.211 (23)	0.321 (35)	0.266 (29)	ns	0.206	0.262	0.239	0.293	ns

Table 4 Genotype and allele frequencies at the *Lap* locus in a mass mating of *Mytilus edulis* sampled at the spat and juvenile stages and reared at different temperatures. For full details see table 1

Expected frequencies		Genotype frequencies							Allele frequencies			
		96/98	96/96	94/96	98/98	94/98	94/94	χ^2	94	96	98	χ^2
		0.360	0.330	0.140	0.090	0.070	0.010		0.115	0.580	0.305	
A [15]	Spat	0.375 (39)	0.289 (30)	0.096 (10)	0.154 (16)	0.067 (7)	0.019 (2)	ns	0.101	0.524	0.375	ns
B [15]	Spat	0.340 (35)	0.223 (23)	0.155 (16)	0.126 (13)	0.117 (12)	0.039 (4)	**	0.175	0.471	0.354	**
C [22]	Spat	0.286 (32)	0.116 (13)	0.268 (30)	0.143 (16)	0.179 (20)	0.009 (1)	***	0.232	0.393	0.375	***
D [8]	Spat	0.333 (35)	0.200 (21)	0.200 (21)	0.086 (9)	0.152 (16)	0.029 (3)	***	0.205	0.467	0.328	***
E [15, 15]	Spat	0.230 (26)	0.274 (31)	0.274 (31)	0.106 (12)	0.115 (13)	0.000 (0)	***	0.195	0.527	0.279	***
	Juvenile	0.218 (17)	0.295 (23)	0.154 (12)	0.192 (15)	0.103 (8)	0.039 (3)	***	0.167	0.481	0.353	*
F [15]	Spat	0.442 (46)	0.164 (17)	0.192 (20)	0.125 (13)	0.077 (8)	0.000 (0)	**	0.135	0.481	0.385	*

Table 5 Numbers of samples of cultures of spat or juvenile *Mytilus edulis* reared in the laboratory in which observed *Lap* genotype frequencies are higher than, or lower than expected frequencies. Genotypes *Lap*^{92/94} and *Lap*^{92/96} are not included because these genotypes were only present in two cultures. Under the null hypothesis of equal occurrences of frequencies above or below expectations, the sign test compares the ratio of instances higher than or lower than expected frequencies for each genotype.

<i>Lap</i> Genotype		Number of samples	Number above expected frequency	Number equal to expected frequency	Number below expected frequency	Sign test
Homozygotes	94/94	7	4	—	3	ns
	96/96	24	2	1	21	**
	98/98	7	6	—	1	ns
Heterozygotes	94/96	24	21	1	2	**
	94/98	18	14	—	4	*
	96/98	18	4	—	14	*

* = $P < 0.05$.

** = $P < 0.01$.

ns = non-significant.

Table 6 Numbers of samples of cultures of spat or juvenile *Mytilus edulis* reared in the laboratory in which observed *Lap* allele frequencies are higher than or lower than expected frequencies. *Lap*⁹² is not included. For details of sign test see table 5.

Allele	Number of samples	Number above expected frequency	Number equal to expected frequency	Number below expected frequency	Sign test
<i>Lap</i> ⁹⁴	26	23	1	2	**
<i>Lap</i> ⁹⁶	26	4	1	21	**
<i>Lap</i> ⁹⁸	18	8	—	10	ns

* = $P < 0.05$.

** = $P < 0.01$.

ns = non-significant.

to *Lap* genotypes then equal numbers of samples would be expected to show higher than, or lower than, expected genotype frequencies. The number of instances where observed frequencies are higher than, or lower than expected are tested using the sign test (Sokal and Rohlf, 1981). The genotypes *Lap*^{94/96} and *Lap*^{94/98} occurred at a higher frequency than expected in significantly more than half of the samples, while the reverse was true for the *Lap*^{96/98} and *Lap*^{96/96} genotypes. In table 6 the *Lap* allele frequency data is tested against the same hypothesis that equal numbers of samples should show higher or lower frequencies than expected. *Lap*⁹⁴ frequencies were higher than expected in significantly more than half the samples and this was balanced principally by reduced frequencies of *Lap*⁹⁶, but not *Lap*⁹⁸.

DISCUSSION

Because we have used single pair, or restricted matings we can be sure that deviations from expected genotype frequencies are not due to the Wahlund effect. Moreover, bearing in mind the

dispersal potential of mussels, it is extremely unlikely that any of the adults used in our trials were closely related and inbreeding effects can therefore also be discounted. Deviations from expected genotype frequencies in wild populations can be produced by the presence of null alleles. However, only one parent from one of the single families (M2) could possibly have been heterozygous for a null allele (all others were heterozygous for known *Lap* alleles) and this would have resulted in some M2 offspring appearing to be *Lap*⁹⁴ homozygotes. No such offspring were observed in that family. Null alleles could have gone undetected in the mass mating, but, in more extensive studies of *M. edulis*, no evidence has been found for the existence of null *Lap* alleles (Koehn *et al.*, 1976; Skibinski *et al.*, 1983).

We conclude therefore that the *Lap* genotype specific mortalities observed are most likely due to selection. However, it is important to establish whether selection is occurring at the *Lap* locus or at other linked loci. In the case of a single family, electrophoretically detected loci may act as markers for much of, or entire chromosomes. However, when comparable trends in genotype

frequencies at a locus are seen across progeny from unrelated families and also in offspring from a mass mating then that locus is less likely to be acting as a marker for more than a small portion of the chromosome tightly linked to the locus. Beaumont *et al.* (1983, 1988) have reported data in which significant variations in genotype frequencies at a number of loci occur between unrelated families of *M. edulis*. However, this is not the case at the *Lap* locus in the cultures described here where particular *Lap* genotypes occur consistently at lower or higher frequencies than expected.

There is strong genetic, physiological and biochemical evidence for direct selection at the *Lap* locus in relation to salinity (Hilbish and Koehn, 1985). However, the fact that *Lap* genotypes may have different fitnesses in relation to salinity does not rule out the possibility that linked loci may be involved in expressing the apparent differential fitnesses at the *Lap* locus detailed here. *Lap* alleles or genotypes may impose no fitness constraints at oceanic salinity and therefore may be effectively neutral in situations other than at low salinity. On the other hand, it seems probable that fitness constraints imposed by a locus, or loci closely linked to *Lap* would have effects over a range of salinities rather than being restricted to high salinity. Indeed effects are more likely under the stresses imposed by low salinity. It is difficult to reconcile this with the strong biochemical and physiological evidence which points to direct selection at the *Lap* locus in low salinities.

One further possibility stems from the work of Day *et al.* (1982) who demonstrated that the alcohol dehydrogenase (*Adh*) locus is associated with a chromosome inversion polymorphism in the seaweed fly *Coelopa frigida*, such that consistent significant differences from Hardy-Weinberg expectations recorded at the *Adh* locus are most likely due to the effect of linked loci within the inversion rather than to the *Adh* locus itself. Mussels do not possess polytene chromosomes and therefore the presence of chromosomal inversions can best be detected by observing bivalents during meiosis. As far as we know, no systematic search has yet been undertaken for evidence of inversions during meiosis in mussels although mitotic chromosomes are beginning to be studied in more detail (Dixon and Clarke, 1982; Dixon *et al.*, 1986). We cannot therefore rule out the possible association of the *Lap* locus with other linked loci within a chromosome inversion.

However, in the light of our earlier arguments, and in the absence of evidence of inversion poly-

morphisms, we feel that there is a high probability that the apparent differences in fitness of *Lap* genotypes observed in our experiments are directly related to the *Lap* locus rather than to other closely linked loci in general.

The results clearly show that, in general, the *Lap*^{94/96}, *Lap*^{94/98} and *Lap*^{98/98} genotypes are more frequent, while the *Lap*^{96/96} and *Lap*^{96/98} are less frequent than expected. There is no suggestion in the data that the temperature regime to which a culture was exposed had any significant effect on genotype frequencies.

Using the genotype frequency data it is possible to estimate approximate relative fitnesses for the six common *Lap* genotypes. Estimates were obtained by calculating for each genotype, the mean percentage, over all families and the mass mating, by which observed frequencies deviated from expected frequencies. In the case of *Lap*^{94/94}, which was only present in the mass mating, the mean proportion of *Lap*^{94/94} individuals (0.018 + / - 0.010, 95 per cent CI) over all cultures was not significantly different from the expected proportion (0.01). This genotype was therefore given a fitness of 1. The relative fitnesses of all six *Lap* genotypes are given in Table 7.

Table 7 Relative fitnesses of six *Lap* genotypes estimated from observed frequencies in up to 24 cultures from four single families and one mass mating

Lap genotype	94/94	96/96	98/98	94/96	94/98	96/98
Relative fitness	1.000	0.701	1.480	1.406	1.350	0.725

Hilbish and Koehn (1985) have proposed that a dominance model is most appropriate to explain their data on salinity related selection at the *Lap* locus in Long Island Sound. Individuals which were either homozygous or heterozygous for *Lap*⁹⁴ exhibited similar biochemical and physiological phenotypes that were equally less fit compared to non-*Lap*⁹⁴ genotypes. Furthermore, analyses of heterozygote deficiencies in genotype frequency data produced results which most closely fitted a selection model incorporating dominance. The relative fitnesses at the *Lap* locus estimated from our data would suggest overdominance rather than dominance, with regard to *Lap*⁹⁴ and *Lap*⁹⁶. *Lap*⁹⁴ and *Lap*⁹⁶ heterozygotes had higher fitnesses than their respective homozygotes. On the other hand the *Lap*⁹⁸ heterozygote classes were less fit than the *Lap*^{98/98} homozygote genotype and the intermediate fitnesses of the two heterozygotes indicates semi-dominance in the case of *Lap*⁹⁸.

Theoretical considerations (Crow, 1986) suggest that, if polymorphisms are maintained by selection, then dominance or semi-dominance will lead ultimately to fixation of particular alleles. Only overall overdominance can produce a stable equilibrium between all alleles. The dominance reported by Hilbish and Koehn (1985) for *Lap*⁹⁴ at low salinity depends upon an annual influx of recruits from oceanic populations of mussels with a high frequency of *Lap*⁹⁴. Without such input, *Lap*⁹⁴ would theoretically become lost in estuarine populations of mussels. Beaumont *et al.* (1988) have suggested that there may be selection for, rather than against *Lap*⁹⁴ during the larval stage at low salinity and that ontogenetic changes in fitness may be a feature in the maintenance of the *Lap* polymorphism. However, there is no suggestion of significant ontogenetic differences in fitness of *Lap* genotypes in the data presented here which were collected from cultures reared entirely at oceanic salinities.

Table 8 A model for selection at a locus with three alleles: *A*₁, *A*₂ and *A*₃

Genotype	<i>A</i> ₁ <i>A</i> ₁	<i>A</i> ₂ <i>A</i> ₂	<i>A</i> ₃ <i>A</i> ₃	<i>A</i> ₁ <i>A</i> ₂	<i>A</i> ₁ <i>A</i> ₃	<i>A</i> ₂ <i>A</i> ₃
Fitness	<i>W</i> ₁₁	<i>W</i> ₂₂	<i>W</i> ₃₃	<i>W</i> ₁₂	<i>W</i> ₁₃	<i>W</i> ₂₃
Frequency	<i>p</i> ₁ ²	<i>p</i> ₂ ²	<i>p</i> ₃ ²	2 <i>p</i> ₁ <i>p</i> ₂	2 <i>p</i> ₁ <i>p</i> ₃	2 <i>p</i> ₂ <i>p</i> ₃

Using a standard model for selection at a locus with three alleles (table 8, Crow, 1986) it is possible to calculate what the frequencies of the three alleles would be at equilibrium in a panmictic population in Hardy-Weinberg equilibrium, assuming that the relative fitnesses proposed were applicable and were constant. The equilibrium frequencies of *Lap*⁹⁴ (*p*₁), *Lap*⁹⁶ (*p*₂) and *Lap*⁹⁸ (*p*₃) are calculated as follows (Crow, 1986).

$$p_1 = \frac{D_1}{D}, \quad p_2 = \frac{D_2}{D} \quad \text{and} \quad p_3 = \frac{D_3}{D}$$

where

$$\begin{aligned} D_1 &= (W_{12} - W_{22})(W_{13} - W_{33}) \\ &\quad - (W_{12} - W_{23})(W_{13} - W_{23}) \\ D_2 &= (W_{23} - W_{33})(W_{12} - W_{11}) \\ &\quad - (W_{23} - W_{13})(W_{12} - W_{31}) \\ D_3 &= (W_{13} - W_{11})(W_{23} - W_{22}) \\ &\quad - (W_{13} - W_{12})(W_{23} - W_{12}) \end{aligned}$$

and $D_1 + D_2 + D_3 = D$. W_{11} = fitness of *Lap*^{94/94}, W_{12} = fitness of *Lap*^{94/96} etc. A further quantity, *T*,

is defined as

$$T = W_{11} - W_{13} - W_{13} - W_{33}.$$

For an equilibrium to be stable, *D*₁, *D*₂ and *D*₃ must be positive numbers and *T* negative. Using the fitness values estimated here, the calculated allele frequencies at equilibrium are *Lap*⁹⁴ = 0.632, *Lap*⁹⁶ = 0.332 and *Lap*⁹⁸ = 0.036, but although *T* is a negative number, *D*₁, *D*₂ and *D*₃ are also negative. In a panmictic population therefore, these relative fitnesses would lead eventually to fixation of one allele. In a stable equilibrium with three alleles, the mean fitness of the population should exceed that of any homozygote. If such a fit homozygote existed it would eventually become fixed in the population. The mean fitness at equilibrium, $\bar{W}$, can be calculated from the three allele model (table 8) using the expression:

$$\begin{aligned} \bar{W} &= p_1^2 W_{11} + p_2^2 W_{22} + p_3^2 W_{33} + 2p_1 p_2 W_{12} \\ &\quad + 2p_1 p_3 W_{13} + 2p_2 p_3 W_{23}, \end{aligned}$$

where *p*₁, *p*₂ and *p*₃ are the allele frequencies at equilibrium. The calculated value for mean fitness ($\bar{W}$) at the *Lap* locus is 1.148 and this is lower than the fitness of the *Lap*^{98/98} homozygote (1.480, table 7). The model therefore predicts that a panmictic population with these relative fitnesses would lead to fixation of *Lap*⁹⁸. Looking again at the data used to estimate relative fitnesses, the greatest error is likely to have occurred with estimating the fitness of the *Lap*^{98/98} genotype. Firstly, the estimate is derived from the mass mating cultures alone; no single families had this genotype. Secondly, *Lap*^{98/98} was expected at a low frequency (0.09) in the mass mating progeny. Small differences between observed and expected numbers will have a greater effect on percentage differences than in genotypes expected at higher frequencies. The calculated relative fitness for the *Lap*^{98/98} genotype is probably, therefore an overestimate.

Nevertheless, the analysis suggests that the relative fitnesses of *Lap* alleles observed in these laboratory cultures would lead to fixation of *Lap*⁹⁸. Because all three alleles are in fact present in local wild populations, the relative fitnesses estimated from progeny reared in the laboratory must be different from those in the wild if the polymorphism is indeed maintained by selection.

This is perhaps not a surprising conclusion since laboratory conditions are obviously very different from the situation in the wild. What is unexpected, however, is the extent of genotype specific selection at the *Lap* locus in laboratory

conditions which would be expected to be relatively non-stressful. One source of stress in laboratory culture may be nutrition. Algal cells are provided at higher than normal concentrations, but bacteria are very reduced in number and seawater filtration systems tend also to reduce the levels of free amino acids available for direct uptake by larvae or adults (Manahan, 1983; Manahan and Stephens, 1983). The enzyme coded at the *Lap* locus, an α -aminoacyl peptide hydrolase (EC 3.4.11.-) (Young *et al.*, 1979), is involved in the cleavage of N-terminal amino acids from polypeptides and therefore in the composition of the cellular free amino acid pool (Moore *et al.*, 1981). It is possible that a reduction in the amount of amino acids available for direct uptake by larval and juvenile mussels interacts with the cellular free amino acid pool and therefore may be affected by genotype specific differences in biochemical activity at the *Lap* locus.

Other studies of laboratory-reared single families and mass matings of bivalves have also implicated direct or indirect selection at a number of enzyme loci (Beaumont *et al.*, 1983, 1988; Adamkewicz, 1984; Hvilsom and Theisen, 1984; Mallet *et al.*, 1985, 1986; Gaffney and Scott, 1984). Our results suggest that selection at a locus in the laboratory or in a hatchery condition may be very different from that found in nature and that the extrapolation of genetic data obtained in this way to the situation in the wild must be undertaken with caution. It must also be borne in mind that our data only deal with fitness to the juvenile stage and that changes in relative fitnesses may occur as the mussels grow. However, where data are available (tables 1 and 2), relative fitnesses of *Lap* genotypes generally do not appear to change between spat and juvenile sampling within cultures, and, since mussels are sedentary and experience no major ecological changes while growing from juveniles to adults, we would not expect to observe major changes in relative fitnesses of the *Lap* alleles during this period.

Further suggestive evidence that *Lap* genotype specific mortalities in laboratory conditions may differ significantly from those in the wild comes from analysis of the overall deficiencies of *Lap* heterozygotes. In wild populations of *M. edulis* and most other bivalves studied, *Lap* heterozygotes are generally deficient against Hardy-Weinberg expectations (Koehn *et al.*, 1976; Skibinski *et al.*, 1983; Colgan, 1987) and a number of explanations have been advanced to account for this observation (Zouros and Foltz, 1984; Colgan, 1987). Taking *Lap* genotype data from 37 samples of single family

and mass mating cultures (excluding M4 where all *Lap* genotypes were heterozygous, but including cultures reared at varying salinities (Beaumont *et al.*, 1988), 27 samples showed an excess of heterozygotes while only nine had too few heterozygotes. According to the null hypothesis that equal numbers of excesses and deficiencies are expected, the sign test confirms that there is a significant excess of heterozygotes in these laboratory cultures. This therefore contrasts with the deficiencies of *Lap* heterozygotes displayed by wild populations. However, direct comparison between heterozygote excesses caused by selection in laboratory conditions and heterozygote deficiencies in wild populations may not be entirely valid since the latter phenomenon may be caused by factors other than selection (Zouros and Foltz, 1984). Although there is an apparent anomaly at the *Lap* locus, overall data from all loci studied do show a significant deficiency of heterozygotes in samples taken at the juvenile stage (unpublished data). Thus the overall trend in laboratory cultures is similar to the situation in the wild and the *Lap* locus may be unusual in this respect.

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