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Genotypic and phenotypic differences between Baltic and North Sea populations of *Mytilus edulis* evaluated through reciprocal transplantations. II. Genetic variation

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ABSTRACT: There are large differences in allozyme frequencies between Baltic and North Sea populations of *Mytilus edulis*. These differences have recently been ascribed to the presence of 2 species, viz. *M. edulis* L. in the North Sea and *M. trossulus* Gould in the Baltic. Our study supports the earlier findings of an extensive differentiation between North Sea and Baltic mussels. However, in reciprocal transplantations of mussels between a North Sea (20 to 30 ‰ S) and a Baltic (6 to 7 ‰ S) site, 96 % of the North Sea mussels transferred to the Baltic site died immediately. Baltic mussels transferred to the North Sea site survived the first year but 99 % suddenly died after 16 mo. The deaths altered the allele frequencies of the transplanted populations drastically at the 2 loci *Pgm* (phosphoglucomutase) and *Pgi* (phosphoglucose isomerase). The surviving *Pgi* genotypes were very similar to those of mussels native to each site, and this was true also for surviving *Pgm* genotypes of Baltic mussels transplanted to the North Sea. The genotype distribution of the surviving mussels also suggests that *Pgi* and *Pgm* are in linkage-disequilibrium. Allele frequencies at the *Ap* (aminopeptidase) locus did not differ between Baltic and North Sea populations, possibly as a result of a gene flow between Baltic and North Sea mussels. Rare alleles of 'wrong' types being present in both North Sea and Baltic populations at the loci *Lap* (leucine aminopeptidase, also called *Lap-2*), *Pgm* and *Pgi* supports the lack of a reproductive barrier. This study shows that substantial genetic differentiation may be maintained by selection, and we suggest that Baltic and North Sea mussels might well be of the same evolutionary lineage.

INTRODUCTION

Mytilus edulis has a high potential for dispersal during a planktonic larval stage of several weeks. It is therefore not surprising that the species in some areas seems to be genetically rather homogeneous, as, for example, in the North Sea (Fevolden & Garner 1986), in the Baltic (Bulnheim & Gosling 1988), and around the parts of the British and Irish coasts where *Mytilus galloprovincialis* Lmk is absent (Skibinski et al. 1983). More surprising, perhaps, is the finding of a substantial amount of variation in some allozyme loci over relatively short distances, on the order kilometres or less (Koehn et al. 1976, Theisen 1978, Gartner-Kepkay et al. 1983, Koehn et al. 1984).

Reproductive isolation between populations acts as a barrier to genetic exchange and will cause genetic differentiation over time as a result of stochastic processes in neutral loci. Genetic differentiation may alternatively be a consequence of selection at assayed loci, or at loci which are coupled to the selected loci. It is difficult to distinguish between genetic drift and selection in a descriptive study if the 2 genetically distinct groups are found allo- or parapatrically, because selection may differ between the 2 environments and genetic differences may arise despite a substantial gene flow (but see Hilbish & Koehn 1985a). The possibility of differential selection may, however, be examined through reciprocal transplants of non-sympatric populations.

In a series of descriptive and experimental studies of *Mytilus edulis*, Koehn, Hilbish and co-workers have

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worked out the molecular mechanism of selection acting on the highly polymorphic *Lap* locus which shows a clinal variation along a salinity gradient in Long Island Sound (Koehn 1978, Koehn et al. 1980, Hilbish et al. 1982, Hilbish & Koehn 1985b). The genetic variation is extensive; the Lap^{94} allele, for example, changes its frequency from 0.55 to 0.12 over a distance of 30 km (Koehn et al. 1976) and a salinity change of 3.5 ‰. In the Baltic proper the surface salinity is 6 to 8 ‰, while in the Kattegat part of the North Sea it is about 20 ‰ and sharp salinity gradients are built up in the Öresund and the Belts. Theisen (1978) found gradual shifts in allele frequencies of the 3 loci *Pgi, Pgm* and *Lap* over these salinity gradients. Bulnheim & Gosling (1988) confirmed Theisen's findings of a rather homogeneous situation in the Baltic and in the North Sea and steep genetic clines in between. Theisen (1978) transplanted mussels from a site of 8 to 10 ‰ S to a site of 14 to 17 ‰ S and found that different genotypes of the transplanted mussels had different survival rates, indicating selection at the 2 loci *Pgi* and *Pgm*, and to a less extent at *Lap*.

Bulnheim & Gosling (1988) found substantial differences at the loci *Pgm, Pgi, Lap, Odh,* and *Est-D* (= *Est*). No differences were, however, found at the polymorphic *Ap* locus. Varvio et al. (1988) similarly revealed large differences between North Sea and Baltic mussels at *Pgm, Gpi* (= *Pgi*), *Lap* and *Mpi*, but not at the polymorphic loci *Odh, Ap* and *Aap* (= *Lap-1*). At *Pgm* and *Pgi* the Baltic mussels were more similar to mussels from certain areas of Canada (Group III of Koehn et al. 1984) than to North Sea mussels, while at *Ap* the Baltic mussels were most closely related to the North Sea population, and at *Lap* the Baltic, North Sea and Group III mussels were all different from each other. In both studies the conclusion was that the Baltic mussels represent an evolutionary lineage separate from the North Sea mussels. McDonald & Koehn (1988) have since revived the species name *Mytilus trossulus* for Baltic mussels, and for mussels from part of the Atlantic coast of Canada (intermingled with *M. edulis*), the Pacific coast of North America from northern California (where it hybridizes with *M. galloprovincialis*) to Alaska, and the Pacific coast of the Soviet Union.

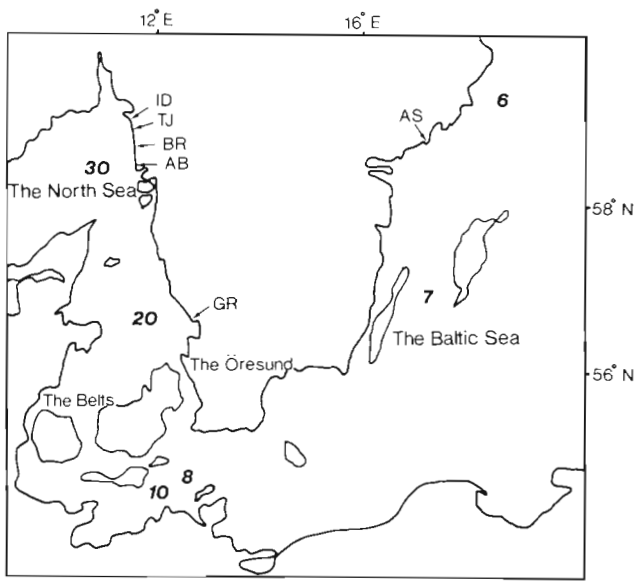

Fig. 1. Locations of sampled populations of *Mytilus edulis* along the Swedish North Sea coast and in the Baltic Sea. Surface salinities are indicated in bold italics

The transplant experiment by Theisen (1978) suggests, however, the possibility that the allozymic differences found between Baltic and North Sea populations are due to differential selection over the steep salinity gradient between the 2 regions. In this study we examine this possibility further by analyzing geographical and temporal variation along the Swedish west coast (North Sea sites), and by making reciprocal transplants of mussels from a site well within the Baltic proper and a site on the Swedish North Sea coast.

This study is accompanied by a study, which deals with growth and morphology (Kautsky et al. 1990), and another which deals with physiology (Tedengren et al. 1990) of the mussels transplanted between the Baltic and the North Sea sites.

MATERIAL AND METHODS

Populations studied. Geographical and temporal allozyme variation were assayed in up to 4 different year classes of mussels from 5 North Sea and 1 Baltic site (Fig. 1; Table 1). Most populations were from small commercial farms of rope-cultured mussels. These populations are recruited from the natural pool of pelagic larvae. The advantage of sampling cultured mussels is that their ages are known. The salinity of the Baltic site is 6 to 7 ‰, while salinities at the North Sea sites are 10 to 30 ‰, with more pronounced fluctuations and lower average values in the southern parts and in estuaries.

A rope with about 600 small mussels settled in June 1984 was transferred from a North Sea site (20 to 30 ‰ S) to the Baltic site at the end of September 1984. Large numbers (ten thousands) of Baltic mussels of different year classes attached to ropes were transplanted to the North Sea site on 4 occasions during the period 1984 to 1986. The transferred mussels were stepwise acclimatized to the ambient salinity of the new site over about a week in the laboratory (for details see Kautsky et al. 1990). Thereafter they were transferred to the sea (still on their ropes) and placed at a depth of about 9 m where settling rate of native mussel larvae generally is

Table 1. Description of sampled localities. The benthic mussels were asigned an approximate age based on the length distribution of the samples and the time of sampling

Sample	Locality		Year class	Substratum	Depth	Salinity
North Sea populations:						
ID83, -87	Idefjorden	59° 6′ N, 11° 17′ E	1983, 87	Benthic	8–10 m	5–25 ‰
TJ82, -84, -85, -87	Tjärnö	58° 53′ N, 11° 9′ E	1982, 84, 85, 87	Rope	0–9 m	19–30 ‰[a]
TJ84• (transferred sample from Tjärnö; see Table 4)						
BR82, -83	Brattö	58° 34′ N, 11° 16′ E	1982, 83	Rope	0–9 m	17–29 ‰[a]
AB82, -83	Åbyfjorden	58° 24′ N, 11° 24′ E	1982, 83	Rope	0–9 m	16–27 ‰[a]
GR82	Grötvik	56° 38′ N, 12° 49′ E	1982	Benthic	0.5 m	9–19 ‰[b]
Baltic populations:						
AS83	Askö	58° 49′ N, 17° 38′ E	1983	Rope	9 m	5–7 ‰[b]
AS83•, -84•, -85•, -86• (transferred samples from Askö; see Table 4)						

[a] From Rödström & Loo (pers. comm.)
[b] From Norman (1977)

very low (Romare et al. 1982). The ropes were inspected weekly from June to September by SCUBA-diving and all newly metamorphosed mussels (which were much smaller than the transferred ones) were removed. Settling rate was very low at this depth as judged from settling on empty parts of the rope.

Genetic analysis. Twelve loci were assayed by horizontal starch gel (12.5%) electrophoresis. Two buffer systems were used: (I) continuous tris-citric acid, pH 8.0 (electrode buffer: 0.25 M tris, 0.057 M citric acid; gel buffer: 25:1 dilution of electrode buffer); (II) discontinuous tris-citrate-borate, pH 8.6 (electrode buffer: 0.3 M boric acid, 0.1 M sodium hydroxide; gel buffer: 0.066 M tris, 0.007 M citric acid, mixed 9:1 with electrode buffer). Parts of the hepatopancreas were homogenized in 0.1 M tris-HCl, pH 8.0 and absorbed onto filter paper for subsequent electrophoresis. Enzyme stains were modified after Shaw & Prasad (1970) and Harris & Hopkinson (1976). Esterases were stained using 1-naphthyl acetate and fast blue BB. Three (isocitrate dehydrogenase, *Idh*, EC code 1.1.1.42; mannose phosphate isomerase, *Mpi*, 5.3.1.8; and esterase, *Est*, 3.1.1.1) gave poor resolutions and were not included in the evaluation of data. Five loci revealed none or very little genetic variation (malate dehydrogenase, *Mdh*, 1.1.1.37; xanthine dehydrogenase, *Xdh*, 1.2.3.2; α-glycerophosphate dehydrogenase, *α-Gpdh*, 1.1.1.8; superoxide dismutase, *Sod*, 1.15.1.1; and aspartate aminotransferase, *Aat*, 2.6.1.1), while the remaining 4 (leucine aminopeptidase, *Lap*, 3.4.11.–; aminopeptidase, *Ap*, 3.4.–.–; phosphoglucose isomerase, *Pgi*, 5.3.1.9; and phosphoglucomutase, *Pgm*, 2.7.5.1) were polymorphic and stained well in most runs. *Pgi, Pgm* and *Lap* have been shown to have Mendelian inheritance (Hvilsom & Theisen 1984).

RESULTS

Geographic and temporal genetic variation

The North Sea and Baltic mussels were very different at the loci *Pgi, Pgm* and *Lap*, while there were only minor differences at *Ap* (Fig. 2). Baltic mussels had a predominance of slow-moving allozymes at *Pgi* and fast-moving allozymes at *Pgm* relative to the North Sea mussels. At *Lap*, a fast-moving allozyme was typical of the Baltic population but rare in the North Sea populations, while an allozyme of intermediate mobility was rare in the Baltic and fairly common among the North Sea mussels (Table 2).

The North Sea populations were somewhat different to each other as revealed by a genic contingency chi-square test (Workman & Niswander 1970); at *Pgi* χ^2 = 124 (df = 40, $p < 0.005$), at *Pgm* χ^2 = 118.9 (df = 50, $p < 0.005$), at *Ap* χ^2 = 66.4 (df = 35, $p < 0.005$), and at *Lap* χ^2 = 41.8 (df = 24, $p < 0.025$). A cluster analysis (UPGMA) of Nei's pairwise genetic identities (*I*; Nei 1972), indicated that the population ID83, and to a minor extent ID87 and GR82, contributed the most to this heterogeneity in *Pgi*. At *Pgm* the population ID87, and at *Lap*, ID83, were most different from the other populations. At *Ap* there was no particular population which deviated from the main cluster. ID is a polluted estuarine site with pronounced fluctuations in salinity and GR is the southernmost North Sea site with the lowest salinity of the North Sea sites (Table 1). It was noted that the ID87 sample included a number of individuals with abnormal shell shapes.

There were small but significant variations between year classes at some sites (Table 3), at TJ (for *Pgi*), at AB (for *Pgi* and *Pgm*) and at ID (for *Pgi* and *Pgm*). The polluted estuarine site ID had the greatest between-

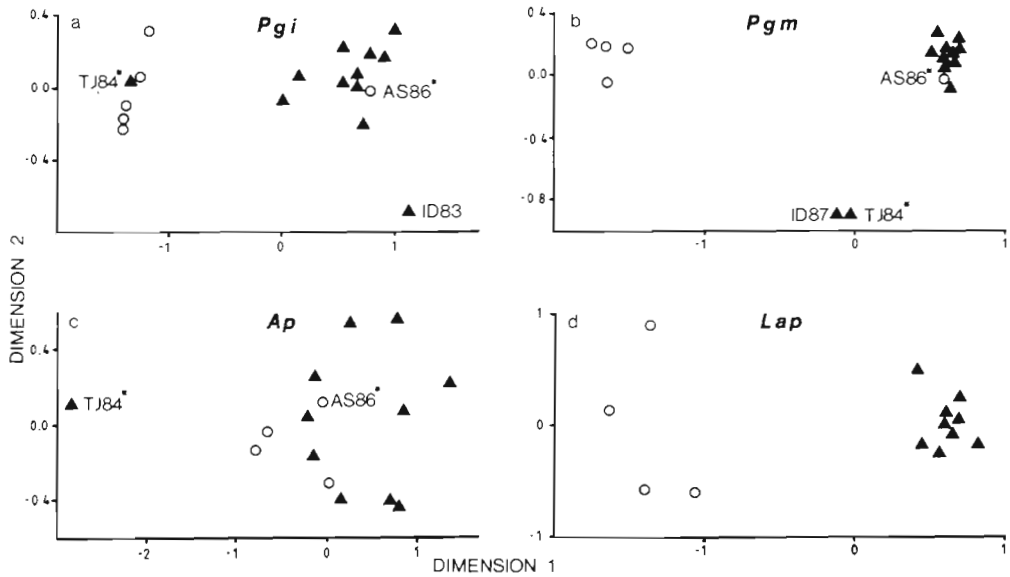

Fig. 2. Multi-dimensional scaling of genetic distance (Nei's *D*) matrices based on allele frequencies at each of 4 loci (a to d). (▲) North Sea; (○) Baltic populations. TJ84* was transferred to AS in the Baltic and AS86* to TJ in the North Sea. Both suffered from very high mortality rates. ID83 and ID87 are from a polluted estuary with pronounced salinity fluctuations

year variation. No population revealed deviations from Hardy-Weinberg expected proportions of genotypes, but this does not rule out the possibility of non-random mating as large samples have to be analysed to test this possibility (Fairbairn & Roff 1981).

Survival and selection of transplanted mussels

The 4 samples of transferred Baltic mussels between 2- and 14-mo-old grew for different periods in the high salinity of the North Sea prior to genetic analysis (Table 4). Three of the samples (AS83*, AS84* and AS85*) which grew 1, 2 and 8 mo, respectively, had survival rates similar to those of native populations. The mussels of AS86* likewise survived well for the first 16 mo, but in late September 1987 most individuals suddenly died (see Tedengren et al. 1990). Only about 200 of the ca 40 000 transferred mussels survived and these were sampled for genetic analysis in August 1988.

The 3 low mortality samples (AS83*, AS84*, AS85*) had allele frequency distributions similar to the untransferred Baltic population (AS83) at *Pgi, Pgm, Ap* and *Lap*. The sample (AS86*) with a mortality rate of 99.5 % became similar to the North Sea populations at *Pgi* and *Pgm* (Table 2; Fig. 2a, b), while at *Ap* there had been no selective mortality (Table 2; Fig. 2c). Unfortunately, *Lap* did not stain accurately in the analysis of this sample.

A few of the alleles of *Pgi* and *Pgm* found at high frequencies in the selected population (AS86*) were not found in the unselected Baltic samples. However, the large number of transferred mussels (ca 40 000), the extremely high mortality rate, and the relatively small sample sizes of the unselected populations (19 to 52) may account for this. The genotype distribution of the selected population indicates, however, that *Pgi* is likely to be in linkage disequilibrium with *Pgm*, as the surviving population have high frequencies of rare alleles at both *Pgi* and *Pgm*. That is, a relatively high number of the surviving mussels carried both allele 2 of *Pgi* and allele 4 of *Pgm*.

One of the transplanted Baltic samples (AS85*) deviated from Hardy-Weinberg expected genotype frequencies with an excess of Pgm^{44} homozygotes ($G = 27.8$, $df = 15$, $p < 0.025$). The reason for this is not obvious. The possibility of a contamination with North Sea mussels is rejected as then an excess of Pgi^{22} homozygotes would also have been present.

The North Sea mussels transferred to the Baltic site (TJ84*) suffered from a high immediate mortality, which is in contrast to the high survival rate of the transferred Baltic mussels during the first year. Of the 600 transferred North Sea mussels of 1 to 40 mm in size (mean 21 mm), only 22 of the smallest (2.0 ± 0.2 mm in size) survived during the week of stepwise adaptation in the laboratory to a salinity of 6 ‰. The survivors were put out for 20 mo, and during this period no further mortality was observed and the mussels grew to an average size of 22.0 ± 1.9 mm.

The initial mortality in TJ84* was coupled to a strong differential selection of genotypes at, particularly, *Pgi*, at *Pgm* and, to a minor extent, at *Ap*. At *Pgi* the resulting allelic distribution was very similar to that of

Table 2. *Mytilus edulis*. Genetic variation in populations from North Sea and Baltic Swedish waters. Population designations indicate locality and year class (see Fig. 1 and Table 1). Five samples (marked '•') were transferred between Baltic and North Sea sites. Alleles numbers refer to the relative mobility of the allozyme in the gel where 1 is the highest mobility

Locus	Allele	North Sea:												Baltic:				
		TJ82	TJ84	TJ85	TJ87	TJ84•	BR82	BR83	AB82	AB83	GR82	ID83	ID87	AS83	AS83•	AS84•	AS85•	AS86•
Pgi	1	0.00	0.04	0.05	0.04	0.00	0.02	0.00	0.00	0.03	0.01	0.04	0.04	0.00	0.00	0.00	0.00	0.04
	2	0.55	0.62	0.55	0.60	0.00	0.53	0.53	0.65	0.56	0.45	0.52	0.46	0.00	0.00	0.00	0.00	0.56
	3	0.22	0.23	0.25	0.22	0.00	0.25	0.32	0.22	0.25	0.18	0.44	0.21	0.00	0.00	0.02	0.00	0.27
	4	0.16	0.11	0.15	0.13	0.91	0.18	0.13	0.08	0.15	0.27	0.00	0.25	0.98	0.84	0.89	0.96	0.12
	5	0.07	0.00	0.00	0.01	0.09	0.02	0.02	0.05	0.01	0.09	0.00	0.04	0.02	0.16	0.09	0.04	0.01
	N	50	52	63	46	22	112	71	85	71	60	46	26	52	43	22	44	74
Pgm	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.06	0.24	0.06	0.00
	2	0.00	0.03	0.01	0.03	0.15	0.01	0.05	0.00	0.04	0.09	0.10	0.15	0.85	0.81	0.68	0.74	0.03
	3	0.21	0.22	0.34	0.26	0.47	0.21	0.22	0.18	0.18	0.20	0.13	0.44	0.06	0.10	0.08	0.08	0.31
	4	0.68	0.72	0.64	0.65	0.38	0.68	0.67	0.65	0.69	0.62	0.72	0.33	0.00	0.04	0.00	0.11	0.64
	5	0.11	0.03	0.01	0.05	0.00	0.09	0.06	0.17	0.09	0.09	0.04	0.04	0.00	0.00	0.00	0.00	0.02
	6	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.01	0.01	0.00	0.04	0.00	0.00	0.00	0.00	0.00
	N	31	58	40	46	17	59	71	66	70	33	45	26	19	42	19	54	74
Lap	1	0.00	0.02	0.00	–	–	0.00	0.03	0.00	0.02	0.05	0.04	–	0.64	0.54	0.66	0.57	–
	2	0.27	0.25	0.33	–	–	0.38	0.31	0.30	0.34	0.26	0.40	–	0.01	0.02	0.16	0.34	–
	3	0.67	0.60	0.59	–	–	0.57	0.61	0.59	0.58	0.58	0.46	–	0.31	0.29	0.18	0.09	–
	4	0.06	0.14	0.09	–	–	0.05	0.05	0.11	0.07	0.12	0.10	–	0.05	0.13	0.00	0.00	–
	N	35	57	23	–	–	62	89	66	52	86	42	–	52	43	22	34	–
Ap	1	0.03	0.00	–	0.02	0.00	0.01	0.03	0.01	0.00	0.00	0.01	0.00	0.00	–	0.00	0.00	0.03
	2	0.15	0.17	–	0.20	0.00	0.23	0.16	0.20	0.26	0.20	0.18	0.20	0.17	–	0.14	0.13	0.17
	3	0.77	0.76	–	0.75	0.98	0.73	0.79	0.70	0.71	0.72	0.79	0.70	0.77	–	0.83	0.84	0.77
	4	0.05	0.07	–	0.00	0.02	0.04	0.03	0.08	0.03	0.08	0.01	0.04	0.06	–	0.02	0.03	0.02
	5	0.00	0.00	–	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.06	0.00	–	0.00	0.00	0.01
	N	50	27	–	46	22	82	35	83	36	60	45	25	24	–	21	63	73

Table 3. *Mytilus edulis*. Temporal heterogeneity in allelic frequencies as revealed by a genic contingency chi-square test (Workman & Niswander 1970) in populations from the North Sea coast of Sweden

Group	Locus	χ^2	df	p
TJ 82/84/85/87	*Pgi*	25.4	12	<0.025
	Pgm	16.1	9	ns
	Ap	13.2	8	ns
	Lap	6.3	6	ns
BR 82/83	*Pgi*	5.6	4	ns
	Pgm	4.8	4	ns
	Ap	3.7	3	ns
	Lap	5.4	3	ns
AB 82/83	*Pgi*	13.7	4	<0.01
	Pgm	10.3	4	<0.05
	Ap	4.2	3	ns
	Lap	4.2	3	ns
ID 83/87	*Pgi*	31.4	4	<0.005
	Pgm	25.9	4	<0.005
	Ap	4.9	4	ns

the native Baltic population (Table 2; Fig. 2a), while at *Pgm* and *Ap*, it became different to both the Baltic and the North Sea populations (Figs. 2b, c).

DISCUSSION

Our results support that of Theisen (1978), Varvio et al. (1988), and Bulnheim & Gosling (1988) in that large genetic differences are present between Baltic and North Sea populations of *Mytilus*. The allele frequency distributions of our study are similar to those of earlier studies although, probably due to small sample sizes, we did not reveal as many rare alleles at *Pgm* and *Pgi* as earlier reported. The genetic differences between Baltic and North Sea populations may be due to: (1) The Baltic mussels descend from a separate evolutionary lineage which has been reproductively isolated from the North Sea populations for a long time during which the large genetic differences observed have been accumulated through stochastic processes. The recent origin of the Baltic Sea suggests that if this is the case the evolutionary lineage which includes the Baltic mussels has evolved outside the Baltic. (2) The brackish-water environment of the Baltic favours different alleles at a number of enzyme loci, and despite distinct genotypes today the 2 populations have a common origin.

Varvio et al. (1988) argued that the genetic similarity between Baltic and e.g Canadian 'Group III' mussels indicates that they belong to the same evolutionary lineage, and McDonald & Koehn (1988) claimed species status for the taxon *Mytilus trossulus* found in the Baltic, eastern Canada, northern California, Oregon and Alaska. McDonald & Koehn (1988) gave no salinity ranges of the sampled localities but mentioned that the '*edulis*-like' mussels (*M. trossulus* and *M. galloprovincialis*) of the Pacific coast 'are common in bays and are also present in the intertidal area of some exposed coast'. In Ireland, *M. galloprovincialis* prefers exposed sites when co-occurring with *M. edulis* (Skibinski et al. 1983) and thus it seems possible that the bay mussels of McDonald & Koehn's Pacific sites are *M. trossulus*. Koehn et al. (1984) described the sites of the 'Group III' mussels from eastern Canada (i.e. *M. trossulus*) as 'open coastal sites', in contrast to the 'Group II' mussels (i.e. *M. edulis*) which inhabited 'more protected or estuarine sites'. However, Gartner-Kepkay et al. (1983) found that estuarine sites with more fluctuating salinities ('head of bay sites') have generally faster-migrating alleles in the *Lap*, *Pep* and *Pgm* loci compared to sites of more stable salinities in the mouth of estuaries, and that genetic similarities correlated with environmental type rather than with geographical distance between populations. This supports the hypothesis of selective differences between populations of estuarine and oceanic salinities. These findings seem contradictory to what Koehn et al. (1984)

Table 4. *Mytilus edulis*. Samples transferred between Baltic (AS) and North Sea (TJ) site. 'Normal' suvival rates are as high as mean survival of rope-growing mussels native to the site – that is, a minor part of the mussels on a rope will normally fall off due to space and food competition. The survival rate of AS86* was initially normal but after 12 mo in the North Sea the mortality rate increased dramatically. TJ84* showed a high mortality during the week of acclimation in the laboratory to the salinity of the Baltic site

Sample	Direction of transfer	No. transferred	Age of transferred mussels (mo)	Time in new habitat (mo)	Survival rate
AS83*	AS to TJ	ca 10 000	14	1	normal
AS84*	AS to TJ	ca 10 000	2	8	normal
AS85*	AS to TJ	ca 10 000	12	4	normal
AS86*	AS to TJ	ca 40 000	2	24	0.5 %
TJ84*	TJ to AS	600	4	20	3 %

reported, but their recognition of 'open coast' and 'protected' sites seems to be on a macrogeographic scale as obviously their open coast sites included both the 'head of bay' and 'mouth of bay' sites of Gartner-Kepkay et al. (1983).

Pgi and *Pgm*, two of the most important loci used by Varvio et al. (1988) and McDonald & Koehn (1988) to discriminate between *M. trossulus* and *M. edulis*, reveal large variation between Baltic and North Sea populations and to a lesser extent variation also among the North Sea sites of our study (e.g. ID87 at *Pgm* and ID83 at *Pgi*). The change in allelic frequencies of the transplanted populations indicate that the extensive differentiation between Baltic and North Sea *Mytilus* in *Pgi* and *Pgm* may be attributed to selection. Furthermore, as the mussels of the small and polluted estuarine site ID are recruited by tidal currents from open coast populations, it seems likely that the genetic differences between this and nearby North Sea sites are consequences of selective survival of North Sea *M. edulis* genotypes.

The Baltic and North Sea populations differed also at *Lap*, but at this locus the *Mytilus trossulus* of eastern Canada were more different to the Baltic mussels than were these to the North Sea mussels (Varvio et al. 1988). At *Ap*, Baltic and North Sea genotypes are similar, but on the other hand the east and west Atlantic populations of *M. edulis* differ (Varvio et al. 1988). Similarly, *Aap* differed between Baltic and North American populations (McDonald & Koehn 1988), but not between Icelandic and Baltic populations (Varvio et al. 1988). At *Odh*, no differences were found between *M. trossulus* and *M. edulis* by McDonald & Koehn (1988) or by Varvio et al. (1988), although Bulnheim & Gosling (1988) found differentiation between Baltic and North Sea samples at this and at *Est-D*. Thus of 8 polymorphic loci, some 5 (*Pgi, Pgm, Lap, Est* and *Mpi*) differ between Baltic and North Sea mussels. However, three of these loci (*Pgm, Pgi* and *Lap*) experience selection over salinity gradients (Theisen 1978, Hilbish & Koehn 1985a,b, this study), while this possibility has not been examined in *Mpi* and *Est*. Watt (1985) argues that enzymes which are central to cellular processes are more likely than other enzymes to be under selection. Indeed, at both *Lap* and *Pgi* different allozymes have different catalytic efficiency (Koehn & Siebenaller 1981, Hall 1985).

The taxonomic status of the Baltic mussels is essentially set by the amount of gene flow over the zone of contact, and this is independent of whether the cline is primary or secondary. If, however, a reproductive barrier is present, it seems more likely to have evolved during isolation of 2 allopatrically developing lineages than between 2 ecotypes, in view of the short evolutionary history of the Baltic. Varvio et al. (1988) reported that the Baltic and North Sea populations 'commonly interbreed'. Our study indicates that post-settlement Baltic mussels may survive for rather long periods in the North Sea, and possibly participate in reproduction, although, as shown by Hilbish & Zimmerman (1988) a difference in allozyme frequencies at one locus *(Lap)* may be enough to displace the timing of the reproduction in 2 genotypes of *Mytilus edulis*. The polymorphic locus *Ap* shows almost no differentiation between Baltic and North Sea populations, or between *M. trossulus* and *M. edulis* populations of Canada, while significant differentiation has been established over the Atlantic Ocean (Bulnheim & Gosling 1988, Varvio et al. 1988). The simplest explanation for the variation in *Ap* is that this locus is selectively neutral over the environments encountered by these *Mytilus* populations, and this suggests furthermore that there is a gene flow between Baltic and North Sea populations. That alleles of *Pgi, Pgm* and *Lap* typical of the Baltic mussels are present, albeit often at low or very low frequencies, in the North Sea, and vice versa supports this suggestion. In fact, no locus is diagnostic between the Baltic and North Sea populations (Bulnheim & Gosling 1988, Varvio et al. 1988) although McDonald & Koehn (1988) claim that *Mpi* has a unique '*trossulus*-allele'; this allele is present at low frequencies in European and Canadian populations of *M. edulis* (Varvio et al. 1988).

Despite a certain gene flow, hybrids may, however, be more or less non-viable due to genetic incompatibility at several loci. Hybrid non-viability impedes gene flow, but as shown by Barton & Bengtsson (1986), for gene flow to be significantly reduced over much of the genome, hybrids must be substantially less fit than non-hybrids, and the number of genes which make the barrier must be so large that most other genes are closely linked to one or another of the loci under selection. Furthermore, even with a strong barrier to neutral genes a gene which is slightly favourable on both sides of the barrier will easily cross the hybrid zone (Barton 1979), and as argued by Barton & Hewitt (1985) as long as this occurs it is relevant to consider the 2 populations as belonging to the same biological species.

Substantial genetic differentiation may evolve rapidly in traits under selection (Endler 1977, 1986). In the marine snails *Littorina saxatilis*, for example, sharp genetic gradients are found over distances in the range of metres only (this species lacks a pelagic larva), when the micro-environment changes accordingly (Janson 1982, 1983, Janson & Ward 1985, Johannesson & Johannesson 1989), while no or small differentiation occurs in neutral enzyme loci (Janson & Ward 1984, Johannesson & Johannesson 1989).

The genetic differences found between Baltic and North Sea *Mytilus* are according to our study, at least at

some of the loci, deterministic rather than stochastic and thus not conclusive in a phylogenetic analysis. Gene flow, albeit restricted by selection in certain loci, is most certainly present between Baltic *Mytilus* and *M. edulis* of the North Sea, although no one has as yet estimated the amount of gene flow in characters shown to be unaffected by selection. As there is no a priori minimum level of gene flow at which one should consider taxa to be incipient species, the decision will always be arbitrary when to consider 2 taxa as conspecific. The situation is as complex in the case of the partly sympatric taxa *M. edulis* and *M. galloprovincialis* Lmk which hybridize to different degrees around the British Isles (Skibinski et al. 1983). While some authors consider the genetic differences large enough to warrant the discrimination of 2 distinct species (McDonald & Koehn 1988) others argue that they are to be considered conspecific (Skibinski et al. 1983, Gosling 1984). An immunological tests of the 3 *Mytilus* taxa, *edulis, trossulus* and *galloprovincialis* (Brock 1985), supports the conclusion of 3 conspecific taxa. We argue that allozyme differences, as well as genetic differences in morphological and physiological characters (Kautsky et al. 1990, Tedengren et al. 1990), which may be caused by differential selection in different environments (of micro- and macro-scale) should not be used as indicators of taxonomic ranks. Furthermore, it is premature to reject the possibility of the Baltic mussels being a specialized brackish water ecotype of North Sea *M. edulis*, that is, the 2 taxa originating from the same ecolutionary lineage.

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