Genetic differentiation of deep-sea hydrothermal vent alvinellid populations (Annelida: Polychaeta) along the East Pacific Rise

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The alvinellid polychaetes, which live in the hottest part of the deep-sea hydrothermal environment, have a nested island-like distribution and locally are subjected to extinctions. They are sedentary and exhibit a peculiar reproductive behaviour and a development which may result in little or no planktonic stage (i.e. larval dispersal). The genetic variation within and among populations of the three main species (*Alvinella pompejana*, *Alvinella caudata* and *Paralvinella grasslei*) inhabiting vents along the East Pacific Rise was examined at a hierarchy of spatial scales using allozyme electrophoresis. The genetic diversity of *P. grasslei* is high ($H_o = 0.24$), about twice that of both the *Alvinella* species ($H_o = 0.10$). The three species show a strong tendency towards a heterozygote deficiency which systematically occurs at the same loci in nearly all the populations. These structures are particularly obvious in the genus *Alvinella* and might be explained by differential allozyme fitness. Populations display considerable genetic differentiation at the microgeographical scale, which could be explained by repeated founder effects in populations, but it varies from species to species according to their possible ability to be transported by crabs from vent to vent. However, the genetic variation among populations separated by at least 1000 km is of the same magnitude as that found within the 13°N/EPR segment. These results demonstrate that each species maintains its genetic identity along the oceanic rifts despite the evidence for founder effects. To explain this phenomenon, we hypothesize that in such a harsh environment, genetic drift in alvinellid populations could be balanced by a uniform selective pressure stemming from the vent chemistry.

Keywords: allozymes, Alvinellidae, F_{ST} , genetic variation, hydrothermal vents.

Introduction

Genetic variation in subdivided populations at equilibrium results from a balance between gene flow, natural selection and genetic drift. In sessile marine invertebrates, numerous studies have reported a correspondence between the ability of larvae to disperse and the amount of genetic differentiation between populations. It is generally admitted that a long larval planktonic phase favours extensive gene flow resulting in little differentiation among populations over large geographical distances, whereas a short

dispersal period or direct development are the main grounds for significant differences between marine populations (Avice, 1994). Nevertheless, whatever the dispersal strategy adopted by organisms, local genetic differentiation in populations may also be a result of selection and/or some form of drift derived from bottleneck effects.

Fauna inhabiting deep-sea hydrothermal vents represent ideal biological material with which to study the relative contributions of these influences. These endemic organisms experience natural radioactivity (Cherry *et al.*, 1992), hypoxia and high concentrations of sulphide, ammonia and heavy metals (Edmond *et al.*, 1982) which greatly exceed any recorded for either

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marine or terrestrial environments. They exhibit a marked linear hierarchy in the spatial arrangement of their populations. Vents are clustered to form hydrothermal fields of a few hundred metres in length, fields are linearly clustered for tens of kilometres within active spreading segments, and ridge segments are separated by transform faults and by distances which can be hundreds of kilometres. At the highest scale, the separate ridges are spaced thousands of kilometres apart (Tunnicliffe, 1991). As a consequence, this island-like arrangement of vents along the ridge axes results in a peculiar, nested distribution of many vent-endemic species. As the geographical distance separating vents increases, the number of shared species seems to decrease (Tunnicliffe, 1988; Hessler & Lonsdale, 1991). At the population level, gene flow estimates should also decrease with geographical distance as predicted by the one-dimensional stepping stone model of Kimura & Weiss (1964). In addition, the environment is characterized by great spatial and temporal instability as a result of tectonic and magmatic events or the dynamic properties of the hydrothermal convection through the basaltic crust of the oceanic rifts (Hessler *et al.*, 1988; Watremez & Kervevan, 1990; Jollivet, 1993; Haymon *et al.*, 1993). The hydrothermal activity shifts along the spreading axes and generates numerous short-lived vents (1–100 years: Lalou, 1991), thus causing catastrophic and chaotic extinctions in populations and inducing the need of a continual colonization of the new active vents (Hessler *et al.*, 1988; Jollivet, 1993). Such an ecological situation may alter the expectations of the one-dimensional stepping stone model which assumes that local populations must be sufficiently stable over time to reach an equilibrium between drift and gene flow.

Several population genetic studies have been conducted on deep-sea hydrothermal vent molluscs (Grassle, 1985; Denis *et al.*, 1993; Vrijenhoek *et al.*, 1993), vestimentiferan tube-worms (Bucklin, 1988; Black, 1991) and amphipods (France *et al.*, 1992). Surprisingly, the results sometimes show a significant variation, and sometimes a lack of variation between well-separated populations which is not clearly related to their dispersal ability but could be explained by selection and genetic drift.

The alvinellid polychaetes are restricted to deep-sea hydrothermal vents. Most of them, especially species belonging to the genus *Alvinella*, live on the hydrothermal chimneys where *in situ* ambient temperatures can occasionally reach up to 105°C (Chevaldonné *et al.*, 1992). Previous studies on their reproduction and larval development (Desbruyères & Laubier, 1986; McHugh, 1989) show that these species have lecithotrophic erpochaete larvae which could reduce their

ability to disperse. More recently, a study has shown that these organisms use internal fertilization to reproduce (Zal *et al.*, 1994) and exhibit an unusual mating behaviour (Chevaldonné & Jollivet, 1993) which could be interpreted as a mechanism to protect offspring against unstable and extreme environmental conditions. Such reproductive behaviour is known to limit dispersal. Both a restricted dispersion and a high rate of extinction in populations raises the question of how alvinellids colonize their environment and maintain the cohesion of the species' gene pool.

The aim of this study was to examine the genetic variability within and among populations of the three main alvinellid species found along the East Pacific Rise and to test the hypothesis that gene flow decreases as a function of the geographical distance under the neutral assumptions of the stepping stone model.

Materials and methods

Samples

Samples of *Alvinella pompejana*, *Alvinella caudata* and *Paralvinella grasslei* were collected at hydrothermal vents along the East Pacific Rise and on the Galapagos Rift (Table 1) using the deep-sea manned submersibles Nautile and Alvin. Collections were made during several cruises supported by the University of Rutgers, New Jersey, the University of California, Santa Barbara and the IFREMER and Woods Hole Institutes. Most of the samples were collected on the walls of active hydrothermal chimneys where temperatures experienced by the organisms varied from 5°C (PARIGO site, 1987) to 45°C (GENESIS and TOTEM sites, 1990). However, the occurrence of *P. grasslei* on chimneys depended mainly on the environmental conditions and no individuals were found above a temperature of 35°C. *P. grasslei* was also sampled living on the vestimentiferan *Riftia pachyptila* in diffuse venting areas at the GENESIS site in 1987 and the ROSE GARDEN site of the Galapagos Spreading Centre in 1988.

Electrophoretic techniques

The gills and the anterior part of the body were homogenized in an equal volume of a special extraction buffer described by Denis *et al.* (1993). This allowed us to avoid inclusion of both the epibiotic bacteria found in the dorsal part of the *Alvinella* spp. (Desbruyères & Laubier, 1980) and the chelated heavy metals found in the tissues of these polychaetes (Gaill *et al.*, 1984). Tissue homogenates were centrifuged at 25 000 g for 30 min at 4°C and electrophoresis was conducted for 4–6 h at 80 mA using 11 per cent starch gel. The stain-

Table 1 Location of the different alvinellid polychaete populations sampled along the East Pacific Rise (EPR) using the deep-sea manned submersibles Alvin and Nautile since 1984

Species	Field	Site	Year	Code	N	Location	Depth
<i>A. pompejana</i>	13°N (EPR)	POGONORD	1987	PO87	20	12°48 61'N-103°56 62'W	2635 m
		PARIGO	1987	PA87	60	12°48 52'N-103°56 48'W	2630 m
		ELSA	1987	EL87	7	12°48 08'N-103°56 34'W	2635 m
		TOTEM	1987	TO87	27	12°48 72'N-103°56 53'W	2630 m
		TOTEM	1990	TO90	18	12°48 72'N-103°56 53'W	2630 m
		GENESIS	1990	GE90	32	12°48 58'N-103°56 47'W	2635 m
	21°N (EPR)	HANGING GARDEN	1990	HG90	21	20°50 30'N-109°06 40'W	2595 m
<i>A. caudata</i>	13°N (EPR)	POGONORD	1987	PO87	19	12°48 61'N-103°56 62'W	2635 m
		PARIGO	1987	PA87	32	12°48 52'N-103°56 48'W	2630 m
		TOTEM	1987	TO87	50	12°48 72'N-103°56 53'W	2630 m
		TOTEM	1990	TO90	23	12°48 72'N-103°56 53'W	2630 m
		GENESIS	1990	GE90	19	12°48 58'N-103°56 47'W	2635 m
	21°N (EPR)	HANGING GARDEN	1990	HG90	33	20°50 30'N-109°06 40'W	2595 m
<i>P. grasslei</i>	13°N (EPR)	PARIGO	1987	PA87	22	12°48 52'N-103°56 48'W	2630 m
		GENESIS	1987	GE87	32	12°48 58'N-103°56 47'W	2635 m
		GENESIS	1990	GE90	34	12°48 58'N-103°56 47'W	2635 m
	21°N (EPR)	HANGING GARDEN	1990	HG90	33	20°50 30'N-109°06 40'W	2595 m
	11°N (EPR)	UNNAMED VENT	1990	VE90	40	11°26 30'N-103°47 30'W	2600 m
	Galapagos	ROSE GARDEN	1988	RG88	9	00°48 25'N-86°13 48'W	2450 m
	Guaymas	UNNAMED VENT	1985	GU85	12	27°00 35'N-111°24 58'W	2010 m

N: sample size.

ing recipes were those provided in Harris & Hopkinson (1976) and Pasteur *et al.* (1987). The assayed enzymes and the buffer media are listed in Table 2. The *A. pompejana* specimens collected at the site POGONORD in 1987 were arbitrarily chosen as our reference population for numbering alleles according to their relative mobility.

Analysis

For each alvinellid colony, the BIOSYS-1 program (version 1.6) of Swofford & Selander (1989) was used to compute the allele frequencies at a given locus, to test for deviations from Hardy-Weinberg equilibrium and to estimate the levels of polymorphism and heterozygosities. Expected genotype frequencies were estimated using Levene's formula (1949) and tested by employing either χ^2 , Yates's χ^2 and G-tests or the exact significance probabilities (Vithayasai, 1973).

We analysed heterozygote deficiencies of each population using the *F*-statistic F_{IS} (Wright, 1965) estimated according to Nei (1977). This statistic measures the deviation of genotype frequencies from the Hardy-Weinberg model within a subpopulation and takes a positive value when a heterozygote deficiency occurs.

To analyse the differentiation across alvinellid populations, we performed both a pairwise analysis using three estimators of gene flow and a spatial hierarchical analysis using *F*-statistics.

To estimate gene flow, we calculate Nm (the effective number of migrants exchanged per generation across two populations) from pairwise combinations of samples within each species. Assuming that the estimator G_{ST} (Nei, 1977) and Weir & Cockerham's (1984) coancestry θ provide reliable estimates of F_{ST} (Slatkin and Barton, 1989), we calculated Nm using Wright's (1965) infinite island model formula: $Nm = (1/F_{ST} - 1)/4$, which relies on the assumptions that alleles are neutral and that mutation and migration are in equilibrium. First, we used genotype frequency data to calculate G_{ST} employing the computer program BIOSYS-1 (Swofford & Selander, 1989). Secondly, we calculated θ from the same set of data employing the program DIPLOID.FOR (Weir, 1990). The third approach to estimate Nm was Slatkin's (1985) 'private allele' method because rare alleles appear to be strongly involved in the genetic variation of alvinellid populations. Following Slatkin's simulation models, Nm is given by the formula: $\ln[p(1)] = a \ln(Nm) + b$ where $a = -0.505$, $b = -2.440$ and $[p(1)]$ corresponds to the mean frequency of private alleles.

Table 2 Enzymes assayed for the alvinellid species and buffer conditions used for the electrophoretic separations

Enzyme	E.C. No.	Locus	Buffer
Aspartate aminotransferase	2.6.1.1	<i>Aat-1</i>	TC 8.0
Aconitase	4.2.1.3	<i>Acoh-1</i>	TC 6.7
		<i>Acoh-2</i>	
Acid phosphatase	3.1.3.2	<i>Acp</i>	TC 6.7
			LiOH 7.0
Adenylate kinase	2.7.4.3	<i>Ak</i>	THCl 8.5
Diaphorase	1.8.1.4	<i>Dia-1</i>	TBE 8.6
		<i>Dia-2*</i>	TC 8.0
		<i>Dia-3**</i>	
Fumarase	4.2.1.2	<i>Fumh*</i>	TC 8.0 ^a
α -Glycerophosphate dehydrogenase	1.1.1.8	<i>α Gpdh*</i>	TC 8.0 ^a
			THCl 8.5
Glucosephosphateisomerase	5.3.1.9	<i>Gpi</i>	TC 8.0
			TCB 8.7
Hexokinase	2.7.1.1	<i>Hk-1**</i>	THCl 8.5
Isocitrate dehydrogenase	1.1.1.42	<i>Idh-1</i>	TC 8.0 ^b
Leucine aminopeptidase (Cytosol)	3.4.11.1	<i>Cap-1</i>	TCB 8.7
		<i>Cap-2</i>	TC 6.7
Malate dehydrogenase	1.1.1.37	<i>Mdh-1</i>	TC 6.7 ^a
		<i>Mdh-2</i>	TC 8.0 ^a
Mannosephosphateisomerase	5.3.1.8	<i>Mpi</i>	TC 8.0 ^a
Phosphoglucumutase	5.4.2.2	<i>Pgm</i>	TC 8.0

The buffer abbreviations correspond to those described by Pasteur *et al.* (1987).

^a 25 mg of NAD added to the gel and the cathodal reservoir.

^b 25 mg of NADP added to the gel and the cathodal reservoir.

*Allozyme pattern only resolved for the genus *Alvinella*.

**Allozyme pattern only resolved for *Paralvinella grasslei*.

The genetic structure of the alvinellid polychaetes was assessed on a hierarchy of spatial scales. The statistics G_{DS} and θ_{DS} (D: deme, S: spreading segment) were obtained from the genotype frequencies of demes within a 2 km portion of the 13°N/EPR hydrothermal segment, the statistics G_{DR} and θ_{DR} (D: deme, R: oceanic ridge) from the genotype frequencies of populations separated by at least a few hundred kilometres along the East Pacific Rise, and, finally, the single statistics G_{DT} and θ_{DT} (D: deme, T: total) were obtained only for *P. grasslei* from the overall genotypic frequencies. These frequencies include the samples collected on the Galapagos Rift and the Guaymas Basin, which are isolated from the East Pacific Rise by both edaphic and bathymetric characteristics (Guaymas Basin) and the Hess Deep depression (Galapagos Rift) at distances which were commonly greater than 1000 km (Fig. 1).

We tested the significance of the F_{IS} and F_{ST} derived from G_{ST} at each locus according to Brown (1970) and

Workman and Niswander (1970), respectively. A jack-knife procedure was used to combine the information across loci within a population or across populations within a locus, which generated a mean and a standard deviation from the single F -statistic estimates. The averages of the F -statistics were then compared to zero using a t -test (Sokal & Rohlf, 1981). In addition, Dixon's test for detecting outliers in a normal distribution (Sokal & Rohlf, 1981) was used on the set of single-locus G_{ST} and θ estimates. Indeed, the occurrence of a locus displaying large differences between populations among loci presenting low variation within populations could suggest that one or several alleles at this locus were under natural selection. The correlation of gene flow estimates obtained from θ with the geographical distance was tested using Mantel's test of the association of two parameters in data matrices with internal correlation (Manly, 1985). This test was performed using one of the procedures of the GENETIX 0.0 package (Bonhomme *et al.*, 1993).

Fig. 1 Location map showing the occurrence of alvinellid species in the Eastern Pacific at two spatial scales: (a) along the East Pacific Rise (EPR) and the Northern Pacific ridges where fields are separated by a few hundred kilometres to a few thousand kilometres (empty stars correspond to the vent collecting sites), (b) within the 13°N/EPR active spreading segment where sites are separated by a few metres to a few tens of metres (black squares correspond to the vent collecting sites).

Results

Genetic diversity of alvinellids

The genetic variability of the three alvinellid species is based on 18 putative enzyme loci including eight, nine and four loci monomorphic in all populations of *A. pompejana*, *A. caudata* and *P. grasslei*, respectively. Most loci of the three species are polymorphic using the 0.99 criterion but the number of observed alleles is much greater for *P. grasslei* than for the *Alvinella* species (see Appendix). The percentage of polymorphic loci, the observed (direct count) and unbiased expected heterozygosities, and the observed number of alleles for the three species are shown in Table 3. This shows evidence for the occurrence of a high genetic variability in *P. grasslei* ($P_{95\%} = 63.5$ and $H_o = 0.237$)

which is about twice that of both the *Alvinella* species. This difference is emphasized by the fact that for each species, populations display the same level of genetic variability (low standard errors).

Population structure

For most of the loci, one allele often predominates in nearly all populations whereas the alternative alleles have frequencies which vary greatly at both the segment and ridge spatial scales (see Appendix). The percentage of alleles whose frequency did not exceed 0.1 ranged from 28 per cent (*P. grasslei*) to 42 per cent (*A. pompejana*). A few loci such as *Aat-1* for *P. grasslei*, *Mpi* for *A. caudata* and *Pgm* for *A. pompejana*, exhibited at least two alleles whose frequencies varied slightly

Table 3 Genetic variability of the three alvinellid species observed along the East Pacific Rise

Species	N	$P_{95\%}$	H_o	H_e	N_o
<i>Alvinella pompejana</i>					
Mean	25.0	36.4	0.094	0.107	1.7
SE	(± 5.1)	(± 4.3)	(± 0.032)	(± 0.034)	(± 0.2)
<i>Alvinella caudata</i>					
Mean	27.6	31.7	0.110	0.118	1.8
SE	(± 3.9)	(± 3.6)	(± 0.038)	(± 0.040)	(± 0.2)
<i>Paralvinella grasslei</i>					
Mean	23.9	63.5	0.237	0.253	2.5
SE	(± 4.0)	(± 1.6)	(± 0.047)	(± 0.052)	(± 0.3)

N , average number of individuals collected per population.

$P_{95\%}$, average percentage of polymorphic loci obtained at the 5% criterion per population.

H_o , average observed heterozygosity per population (direct count).

H_e , average expected heterozygosity per population (unbiased estimate).

N_o , average observed number of alleles per locus in each population. (Values in brackets correspond to the standard error of the mean: SE.)

in samples whereas the occurrence of the other alleles mainly depended on the location of the population (see Appendix).

The *Acoh-1*, *Acoh-2* and *Acp* loci displayed significant departures from the Hardy-Weinberg equilibrium using an exact probability test (see Appendix) for *A. pompejana* and *P. grasslei* at the 5 per cent level of significance. However, except for one (*Acoh-1*, TO87), this result was not supported by the Bonferroni procedure (Hochberg, 1988). Assuming that $F_{IS}\sqrt{N}$ has a normal distribution under the null hypothesis of the Hardy-Weinberg equilibrium (Brown, 1970), this departure corresponds to a significant heterozygote deficiency. Most of the highest single-locus F_{IS} estimates were positive and systematically found at the same loci throughout the demes for all these species (Fig. 2). As a consequence, most of the average multi-locus F_{IS} estimates per population were positive for the three species (Table 4), all the positive values being significant (Student's *t*-test; d.f. = 12; $P < 0.05$) for *A. pompejana* as well as half of those found for *A. caudata* and *P. grasslei*.

Genetic differentiation between populations

Pairwise analysis The genetic differentiation between populations is mostly due to the presence/absence of numerous alleles (see Appendix). However, caution must be exercised when pairwise comparisons are made with EL87 (*A. pompejana*), RG88 and GU85 (*P. grasslei*) as the presence or absence of rare alleles

depends critically on the sample size. Results are presented for each species as a log-scale diagram showing the Nm variation against the geographical distance (Fig. 3). The three methods provide roughly the same distribution of the Nm values. However, the values obtained using the 'private allele' method are lower than those calculated from θ and G_{ST} . When EL87 is removed (small sample size), the Nm values obtained for *A. pompejana* within the 13°N/EPR segment are low if we consider the average distance between two vents but do not fall below the critical value of 1, beneath which genetic differentiation of conspecific populations may occur only by random processes (genetic drift). This is not the case for *A. caudata* and *P. grasslei* for which Nm values appear to be higher. Assuming that alvinellid polychaetes have a low capacity to disperse, these findings do fit the expected theoretical one-dimensional stepping stone model of Kimura and Weiss (1964) at both segment and ridge scales. The curves exhibit patterns which are different according to the species. For *A. pompejana*, Nm values are negatively correlated with geographical distance within the segment 13°N/EPR (Fig. 3a) but no clear relationship is shown at the ridge scale (i.e. when the HG90 sample is added; Mantel's test; $g = 0.063$; $P = 0.438$). Conversely, Nm values obtained for *A. caudata* do not seem to decrease with increasing distances within the segment 13°N/EPR (Fig. 3b) but a positive relationship emerges at the ridge scale (i.e. when the HG90 sample is added; Mantel's test; $g = 0.781$; $P = 0.001$). For *P. grasslei*, Nm values show a slight decrease with the geographical distance (Fig. 3c)

Fig. 2 Histograms showing the genetic structure of the three alvinellid species using Wright's fixation index (F_{IS}). White bars correspond to a heterozygote deviation which systematically occurs at a locus in the same direction within and between species. Sample EL87 was excluded because of its small size. *Corresponds to a significant value of the mean fixation index at the 1 per cent level (t -test).

Table 4 Wright's fixation index (F_{IS}) averaged across loci for each population (within populations) and across populations of each species (across populations) with its jackknife estimate of the standard error

<i>Alvinella pompejana</i>		<i>Alvinella caudata</i>		<i>Paralvinella grasslei</i>	
<i>N</i>	F_{IS}	<i>N</i>	F_{IS}	<i>N</i>	F_{IS}
Within populations					
50	0.116 ± 0.037***	30	0.018 ± 0.029	29	-0.027 ± 0.039
20	-0.044 ± 0.017***	19	0.031 ± 0.037*	20	-0.004 ± 0.033
27	0.052 ± 0.050*	43	0.004 ± 0.020	30	0.013 ± 0.028
32	0.069 ± 0.035***	22	0.003 ± 0.031	32	0.070 ± 0.038***
18	0.132 ± 0.081***	19	0.015 ± 0.048	39	0.058 ± 0.038***
21	0.093 ± 0.074*	33	0.035 ± 0.028**	9	-0.015 ± 0.049
7	-0.014 ± 0.032			12	0.006 ± 0.040
Across populations					
	0.069 ± 0.022***		0.015 ± 0.014*		0.011 ± 0.023

All the means are based on 13 or 12 putative enzyme loci and compared to zero using a t -test (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$).

N corresponds to the number of individuals scored to perform analyses.

(Mantel's test; $g = 2.138$; $P = 0.015$). These relationships do not support the hypothesis of genetic isolation with geographical distance.

Hierarchical analysis The F -statistics obtained from distinct spatial scales are given in Tables 5, 6 and 7. The G_{ST} and θ values give the same trend indicating that genetic differentiation across the alvinellid populations is heterogeneous among loci. Indeed, the values obtained at the segment scale (G_{DS} and θ_{DS}) at the locus *Acoh-2* were four times greater than any other estimates for both *A. pompejana* and *P. grasslei* and depart significantly from the distribution of the other single-locus estimates (Dixon's test: $P < 0.05$). Two jackknife procedures were performed with and without these outliers. In both cases, the average multi-locus F -statistics obtained for all the spatial scales (segment: DS, ridge: DR and total: DT) are clearly significantly different from zero (Tables 5, 6 and 7) indicating that populations are sufficiently differentiated to support the hypothesis that these organisms have a low dispersal ability. However, pairwise comparisons between the overall multilocus G_{DS} or θ_{DS} and G_{DR} or θ_{DR} do not show this differentiation to increase with geographical distance along the 1000 km of the East Pacific Rise for either *A. pompejana* or *P. grasslei* (Student's t -test: $t_{\max} = 2.1$, d.f. = 11, $P > 0.05$) in contrast to the situation in *A. caudata* (Student's t -test: $t_{\min} = 4.4$, d.f. = 11, $P < 0.01$). Nevertheless, the level of differentiation among populations of *P. grasslei* significantly increases when comparisons are made between the overall multilocus G_{DS} or θ_{DS} and G_{DT} or θ_{DT} (Student's t -test: $t_{\min} = 4.9$, d.f. = 12, $P < 0.001$). Although these latter comparisons could be biased by the small sample size of RG88 and GU85, this result may indicate that the genetic isolation of the populations of *P. grasslei* is mostly the result of bathymetric and edaphic gaps between ridges rather than the geographical distance itself. This is clearly shown by the percentages of the alleles which display a significant value of F_{ST} from each set of populations grouped according to the

Fig. 3 Relationship between the number of migrants exchanged between two populations per generation (N_m) and the geographical distance separating their corresponding collection sites at 13°N/EPR for both *Alvinella pompejana* (a) and *Alvinella caudata* (b) and, along the East Pacific Rise for *Paralvinella grasslei* (c). N_m is estimated (1) by Slatkin's 'private allele' method (empty diamond: Slatkin, 1985), and (2) by Wright's formula (Wright, 1965) using F_{ST} derived from G_{ST} (black diamond: Nei, 1977) and θ coancestry (empty circle: Weir & Cockerham, 1984). Bars represent the standard deviation obtained when more than two populations are separated by the same geographical distance.

Table 5 F_{ST} values estimated among distinct segments of the East Pacific Rise (G_{DR} and θ_{DR}) and among sites within the 13°N/EPR segment (G_{DS} and θ_{DS}) for *Alvinella pompejana*

Locus	G_{DS}	$\theta_{DS} \pm SD$	G_{DR}	$\theta_{DR} \pm SD$
<i>Aat-1</i>	—	—	0.020	0.005 $\pm$ 0.005
<i>Acoh-1</i>	0.051	0.016 $\pm$ 0.020	0.044	0.011 $\pm$ 0.016
<i>Acoh-2</i>	0.507***	0.289 $\pm$ 0.162**	0.461***	0.299 $\pm$ 0.124***
<i>Acp</i>	0.025	-0.007 $\pm$ 0.009	0.021	-0.008 $\pm$ 0.007
<i>Ak</i>	0.007	-0.025 $\pm$ 0.012	0.007	-0.026 $\pm$ 0.012
<i>Cap-2</i>	0.014	-0.009 $\pm$ 0.005	0.019	-0.005 $\pm$ 0.007
<i>Fumh</i>	0.027	0.016 $\pm$ 0.030	0.022	0.011 $\pm$ 0.025
<i>α Gpdh</i>	0.060	0.031 $\pm$ 0.031	0.054	0.030 $\pm$ 0.028*
<i>Gpi</i>	0.051	0.032 $\pm$ 0.035	0.055	0.033 $\pm$ 0.035
<i>Idh-1</i>	0.019	-0.014 $\pm$ 0.005	0.029	-0.004 $\pm$ 0.015
<i>Mdh-1</i>	0.069*	0.024 $\pm$ 0.043	0.079*	0.034 $\pm$ 0.042
<i>Mpi</i>	0.106***	0.084 $\pm$ 0.028***	0.100***	0.076 $\pm$ 0.024***
<i>Pgm</i>	0.052	0.018 $\pm$ 0.034	0.049	0.019 $\pm$ 0.025
Mean	0.082***	0.045***	0.075***	0.042***
SD	0.039	0.027	0.033	0.025
Mean§	0.044***	0.020***	0.042***	0.019***
SD§	0.009	0.009	0.008	0.008

Significance levels were determined according to Workman and Niswander (1970) for single values of F_{ST} and by t -tests for jackknife estimates (over populations: θ_{DS} and θ_{DR} and over loci: mean). Table-wide significance levels.

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

§ Jackknife estimates without the *Acoh-2* locus.

Table 6 F_{ST} values estimated among distinct segments of the East Pacific Rise (G_{DR} and θ_{DR}) and among sites within the 13°N/EPR segment (G_{DS} and θ_{DS}) for *Alvinella caudata*

Locus	G_{DS}	$\theta_{DS} \pm SD$	G_{DR}	$\theta_{DR} \pm SD$
<i>Aat-1</i>	0.021	0.000 $\pm$ 0.009	0.019	-0.001 $\pm$ 0.008
<i>Acoh-1</i>	0.038	0.043 $\pm$ 0.036**	0.110***	0.175 $\pm$ 0.087***
<i>Acoh-2</i>	0.012	-0.007 $\pm$ 0.010	0.021	0.002 $\pm$ 0.014
<i>Acp</i>	0.043	-0.007 $\pm$ 0.009	0.042	0.024 $\pm$ 0.014***
<i>Cap-2</i>	0.026	0.002 $\pm$ 0.016	0.028	0.006 $\pm$ 0.015
<i>Fumh</i>	0.014	-0.002 $\pm$ 0.009	0.015	-0.001 $\pm$ 0.009
<i>α Gpdh</i>	0.056*	0.055 $\pm$ 0.052**	0.057*	0.053 $\pm$ 0.045**
<i>Gpi</i>	0.012	0.000 $\pm$ 0.010	0.010	-0.005 $\pm$ 0.007
<i>Idh-1</i>	0.006	-0.007 $\pm$ 0.010	0.058*	0.062 $\pm$ 0.078*
<i>Mdh-1</i>	0.005	-0.017 $\pm$ 0.003	0.008	-0.012 $\pm$ 0.007
<i>Mpi</i>	0.044*	0.015 $\pm$ 0.022*	0.091***	0.082 $\pm$ 0.074**
<i>Pgm</i>	0.053	0.035 $\pm$ 0.038*	0.046	0.025 $\pm$ 0.030*
Mean	0.029***	0.011***	0.042***	0.031***
SD	0.005	0.006	0.009	0.012
Mean§	0.027***	0.010***	0.036***	0.022***
SD§	0.006	0.006	0.008	0.010

Significance levels were determined according to Workman & Niswander (1970) for single values of F_{ST} and by t -tests for jackknife estimates (over populations: θ_{DS} and θ_{DR} and over loci: mean). Table-wide significance levels.

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

§ Jackknife estimates without the *Acoh-1* locus.

Table 7 F_{ST} values estimated among distinct ridges (G_{DT} and θ_{DT}), among distinct segments of the East Pacific Rise (G_{DR} and θ_{DR}) and among sites within the 13°N/EPR segment (G_{DS} and θ_{DS}) for *Paralvinella grasslei*

Locus	G_{DS}	$\theta_{DS} \pm SD$	G_{DR}	$\theta_{DR} \pm SD$	G_{DT}	$\theta_{DT} \pm SD$
<i>Aat-1</i>	0.016	0.012 $\pm$ 0.022	0.023	0.012 $\pm$ 0.009***	0.030	0.011 $\pm$ 0.008***
<i>Acoh-1</i>	0.046	0.047 $\pm$ 0.022***	0.051	0.041 $\pm$ 0.029***	0.070	0.044 $\pm$ 0.024***
<i>Acoh-2</i>	0.106***	0.124 $\pm$ 0.135**	0.076	0.042 $\pm$ 0.056*	0.083	0.051 $\pm$ 0.038***
<i>Acp</i>	0.022	0.007 $\pm$ 0.030	0.031	0.021 $\pm$ 0.016***	0.116*	0.067 $\pm$ 0.044***
<i>Cap-2</i>	0.047	0.042 $\pm$ 0.035***	0.035	0.019 $\pm$ 0.020**	0.177***	0.091 $\pm$ 0.088**
<i>Dia-3</i>	0.019	0.014 $\pm$ 0.017*	0.013	0.004 $\pm$ 0.014	0.128***	0.083 $\pm$ 0.085**
<i>Gpi</i>	0.013	0.007 $\pm$ 0.015	0.011	-0.002 $\pm$ 0.005	0.044	0.008 $\pm$ 0.013
<i>Hk-1</i>	0.038*	0.032 $\pm$ 0.053	0.066***	0.073 $\pm$ 0.064**	0.065**	0.064 $\pm$ 0.055**
<i>Idh-1</i>	0.008	-0.008 $\pm$ 0.005	0.019	0.009 $\pm$ 0.023	0.034	0.011 $\pm$ 0.022
<i>Mdh-1</i>	0.009	-0.008 $\pm$ 0.010	0.020	0.016 $\pm$ 0.018**	0.028	0.013 $\pm$ 0.012**
<i>Mdh-2</i>	0.006	-0.014 $\pm$ 0.009	0.006	-0.014 $\pm$ 0.002	0.009	-0.015 $\pm$ 0.004
<i>Mpi</i>	0.014	-0.003 $\pm$ 0.016	0.016	0.001 $\pm$ 0.005	0.064	0.018 $\pm$ 0.023*
<i>Pgm</i>	0.002	-0.018 $\pm$ 0.003	0.006	-0.009 $\pm$ 0.008	0.051	0.006 $\pm$ 0.020
Mean	0.027***	0.017***	0.029***	0.017***	0.069***	0.037***
SD	0.008	0.011	0.006	0.007	0.013	0.010
Mean§	0.020***	0.009**	0.025***	0.014***	0.068***	0.035***
SD§	0.005	0.007	0.005	0.007	0.014	0.010

Significance levels were determined according to Workman and Niswander (1970) for single values of F_{ST} and by t -tests for jackknife estimates (over populations: θ_{DS} , θ_{DR} and θ_{DT} and over loci: mean). Table-wide significance levels. * $P \leq 0.05$,

** $P \leq 0.01$, *** $P \leq 0.001$. § Jackknife estimates without the *Acoh-2* locus.

Fig. 4 Histograms showing the percentage of alleles which display a significant value of F_{ST} across alvinellid populations nested at distinct spatial scales according to Workman & Niswander (1970) at 5 per cent (scratched bars) and 1 per cent (black bars) levels of significance.

spatial distribution of vents (single-allele F_{ST} : $\chi^2 = 2N.F_{ST}$, Workman & Niswander, 1970) (Fig. 4).

Discussion

Evidence of repeated founder effects in the Alvinella populations

The genetic variability of the deep-sea hydrothermal alvinellid polychaetes is high, especially for *P. grasslei* ($P_{95\%} = 63.5$ per cent, $H_o = 0.235$) in comparison to other invertebrates (Hartl, 1988). This is mainly because of the presence or the absence of numerous alleles whose frequency does not exceed 0.1. This genetic characteristic of alvinellids does not fit the hypothesis that repeated extinctions in populations of hydrothermal vent organisms act to reduce polymorphism, as suggested by Bucklin (1988) to explain the low genetic variability of the deep-sea hydrothermal giant tube-worm *Riftia pachyptila*. Conversely, our values fit well with previous genetic studies conducted on shallow-water polychaetes (Grassle & Grassle, 1976; Nicklas & Hoffman, 1979; Bristow & Vadas, 1991) which reported percentages of polymorphic loci varying from 50 to 70 per cent and heterozygosities which often exceed 0.15. These results are also close to those reported for deep-sea nonhydrothermal organisms (Ayala *et al.*, 1975; Siebenaller, 1978) and contradict the hypothesis that both the stability of the environmental conditions (Gooch & Schopf, 1972) and the high genetic exchanges within nonlimited populations (Soulé, 1976) are responsible for the apparent high genetic variability of these nonhydrothermal deep-sea organisms.

Surprisingly, levels of genetic diversity vary according to the alvinellid genera. We noticed that both the percentage of polymorphic loci and the observed heterozygosity of *P. grasslei* are about twice that of the two *Alvinella* species, and correspond to levels usually found in other paralvinellid species (Jollivet, 1993; Tunnicliffe *et al.*, 1993).

At least three hypotheses could explain this difference: (1) the two *Alvinella* species bear epibiotic bacteria which could favour a low genetic diversity through coevolutionary processes, (2) *Alvinella* spp. live inside a tube and are able to control their micro-environment, (3) genetic exchanges across populations of *Alvinella* spp. may be less important than those across populations of *Paralvinella* spp. because the *Alvinella* species are sedentary and less opportunistic than the other *Paralvinella* species.

The first hypothesis is attractive. The *Alvinella* species as well as the other deep-sea hydrothermal vent symbiont-bearing species must deal with both the

chemoautotrophic symbionts' requirements and their own. As a consequence, such an association may have reduced the host genome to a highly specialized allelic pool. Allozyme studies conducted on deep-sea hydrothermal vent symbiont-bearing species (Grassle, 1985; Bucklin, 1988; Denis *et al.*, 1993; Vrijenhoek *et al.*, 1993) have shown that these organisms display a lower genetic diversity than other endemic vent taxa such as the paralvinellid polychaetes (Jollivet, 1993; Tunnicliffe *et al.*, 1993), the amphipod *Ventiella sulfuris* (France *et al.*, 1992) or some of the limpet species (Vrijenhoek, pers. com.). However, shallow-water species which exhibit endosymbionts such as the lucinids display very high levels of polymorphism (Dwiono *et al.*, 1989).

The second hypothesis assumes that the *Alvinella* species as well as the bivalves or the giant-tube worms which live inside shells or organic tubes are able to control their microenvironment by exhibiting thermoregulatory behaviour (Chevaldonné *et al.*, 1991). In contrast, amphipods and paralvinellid species which are able to move but lack such a protective test have to experience a wider range of environmental conditions which may favour the heterozygote genotypes because of their less energy-demanding maintenance (Koehn & Bayne, 1989).

The third hypothesis is possibly the best explanation for the maintenance of high levels of polymorphism for the paralvinellid species. Unlike the *Alvinella* species, *in situ* observations demonstrate that paralvinellid species crawl over the walls of hydrothermal chimneys in order to feed on deposits (Chevaldonné & Jollivet, 1993) and are able to move from vent to vent using crabs as a vector (Tunnicliffe & Jensen, 1987). Such a phoretic process could increase genetic exchanges across populations, and may avoid bottleneck effects in local populations which are often responsible for reducing polymorphism, as was previously found for subterranean organisms (Nevo *et al.*, 1974).

Genetic structure of alvinellid populations on a microgeographical scale

Genetic structure in marine sedentary invertebrates mostly depends on their ability to disperse and on the heterogeneous conditions that the marine environment provides. The alvinellid polychaetes exhibit a great genetic variation between populations on a microgeographical scale. Such a differentiation mostly arises from the presence or absence of numerous alleles, the frequency of the most common alleles being unaffected. Exposure to high levels of radioactivity may cause such a pattern by increasing the mutation rate as well as the frequency of null alleles, which may account for the systematic heterozygote deficiency

found at many loci. However, no null homozygotes have been observed so far. Nevertheless, the level of differentiation existing between alvinellid populations seems to confirm the low dispersal ability of alvinellids suggested by previous studies conducted on their reproductive system and development (Desbruyères & Laubier, 1986; McHugh, 1989; Zal *et al.*, 1994). Indeed, the tendency towards heterozygote deficiencies in nearly all populations compared with Hardy-Weinberg expectations supports neither a Wahlund effect nor selective mortality in larvae before settlement, as is commonly observed in marine organisms exhibiting a long planktotrophic phase (Zouros & Foltz, 1984) but not in organisms having direct development such as the gastropod *Bembicium vittatum* (Johnson & Black, 1991). Some recent studies have also reported chaotic genetic variabilities among populations at micro- and macro-spatial scales for organisms which do have planktotrophic larvae (Johnson & Black, 1982; Watts *et al.*, 1990; Planes *et al.*, 1994). Such variability has been explained by peculiar hydrodynamic conditions or selection which may vary from habitat to habitat. However, extinction/recolonization could also explain such a differentiation when the environment is unstable.

Wright (1965) claimed that extinction and recolonization alone would enhance genetic differentiation in local populations because the number of individuals which colonize a suitable habitat is lower than the effective size of a local population. *In situ* observations accumulated over the last decade on hydrothermal vent communities have provided evidence for the rapid evolution of vents. The localized cessation of the hydrothermal activity causes strong bottleneck effects in populations of sessile symbiont-bearing species (Hessler *et al.*, 1988; Jollivet, 1993). There are two different models of the way in which colonists might be chosen to found new populations ('migrant pool' and 'propagule pool' models), which have been shown to have very different effects on the rate and the extent of the genetic differentiation of local populations (McCauley, 1991). For alvinellid polychaetes, most of the migrants have to colonize vacant habitats because of the short duration of vents and colonists at a particular vacant site must represent a mix of individuals drawn from one or a few nearby populations ('propagule pool' model). Indeed, Jollivet (1993) reported that the extinction of a vent seems to be associated with the emergence of a new one in its close vicinity, allowing propagules from the dying vent to colonize this vacant habitat. Such a colonization process may result in increasing genetic differentiation in populations and, as long as the number of colonists K is small relative to $2Nm$, may produce the genetic structure we observed (i.e. presence/absence of the less frequent alleles but

less variation in the frequency of the most common alleles).

However, the genetic differentiation between populations could be minimized by the phoretic ability of some of the alvinellid species. Indeed, *in situ* observations have shown that the deep-sea hydrothermal crabs *Bythograea thermydron* and *Cyanograea praedator*, which prey on *P. grasslei* and the *Alvinella* species, respectively, move from vent to vent as soon as the hydrothermal activity ceases at a location. As *B. thermydron* is about 10 times more numerous and more opportunistic than its counterpart *C. praedator* (Fustec *et al.*, 1987), we would expect exchanges across the populations of *P. grasslei* to be more important than those across the *Alvinella* species, as we indeed found.

Genetic differentiation of alvinellid populations along the East Pacific Rise

If repeated founder effects can explain the great genetic differentiation of the *Alvinella* populations on a micro-geographical scale, we cannot explain why the level of differentiation increases so little with geographical distance. At the axis of oceanic ridges, currents are canalized in the central graben and could polarize the larval flux along the rift. However, the average current speeds obtained so far may not be sufficient to support long-range dispersal for the alvinellids (Lonsdale, 1977; A. Vangriesheim, pers. comm.). To explain genetic homogeneity across disjunct populations separated by at least 1000 km, we propose that most of the enzyme loci are exposed to uniform balancing selection mostly driven by the rapid aerobic-anaerobic alternation of the vent conditions, and the outliers we detected using Dixon's test could be the only loci to use in estimating gene flow. Indeed, the hydrothermal environment is conservative and produces a habitat which does not vary spatially along ridges, as was ascertained by comparisons in fluid chemistry between localities (Edmond *et al.*, 1982; Michard *et al.*, 1984). The aerobic-anaerobic alternation of the vent conditions at a location may also be one of the causes for the consistency of apparent heterozygote deficiencies at many loci which are difficult to reconcile with stochastic events. Such an interpretation is somewhat unusual for marine invertebrates for which selection seems to act mostly to produce genetic differences in populations (Johnson & Black, 1991). However, Karl & Avise (1992) argued that allozymes could be exposed to balancing selection to provide an apparent genetic homogeneity in oyster populations in contrast to the results of mtDNA or genomic DNA restriction analyses. Thermostability of allozymes of the AAT and PGM systems vary markedly from genotype to geno-

type within and between alvinellid species (Jollivet, 1993). Such a differential sensitivity of allozymes to temperature may be a genetic response to the temporal instability of the vent emissions and may explain the high levels of polymorphism as well as the genetic homogeneity found among the alvinellid populations along the East Pacific Rise.

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Appendix Allele frequency data for all populations of *Alvinella pompejana*, *Alvinella caudata* and *Paralvinella grasslei*. Locality abbreviations are given in Table 1. Loci allele frequencies in bold correspond to significant deviations from panmixia ($0.01 < P \leq 0.05$) using an exact probability test

A. pompejana						A. caudata						P. grasslei													
Locus	Allele	PA87	EL87	TO87	GE90	TO90	HG90	Locus	Allele	PA87	PO87	TO87	TO90	GE90	HG90	Locus	Allele	GE87	PA87	GE90	VE90	HG90	RG88	GU85	
Aat-1	100	1.000	1.000	1.000	1.000	1.000	0.977	Aat-1	80	—	—	0.021	—	—	0.015	Aat-1	30	0.058	0.068	—	0.118	0.031	0.222	0.200	
	120	—	—	—	—	—	0.023		105	0.984	1.000	0.969	0.955	1.000	0.985		50	0.731	0.773	0.656	0.658	0.594	0.556	0.600	
	75	—	—	0.019	0.016	—	—		130	0.016	—	0.010	0.045	—	—		70	0.212	0.159	0.297	0.224	0.359	0.222	0.200	
Acoh-1	90	0.118	—	0.019	0.063	—	0.075	Acoh-1	120	0.016	—	0.026	0.091	0.026	0.212	Acoh-1	90	—	—	0.047	—	0.016	—	—	
	100	0.853	1.000	0.870	0.875	0.972	0.900		130	0.984	1.000	0.961	0.909	0.974	0.743		115	0.047	0.025	0.241	—	0.125	—	—	
	115	0.029	—	0.092	0.046	0.028	0.025		135	—	—	0.013	—	0.045	120		0.047	—	0.019	0.014	0.047	—	—	0.083	
Acoh-2	80	0.014	—	—	—	—	—	Acoh-2	160	0.500	0.421	0.474	0.455	0.368	0.606	Acoh-2	130	0.766	0.650	0.648	0.868	0.703	0.667	0.875	
	100	0.778	0.950	—	0.943	0.453	0.861		170	0.047	0.105	0.105	0.068	0.026	0.015		135	0.140	0.325	0.092	0.118	0.109	0.333	—	
	120	—	—	—	0.019	0.031	0.056		180	0.453	0.474	0.421	0.477	0.606	0.379		140	—	—	—	—	0.016	—	0.042	
Acp	130	0.180	0.050	1.000	0.019	0.469	0.083	0.625	Acp	105	0.034	0.053	0.081	0.023	0.016	Acp	40	—	—	—	—	0.017	—	—	
	140	0.028	—	—	0.019	0.047	—	120		0.655	0.632	0.779	0.750	0.879	0.833		50	0.737	0.579	0.344	0.461	0.397	0.556	0.750	
	75	0.042	—	—	0.047	—	0.023	135		0.310	0.316	0.140	0.227	0.105	0.167		75	0.263	0.421	0.656	0.539	0.569	0.444	0.250	
Acp	100	0.950	1.000	0.981	0.953	1.000	0.955	Fumh	100	0.016	0.056	0.031	0.023	0.079	0.015	Acp	100	—	—	—	—	0.017	—	—	
	120	0.008	—	—	0.019	—	0.023		106	0.984	0.944	0.969	0.977	0.921	0.985		90	—	—	—	—	0.013	0.034	—	
	80	0.008	—	—	—	—	—		90	0.016	0.053	0.011	—	—	—		105	0.016	0.045	—	—	0.125	—	0.278	
Ak	100	0.992	1.000	1.000	1.000	1.000	1.000	αGpdh	100	0.046	—	0.032	0.024	0.158	0.015	Ak	120	0.140	0.023	0.219	0.039	0.190	0.166	0.375	
	100	1.000	0.975	1.000	0.963	0.952	0.917		0.955	103	0.938	0.947	0.957	0.976	0.842		0.985	135	0.172	0.250	0.094	0.145	0.206	0.334	0.208
	106	—	0.025	—	0.037	0.048	0.083		0.045	Cpi	50	0.016	0.026	0.010	0.023		—	150	0.672	0.682	0.687	0.803	0.570	0.222	0.250
αGpdh	90	0.024	—	—	—	—	—	Dia-3	70	0.953	0.974	0.990	0.997	1.000	0.970	Dia-3	100	0.953	1.000	0.984	0.985	0.987	0.778	1.000	
	100	0.810	0.900	1.000	0.926	0.750	0.861		0.909	100	0.031	—	—	—	0.015		110	0.047	—	0.016	0.015	0.013	0.222	—	
	103	0.118	0.075	—	0.036	0.250	0.139		0.068	Idh-1	50	—	—	0.023	—		—	35	0.016	0.023	0.063	0.053	—	—	—
Gpi	109	0.048	0.025	—	0.019	—	0.023	Idh-1	70	0.234	0.211	0.302	0.250	0.237	—	Gpi	50	0.938	0.954	0.874	0.908	0.940	1.000	0.792	
	50	—	—	—	—	—	—		95	0.766	0.789	0.688	0.727	0.763	1.000		80	0.046	0.023	0.063	0.039	0.045	—	0.166	
	100	0.983	1.000	1.000	0.963	0.984	0.889		1.000	110	—	—	0.010	—	—		—	98	—	—	—	0.015	—	0.042	—
Idh-1	120	0.017	—	—	0.019	0.016	0.111	Cap-2	92	0.969	0.895	0.948	0.932	1.000	0.985	Hk-1	108	0.703	0.773	0.554	0.895	0.788	0.889	0.727	
	100	0.909	0.925	1.000	0.923	0.887	0.944		1.000	96	0.031	0.105	0.042	0.068	—		0.015	112	0.297	0.227	0.446	0.105	0.212	0.111	0.273
	120	0.080	0.075	—	0.077	0.113	0.056		—	130	0.016	0.026	0.018	0.023	—		—	70	0.016	—	0.048	—	0.031	—	—
Cap-2	140	0.011	—	—	—	—	—	Mdh-1	100	0.984	0.974	0.982	0.977	1.000	1.000	Idh-1	85	0.906	0.955	0.904	1.000	0.922	1.000	0.875	
	96	0.040	0.050	—	0.056	0.016	0.056		—	70	1.000	1.000	1.000	1.000	1.000		100	0.078	0.045	0.048	—	0.047	—	0.125	
	100	0.930	0.925	1.000	0.944	0.968	0.944		1.000	100	0.281	0.158	0.319	0.159	0.395		0.015	54	0.048	0.023	0.017	0.056	—	0.389	—
Mdh-1	104	0.030	0.025	—	—	0.016	—	Mpi	104	0.719	0.842	0.681	0.841	0.605	0.985	Cap-2	58	0.032	0.204	0.017	0.028	0.048	0.333	0.042	
	130	0.875	1.000	0.857	0.981	0.844	1.000		1.000	38	0.016	0.026	—	0.023	—		0.015	64	0.775	0.750	0.914	0.833	0.871	0.278	0.875
	160	0.025	—	0.143	—	0.156	—		—	46	0.016	—	—	—	—		0.015	72	0.145	0.023	0.052	0.083	0.081	—	0.083
Mdh-2	130	0.100	—	—	—	—	—	Pgm	50	0.937	0.842	0.969	0.977	1.000	0.955	Mdh-1	115	0.100	0.067	0.071	—	0.130	—	—	
	160	0.025	—	—	0.019	—	—		—	62	0.031	0.132	0.031	—	0.015		130	0.880	0.866	0.929	0.934	0.826	0.889	0.955	
	100	1.000	1.000	1.000	1.000	1.000	1.000		1.000	—	—	—	—	—	—		145	0.020	0.067	—	0.066	0.044	0.111	0.045	
Mpi	92	—	—	0.071	0.019	—	—	Mdh-2	95	0.020	—	—	—	—	—	Mdh-2	95	0.020	—	0.036	0.026	0.043	—	—	
	96	0.096	0.050	0.357	0.259	0.323	0.083		0.125	130	0.960	0.967	0.964	0.948	0.957		145	0.960	0.967	0.964	0.948	0.957	0.944	0.955	
	100	0.904	0.950	0.572	0.722	0.677	0.917		0.875	145	0.020	0.033	—	0.026	—		0.026	—	0.020	0.033	—	0.026	—	0.056	0.045
Pgm	78	0.009	0.075	—	—	0.031	—	0.068	Mpi	38	0.023	0.023	0.016	0.013	—	Mpi	38	0.023	0.023	0.016	0.013	—	0.056	—	
	90	0.429	0.350	0.500	0.352	0.453	0.167	0.273		44	—	—	—	—	—		44	—	—	0.047	0.066	0.016	—	0.042	—
	100	0.536	0.550	0.500	0.648	0.500	0.833	0.659		46	—	—	—	—	—		46	—	—	0.136	—	0.105	—	—	—
Pgm	112	0.026	0.025	—	—	0.016	—	Pgm	48	0.682	0.591	0.656	0.632	0.734	0.833	Pgm	48	0.682	0.591	0.656	0.632	0.734	0.833	0.416	
	50	0.205	0.227	0.188	0.171	0.172	0.500		50	0.205	0.227	0.188	0.171	0.172	0.500		50	0.205	0.227	0.188	0.171	0.172	0.111	0.500	
	56	0.090	0.023	0.093	0.013	0.078	—		56	0.090	0.023	0.093	0.013	0.078	—		56	0.090	0.023	0.093	0.013	0.078	—	0.042	—
Pgm	72	0.094	0.071	0.104	0.038	0.106	—	0.250	72	0.094	0.071	0.104	0.038	0.106	—	72	0.094	0.071	0.104	0.038	0.106	—	0.250	—	
	78	0.844	0.881	0.854	0.910	0.864	1.000	0.708	78	0.844	0.881	0.854	0.910	0.864	1.000	78	0.844	0.881	0.854	0.910	0.864	1.000	0.708		
	90	0.047	0.024	0.042	0.038	0.015	—	0.042	90	0.047	0.024	0.042	0.038	0.015	—	90	0.047	0.024	0.042	0.038	0.015	—	0.042	—	
Pgm	100	0.016	0.024	—	—	—	—	Pgm	100	0.016	0.024	—	—	—	—	Pgm	100	0.016	0.024	—	—	0.014	0.015	—	