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Differences in allele frequencies of *Aat* between high- and mid-rocky shore populations of *Littorina saxatilis* (Olivi) suggest selection in this enzyme locus

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Summary

Samples of the intertidal prosobranch *Littorina saxatilis* were collected along vertical transects from high- to mid-store levels at five different geographic locations of western Europe. Electrophoretic screening of ten metabolic enzymes revealed five highly polymorphic loci. Four of these showed no or few significant differences in allele frequencies between high- and mid-shore samples of *Littorina saxatilis*. The fifth locus, *Aat* (aspartate aminotransferase, EC 2.6.1.1), showed clinal variation in allele frequencies over the few metres of each transect, suggesting that this locus, or a coupled locus, is under selection with a slow allele (Aat^{100}) favoured in mid-shore habitats and a faster allele (Aat^{120}) selected for in the high littoral fringe.

1. Introduction

Heterogeneity within a locus may be established and maintained by selection or by stochastic forces, and it is of fundamental importance to know which is the case before interpreting allozyme data of natural populations. Gene flow, founder effects and non-random mating, may, for example, be inferred from allele frequency distributions of neutral polymorphisms, while selected loci are less useful in this sense. One way to test the neutral theory is by statistical studies of genetic variation over large numbers of loci (e.g. Nei, 1983). To prove neutrality of a particular locus is, however, in principal almost impossible, as all alternative hypotheses of selection must have been rejected. Selection can, on the other hand, be inferred from field data through correlation with environmental parameters (Endler, 1986), although historical factors may sometimes lead to correlations between selectively neutral polymorphisms and environmental clines. Detailed experimental studies may be required to determine the actual locus under selection (see Koehn & Immerman, 1981; Koehn & Siebenaller, 1981), as loci in linkage disequilibrium will reveal similar patterns to the locus under selection.

Allele frequencies have in some marine species been found to parallel environmental gradients of, for example, salinity and temperature (reviewed by Koehn *et al.* 1983). A number of species of the marine gastropod genus *Littorina* have been studied electrophoretically and most of them are found to be highly polymorphic (e.g. Ward & Warwick, 1980; Mastro *et al.* 1982; Janson, 1985*a, b*; Knight *et al.* 1987, but see Janson 1987*a* for an exception). In this genus selection has been suggested as a possible explanation for minor genetic differentiation in *Est*-II (in *L. angulifera* over a scale of kilometres; Gaines *et al.* 1974) and in *Odh* (in *L. saxatilis* over less than 1 km; Janson & Ward, 1984), but in general, no strong indications of selection on enzyme level polymorphism have previously been reported. The present study, however, reports steep allele frequency clines in the enzyme *Aat* in populations of *Littorina saxatilis* distributed over micro-environmental gradients from high- to mid-shore levels.

Littorina saxatilis is mostly a rock dweller confined to the splash zone of shores all around the European and North American Atlantic coasts (Fretter & Graham, 1980), although it may be found also in brackish-water soft-bottom habitats (Janson & Ward, 1985). Populations are generally dense with up to hundreds of individuals per square metre. The species is a direct developer with a sedentary adult stage which results in restricted gene flow over distances of more than tens of metres (Janson, 1983; Janson & Ward, 1984).

2. Materials and methods

Populations from Iceland, Norway, Sweden and Isle of Man were analysed genetically. All populations

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include *Littorina saxatilis* of several exposed pheno-types ('E' *sensu* Janson, 1982), with, in general, different morphological forms of mid- and high-shore snails. The mid-shore form is in all except the Swedish sites similar to what is usually referred to as the species *L. neglecta* (Heller, 1975; Raffaelli, 1982; Fish & Sharp, 1985). However, morphometric and genetic analyses of these populations show that the two forms, at least at our study sites, are conspecific (Johannesson & Johannesson, 1989*a*, *b*). Our study also reconsiders data from earlier studies of *L. saxatilis* to test the hypothesis of selection in *Aat*.

The Swedish population sampled inhabits a west facing rocky shore (Ursholmen) exposed to high wave action in an almost atidal area of the northern Swedish west coast. The splash-zone is wide and *Littorina saxatilis* is found from mean water level up to a vertical height of about 10 m.

Eight samples, mainly from two parallel vertical transects about 10 m apart, were analysed. Three samples were from mean water level: one of them was from within a patch of barnacles, while there were few barnacles at the other two sites. Two additional sites were from about 2 m above mean water level, and the next two were between 2 and 3 m above them. The last sample was from a rock pool at 9 m above mean water level, and here no snails were found on the open rock surfaces.

Vertical transects were likewise studied in Iceland (Akranes and Sandgerði about 50 km apart), in western Norway (Herdla), and in Isle of Man (Port St Mary). Two parallel transects, between 5 and 100 m apart, were sampled at all sites. Each transect included one mid-shore sample from the barnacle zone, one sample from the upper part of the species distribution, that is, the high littoral fringe, and in all transects except Sandgerði 2, one sample intermediate to the two (low littoral fringe) was included. The vertical

distance between the barnacle zone and high littoral fringe samples was about 2 m in all these localities.

About 40 snails from each subpopulation were screened for 10 loci, all loci assayed on the same specimen. Standard procedures of horizontal starch gel electrophoresis and staining were applied as described by Ward & Warwick (1980) on fresh or frozen materials. The interpretation of the zymogram patterns assumed Mendelian inheritance, which had earlier been verified by breeding experiments for nearly all polymorphic enzymes of the present study (Ward *et al.* 1986). The genetics, as well as the morphological characteristics of most populations of this study are further considered in the papers by Johannesson & Johannesson (1989*a*, *b*).

3. Results

Five of the 10 loci are polymorphic with total heterozygosities (H_T) of 0·62 (*Pgm-1*), 0·50 (*Aat-1*), 0·59 (*Mpi*), 0·39 (*Pgi*) and 0·60 (*Np*). The remaining five loci (*Est-1*, *Mdh-1*, *Idh-2*, *Hdh*, and *Xdh*) are fixed for identical alleles in all samples. A genic contingency χ^2 test (Workman & Niswander, 1970) reveals rather few cases of heterogeneity in allele frequencies over the vertical transects in the four loci *Np*, *Pgm*, *Mpi* and *Pgi* (6 of 40 χ^2·s are statistically significant, Table 1). In contrast, the allele frequency distribution at the *Aat* locus differs substantially between high- and mid-shore samples, and this locus is highly heterogeneous over samples within each transect. In each vertical shore transect the frequency of Aat^{100} decreases and that of Aat^{120} increases up the shore (Fig. 1).

In the Swedish site, Ursholmen, there is a dramatic shift from dominance of Aat^{100} at mean sea level to dominance of Aat^{120} above a height of approximately 2 m (Fig. 2). The uppermost sample however, the rock pool, has a relatively high frequency of Aat^{100}. The

Table 1. *A genic contingency* χ^2 *test for five European populations of* Littorina saxatilis. *The degree of heterogeneity in allele frequencies over ten vertical transects (barnacle zone to high littoral frine), is indicated by the* χ^2 *values*

Locus	Ursholmen		Akranes		Sandgerði		Herdla		Port St Mary	
	1	2	1	2	1	2	1	2	1	2
Np	8·92	6·75	8·23	5·69	5·25	3·33	7·45	5·30	12·51	7·98
	9 n.s.	6 n.s.	8 n.s.	8 n.s.	8 n.s.	4 n.s.	8 n.s.	8 n.s.	8 n.s.	8 n.s.
Aat	99·04	77·14	136·68	204·1	101·38	25·86	66·31	31·81	21·01	21·24
	3**	3**	2**	2**	2**	1**	2**	2**	2**	2**
Pgm	5·54	4·37	4·14	51·05	4·09	0·04	13·24	6·71	20·74	8·36
	6 n.s.	6 n.s.	6 n.s.	6**	6 n.s.	3 n.s.	6*	6 n.s.	6**	6 n.s.
Mpi	0·62	6·27	27·02	7·34	15·99	2·37	13·86	8·68	2·98	0·61
	3 n.s.	6 n.s.	8**	8 n.s.	8*	4 n.s.	8 n.s.	8 n.s.	8 n.s.	8 n.s.
Pgi	2·02	3·53	2·81	12·08	2·02	1·14	4·58	3·98	13·57	1·90
	3 n.s.	6 n.s.	6 n.s.	6 n.s.	6 n.s.	3 n.s.	6 n.s.	6 n.s.	6*	6 n.s.

Degrees of freedom and level of significance are indicated as n.s. ($P > 0.05$); * ($0.005 < P < 0.05$); ** ($P < 0.005$). For allele frequencies and sample sizes see Johannesson & Johannesson (1989*b*).
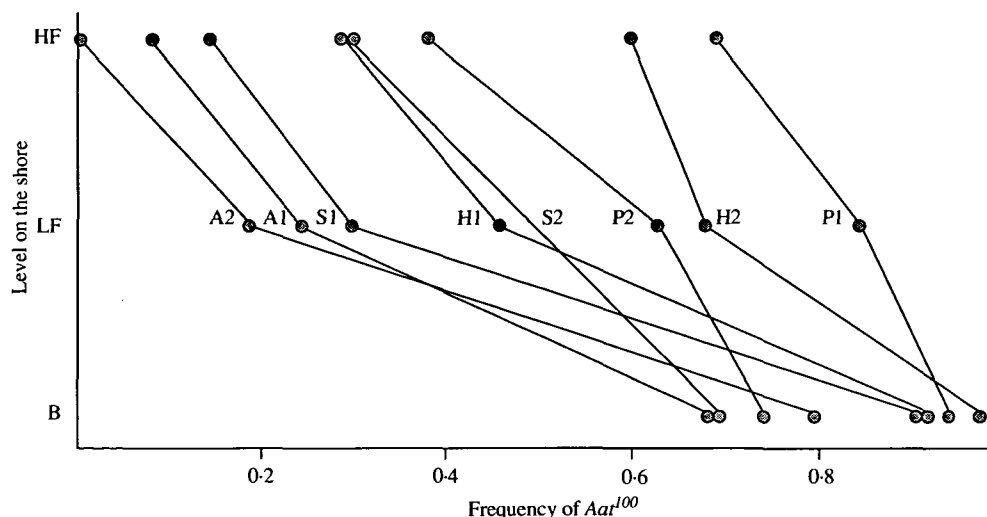
Fig. 1. Clinal variation in the slow moving allele *Aat^{100}* in populations of *Littorina saxatilis*. Each dot represents a sample (sample size = 40) in a vertical transect of a rocky shore, and each transect includes three (or in S2 two) samples, one mid-shore from the barnacle zone (B), one intermediate from the low littoral fringe (LF), and one high-shore from the high littoral fringe (HF). Two parallel transects, 5–100 m in between, are analysed from each locality, Sandgerði (S) and Akranes (A) in Iceland, Herdla (H) in Norway, and Port St Mary (P) on Isle of Man.
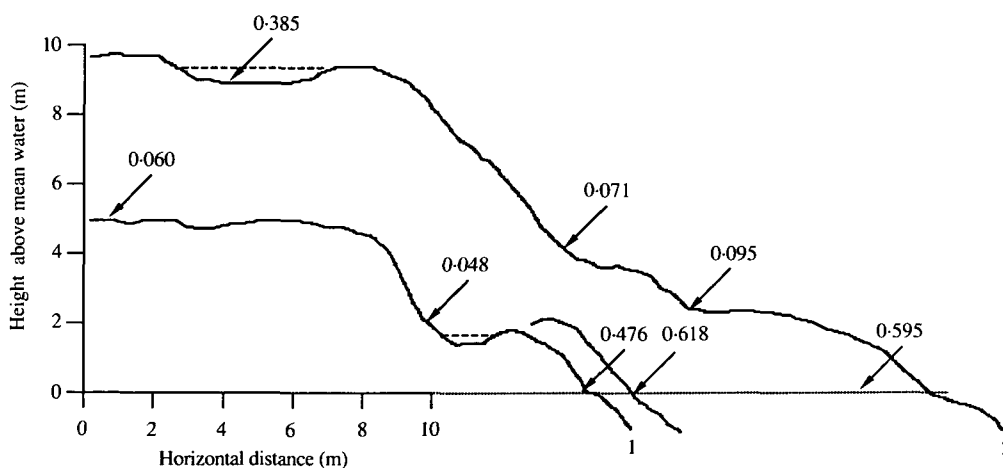

Fig. 2. Frequencies of the slow moving allele *Aat^{100}* in samples from different shore levels at a rocky shore, Ursholmen, Sweden. The two transects 1 and 2 are about 10 m apart. Each transect includes samples (sample size = 40) from the barnacle zone (at mean water level), from the low littoral fringe (at 2 m height), and from the high littoral fringe (at 5 m height). *Aat^{100}* frequencies for two additional samples, one from the barnacle zone about 2 m from transect 1, and one from a rock pool at a height of 9 m, are also indicated.

transects from Iceland, Norway and Isle of Man all reveal a more gradual variation in *Aat* compared to the Swedish transects (Fig. 1), although the intermediate samples (low fringe) in all cases were taken approximately halfway in between the two ends of each transect.

4. Discussion

Small, or no differences in allele frequencies of four highly polymorphic loci (*Pgm, Pgi, Mpi* and *Np*) in five widespread sites suggest that these are roughly neutral to selection over the environmental cline studied. In contrast, the marked clinal variation in the locus *Aat*, which parallels the environmental gradients, suggests strong selection in *Aat*, or in a closely linked locus (e.g. Endler, 1986).

The frequency of *Aat^{100}* and *Aat^{120}* may differ considerably between samples of *L. saxatilis*, as indicated by earlier studies where the fast allele *Aat^{120}* ranges from 0·05 to 0·63 (Janson & Ward, 1984), from 0·03 to 0·72 (Ward & Warwick, 1980), and from 0·00 to 1·00 (Knight *et al.* 1987). Although Janson & Ward (1984) discussed the differentiation of *Aat* in relation to a horizontal gradient of exposure, they concluded that the pattern of variation was not fully consistent with selection.

We have reconsidered data from some earlier studies where it has been possible to recognize the approximate level of sampling on the shore (Table 2). A

Table 2. *Compiled data of* Aat^{100} *frequency distributions within populations of* Littorina saxatilis *from earlier studies. The approximate level on the shore of sampling is indicated. The mean frequency of* Aat^{100} *from* n *populations of similar environments studied is indicated*

Locality	Level on the shore	*n*	Mean of *Aat^{100}*	Reference
Saltö, Sweden	High littoral fringe, exp.	3	0·531	Janson & Ward, 1984
Saltö, Sweden	Low littoral fringe, exp.	5	0·731	Janson & Ward, 1984
Saltö, Sweden	Low littoral fringe, boulder, exp.	3	0·983	Janson & Ward, 1984
Tjärnö, Sweden	Eelgrass meadow, shelt.	1	0·981	Janson & Ward, 1985
Koster Is., Sweden	Barnacle zone, exp.	13	0·942	Janson, 1987
Oostende, Belgium	Barnacle zone, shelt.	1	0·979	Johannesson & Warmoes, 1989
Oostende, Belgium	Barnacle zone, exp.	1	0.983	Johannesson & Warmoes, 1989
Outer Hebrides, U.K.	On fucoid algae, shelt.	1	1·000	Janson & Ward, 1985
Filey Brigg, U.K.	High littoral fringe, exp.	3	0·101	Ward & Janson, 1985
Filey Brigg, U.K.	Low littoral fringe, exp.	2	0·229	Ward & Janson, 1985
Filey Brigg, U.K.	Low littoral fringe, boulder, shelt.	1	0·726	Ward & Janson, 1985

general pattern is that samples from the eulittoral zone (among barnacles, fucoid algae and eelgrass) have a high frequency of *Aat^{100}*, while high littoral fringe samples have lower frequencies of this allele. The low littoral fringe samples show a less consistent pattern with possible differences between boulder and rock habitats. It seems likely that much of the variation in *Aat* found by Janson & Ward (1984) was due to the fact that different samples were taken at different levels of the shore. Obviously, the same may be true in the data presented by Knight *et al.* (1987) who found large between population differences in *Aat* in populations from a number of European, American and two South African sites.

Allele frequency clines which correlate with environmental parameters have been reported several times before but, in general, on scales of tens of kilometres or more (reviews by Burton, 1983; Koehn *et al.* 1983). The vertical cline in *Aat* in *Littorina saxatilis* is, on the other hand, on a scale of metres only. There will be large differences in selection between mid- and high-shore sites as a number of environmental factors show steep gradients across the shore which may be strong enough to generate allele frequency clines in a selected locus. Thus, for example, wetness, temperature, salinity, and food availability all vary with shore level, which is, in fact one of the most variable of all environments.

Gene flow between high- and mid-shore may in addition be somewhat restricted. This is in fact indicated by the extent of differentiation in some of the other polymorphic loci which in a few cases was statistically significant. A direct development and a sedentary life of juveniles and adults (monthly activity range is within a few square metres; Janson, 1983) support the hypothesis of low gene flow over distances of more than a few metres. Indeed, genetic heterogeneity was found over a horizontal scale of only four metres by Janson & Ward (1984).

A low migration rate, of the order of less than one individual per generation (e.g. Hartl, 1980), may cause differentiation between subpopulations in neutral loci as a consequence random genetic drift. However, it seems unlikely that the high level of differentiation and the consistent nature of the cline in *Aat* is a consequence of stochastic processes, as similar steep clines are not found in the other four highly polymorphic loci. The differentiation in *Aat* is instead likely to be an effect of selection on this, or on a coupled locus. The effect of selection may be reinforced by a rather small migration rate between high- and mid-shore subpopulations.

This study has not considered the actual selective forces responsible, although the fact that the rock pool sample has a rather high frequency of the mid shore allele *Aat^{100}* may suggest that a factor such as wetness is important. Indeed, in many ways an elevated rock pool may be more similar to low shore sites than that it is to exposed surfaces at the same elevation. Furthermore, there seems to be a difference between the atidal Swedish site and the tidal sites of Iceland, Norway and Isle of Man in that there is a more marked allele frequency switch between the barnacle zone and the low littoral fringe in the Swedish transects. The low littoral fringe of the Swedish site is wetted more or less unpredictably, due to the lack of tides (Johannesson, 1989), while the low littoral zone of tidal areas is submerged at all tides except neap tides. However, experimental studies are necessary to sort out what factor(-s) may be responsible for selection at the locus *Aat* or at a locus closely linked to it.

References

Burton, R. S. (1983). Protein polymorphisms and genetic differentiation of marine invertebrate populations. *Marine Biology Letters* **4**, 193–206.

Endler, J. A. (1986). *Natural Selection in the Wild.* Princeton, N.J.: Princeton University Press.

Fish, J. D. & Sharp, L. (1985). The ecology of the periwinkle, *Littorina neglecta* Bearn. In *The Ecology of Rocky Coasts* (ed. P. G. Moore and R. Seed), pp. 143–156. London: Hodder & Stoughton.

Fretter, V. & Graham, A. (1980). The prosobranch molluscs of Britain and Denmark. Part 5 – Littorinacea. *Journal of Molluscan Studies* (Suppl.) **7**, 243–284.

Gaines, M. S., Caldwell, J. & Vivas, A. M. (1974). Genetic variation in the mangrove periwinkle *Littorina angulifera*. *Marine Biology* **27**, 327–332.

Hartl, D. L. (1980). *Principle of Population Genetics.* Sunderland, Mass.: Sinauer Associates.

Heller, J. (1975). The taxonomy of some British *Littorina* species, with notes on their reproduction (Mollusca: Prosobranchia). *Zoological Journal of the Linnean Society* **56**, 131–151.

Janson, K. (1982). Phenotypic differentiation in *Littorina saxatilis* Olivi (Mollusca, Prosobranchia) in a small area on the Swedish west coast. *Journal of Molluscan Studies* **48**, 167–173.

Janson, K. (1983). Selection and migration in two distinct phenotypes of *Littorina saxatilis* in Sweden. *Oecologia (Berl.)* **59**, 58–61.

Janson, K. (1985a). Genetic and morphological variation within and between populations of *Littorina angulifera* from Florida. *Ophelia* **24**, 125–134.

Janson, K. (1985b). Genetic variation in three species of Caribbean periwinkles, *Littorina angustior, L. lineolata,* and *L. ziczac* (Gastropoda: Prosobranchia). *Bulletin of Marine Science* **37**, 871–879.

Janson, K. (1987a). Allozyme and shell variation in two marine snails (*Littorina,* Prosobranchia) with different dispersal abilities. *Biological Journal of the Linnean Society* **30**, 245–256.

Janson, K. (1987b). Genetic drift in small and recently founded populations on the marine snail *Littorina saxatilis. Heredity* **58**, 31–37.

Janson, K. & Ward, R. D. (1984). Microgeographic variation in allozyme and shell characters in *Littorina saxatilis* Olivi (Prosobranchia: Littorinidae). *Biological Journal of the Linnean Society* **22**, 289–307.

Janson, K. & Ward, R. D. (1985). The taxonomic status of *Littorina tenebrosa* Montagu as assessed by morphological and genetic analyses. *Journal of Conchology* **32**, 9–15.

Johannesson, B. & Johannesson, K. (1989a). *Littorina neglecta* Bean, a morphological form within the variable species *Littorina saxatilis* (Olivi)? *Hydrobiologia* (in the press).

Johannesson, K. (1989). The bare zone of Swedish rocky shores – why is it there? *Oikos* **54**, 77–86.

Johannesson, K. & Johannesson, B. (1989b). Genetic variation within *Littorina saxatilis* (Olivi) and *Littorina neglecta* Bean. Is *neglecta* a good species? *Hydrobiologia* (in the press).

Johannesson, K. & Warmoes, T. (1989). Rapid colonization of Belgian breakwaters by the direct developer. *Littorina saxatilis* (Olivi). *Hydrobiologia* (in the press).

Knight, A. J., Hughes, R. N. & Ward, R. D. (1987). A striking example of the founder effect in the mollusc *Littorina saxatilis. Biological Journal of the Linnean Society* **32**, 417–426.

Koehn, R. K. & Immerman, F. W. (1981). Biochemical studies of aminopeptidase polymorphism in *Mytilus edulis.* I. Dependence of enzyme activity on season, tissue, and genotype. *Biochemical Genetics* **19**, 1115–1142.

Koehn, R. K. & Siebenaller J. F. (1981). Biochemical studies of aminopeptidase polymorphism in *Mytilus edulis.* II. Dependence of reaction rate on physical fators and enzyme concentration. *Biochemical Genetics* **19**, 1143–1162.

Koehn, R. K., Zera, A. J. & Hall, J. G. (1983). Enzyme polymorphism and natural selection. In *Evolution of Genes and Proteins* (ed. M. Nei and R. K. Koehn), pp. 115–136. Sunderland, Mass.: Sinauer Associates.

Mastro, E., Chow, V. & Hedgecock, D. (1982). *Littorina scutulata* and *Littorina plena*: sibling species status of two prosobranch gastropod species confirmed by electrophoresis. *Veliger* **24**, 239–246.

Nei, M. (1983). Genetic polymorphism and the role of mutation in evolution. In *Evolution of Genes and Proteins* (ed. M. Nei and R. K. Koehn), pp. 165–190. Sunderland, Mass.: Sinauer Associates.

Raffaelli, D. (1982). Recent ecological research on some European species of *Littorina. Journal of Molluscan Studies* **48**, 342–354.

Ward, R. D. & Janson, K. (1985). A genetic analysis of sympatric subpopulations of the sibling species *Littorina saxatilis* Olivi and *Littorina arcana* Hannaford Ellis. *Journal of Molluscan Studies* **51**, 86–94.

Ward, R. D. & Warwick, T. (1980). Genetic differentiation in the molluscan species *Littorina rudis* and *Littorina arcana* (Prosobranchia: Littorinidae). *Biological Journal of the Linnean Society* **14**, 417–428.

Ward, R. D., Warwick, T. & Knight, A. J. (1986). Genetic analysis of ten polymorphic enzyme loci in *Littorina saxatilis* (Prosobranchia: Mollusca). *Heredity* **57**, 233–241.

Workman, P. L. & Niswander, J. D. (1970). Population studies on southwestern Indian tribes. II. Local genetic differentiation in the Papago. *American Journal of Human Genetics* **22**, 24–49.