THE GENETIC VARIABILITY OF THE PRAWN *PALAEMONETES VARIANS* IN RELATION TO SALINITY

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SUMMARY

(1) The genetic variability of the prawn *Palaemonetes varians* was measured by means of the frequency of alleles and heterozygotes for the enzymes GPI, IDH and PGM.

(2) The animals were collected from eight more or less isolated brackish waters in the Delta area of the rivers Rhine, Meuse and Scheldt (S.W. Netherlands) and one station in the Slack estuary (Ambleteuse, N.W. France). Salinities ranged from 1 to 18 ‰.

(3) The isozymes examined all showed two alleles; the average observed heterozygosity was 0.30, the expected heterozygosity was 0.28, thus a slight excess of heterozygotes occurred.

(4) The frequency of the GPI alleles showed a significant relationship to salinity; the frequency of the fast allele increased with increasing salinity. For PGM, the excess of heterozygotes showed a significant increase with increasing salinity, especially at salinities above 10 ‰.

(5) It is likely that at the higher, unfavourable salinities, animals heterozygotic for PGM and with the fast allele for GPI are better able to survive.

(6) For one station, a relatively high genetic distance from all other stations studied was found. We concluded that the prawns at this station are of a different variety (ecotype).

INTRODUCTION

Strong differences have been found in the life cycle of the prawn *Palaemonetes varians* from inland waters with different salinities in the Dutch Delta area, at the mouth of the rivers Rhine, Meuse and Scheldt. In waters with salinities ranging from 1 to 14 ‰, a normal life cycle with one to two reproduction periods per year (Jeffries, 1958) was found for *P. varians* (Bogaards, 1979; Gijswijt, 1988). At one station, Ellewoutsdijk (B.1 in Figure 1) with salinities from 6 to 14 ‰, a different life cycle was found; no juveniles during some years, slow growth and only one reproduction period per two years. These differences might have been caused by differences in the environment, such as in salinity, or by genetic variability (phenotypic versus genotypic differences).

The aim of this study was to assess the genetic variability of the prawn *P. varians* in the Dutch Delta area. Animals were collected from eight brackish inland waters. As a possible extreme indication of genetic variability, a distant station in the tidal area of the Slack estuary (Ambleteuse, France) was sampled too for *P. varians*.

MATERIALS & METHODS

Adult prawns, with a rostrum-carapace length of 6 mm or longer, were collected from eight inland waters in the Dutch Delta area (Figure 1), and one tidal station in the Slack estuary in France. Forty to forty-six animals were analysed. The animals were homogenised individually for a few seconds in 5 ml of gel buffer. Electrophoresis was carried out in horizontal 12% starch gel at 4 °C for the isoenzymes GPI (Glucose phosphate isomerase, E.C. 5.3.1.9), IDH (Isocitrate dehydrogenase, E.C. 1.1.1.42) and PGM (Phosphoglucomutase, E.C. 2.7.5.1). The buffer systems used were

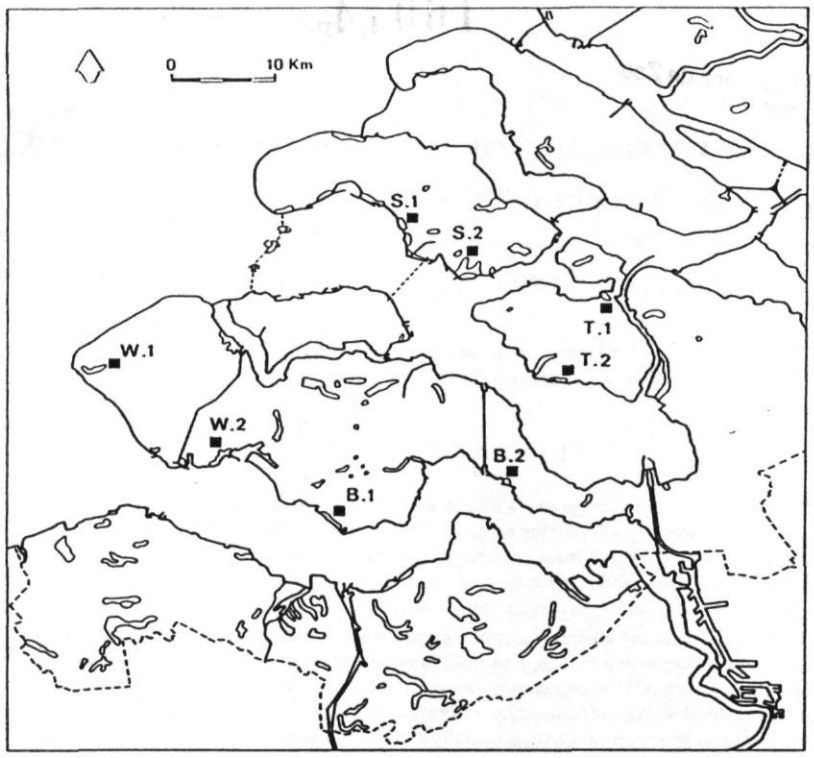

Figure 1. Location of the sampling stations (excluding one tidal station in the Slack estuary in France).

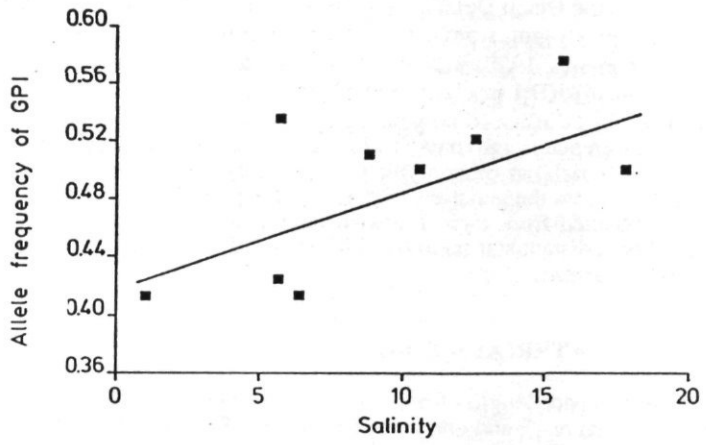

Figure 2. Frequency of the fast allele of GPI in relation to salinity. The line of best fit is indicated.

Tris-citric acid gel buffer (respectively 0.008 and 0.003 M; pH 6.7) and Tris-citric acid electrode buffer (respectively 0.223 and 0.086 M; pH 6.3). Staining procedures were run in Bush B buffer according to Menken (1982). The faster allele is called A, the slower B. The expected heterozygosity (He), observed heterozygosity (Ho), heterozygote deficiency (D) and genetic distance were calculated according to Nei (1972, 1975).

Salinity was measured in the simultaneously collected water using the method of Mohr (Strickland & Parsons, 1965).

The homogeneity of the genotype frequencies amongst stations was analysed with the G-statistic, and the contribution of individual stations to the total heterogeneity was analysed (Sokal & Rohlf, 1981). Correlations between salinity and allele frequencies, Ho or D were calculated; the significance of the correlations was read from the Z-table (Sokal & Rohlf, 1981).

RESULTS

The isozymes examined all showed two alleles. The results are compiled in Table I. The frequencies of the genotypes of the isozymes IDH and PGM showed significant heterogeneous distributions amongst stations. For IDH, only station B.1 contributed significantly to this heterogeneity; for PGM it was station B.1 and the most saline station, W.1. For GPI the genotypes were homogeneously distributed amongst all stations.

However, only in the case of GPI did the frequency of the alleles show a significant relationship to salinity; the frequency of the fast allele increased with increasing salinity (Figure 2).

The observed heterozygosity was on average 0.30, the expected heterozygosity was 0.28. Thus, on average, only a slight excess of heterozygotes occurred. The excess of heterozygotes was clear for the three most saline stations, thus at salinities above 10 $^{o}/_{oo}$, and most explicitly with the isozyme PGM (Table I). Significant relationships between salinity and Ho or D were, therefore, found for the isozyme PGM and the average (Table I; Figure 3a,b).

The genetic distance between the prawns from most stations is smaller than 0.01, including the French station (F1, Table II). The only diverging station is B.1 (Ellewoutsdijk), with an average genetic distance of 0.04 to the other stations. The genetic relationships are clarified in a dendrogram (Figure 4).

DISCUSSION

An average observed heterozygosity (Ho) of 0.30 is high. Comparable electrophoretic studies on species of *Palaemonetes* however, are scarce. In a study on two related species, *Palaemon adspersus* and *P. squilla* , a Ho of 0.01 to 0.08 was found on the basis of eighteen monomorphic as well as polymorphic loci (Berglund & Lagercrantz, 1983). Normally, a Ho of 0.10 to 0.20 is found (Berger, 1983). The reason for our high Ho is that we based our value on only three polymorphic loci; if we had included monomorphic loci (with an Ho of 0) they would have lowered our high value. However, monomorphic loci (with only one allele and without heterozygotes) were not relevant to our study where we sought to find a relationship between allele frequencies or observed heterozygosities, and salinity.

Indeed, relationships with salinity were found for allele frequencies (GPI), as well as for the number (and excess) of heterozygotes (PGM and the average). Relationships with salinity have been reported before, not in crustaceans, but in bivalve molluscs, and mostly for the enzyme LAP, but also for PGM and GPI (Boyer, 1974; Koehn *et al.* , 1976; Theisen, 1978; Rose, 1984). The relationship between salinity and the enzyme LAP has been studied in detail for the mussel *Mytilus edulis* (Koehn, *et al.*, 1980; Hilbish, *et al.*, 1982; Deaton, *et al.*, 1984). Animals with the double allele LAP 94 prevailed in water with high salinity. These mussels appeared to have a higher enzyme-activity than the other LAP-genotypes and therefore showed a more energy-consuming osmoregulation, thus being at a disadvantage in low salinities. A comparable difference in the enzyme-activity of GPIa and GPIb is not known.

The excess of heterozygotes in the adult *P. varians* was primarily found at salinities above 10 $^{o}/_{oo}$. Adults tolerate a wide range of salinities, from almost freshwater to hypersaline water (Jeffries, 1958), but for juvenile stages, an optimum salinity for growth and survival was found to be around 9 $^{o}/_{oo}$. Selection of the prawns probably occurrs in the juvenile stages, during which the more heterozygotic animals are better able to survive at the less favourable salinities. This idea fits

Table I.

Genotype (aa, ab, bb) and allele (a,b) frequencies; and expected (He) and observed (Ho) heterozygosity and heterzygote deficiency (D) for *Palaemonetes varians*. The results of the G-statistic (G:) and correlations with salinity (S:) are shown in the last column. (n.s. = non significant: if G was significant then the individual stations causing the heterogeneity are indicated with asterisks).

Station		W.2	S.1	T.1	B.1	T.2	S.2	B.2	F.1	W.1	
Salinity ‰		1.0	5.6	5.7	6.3	8.7	10.5	12.5	15.5	17.7	
										Statistics	
GPI	aa	10	7	8	7	13	15	10	12	11	
	ab	18	25	30	24	21	16	28	22	24	G: n.s.
	bb	18	14	5	15	12	15	8	6	11	
	a	0.41	0.42	0.53	0.41	0.51	0.50	0.52	0.58	0.50	S: p = 0.05
	b	0.59	0.58	0.47	0.59	0.49	0.50	0.48	0.42	0.50	
	He	0.48	0.48	0.49	0.48	0.49	0.50	0.49	0.48	0.50	
	Ho	0.39	0.54	0.69	0.52	0.45	0.34	0.60	0.55	0.52	S: n.s.
	D	-0.19	0.11	0.40	0.08	-0.08	-0.30	0.22	0.13	0.04	S: n.s.
IDH	aa	43	43	43	27	43	5	44	40	45	
	ab	0	0	0	18*	3	1	2	0	1	G: P < 0.001
	bb	0	0	0	1	0	0	0	0	0	
	a	1.00	1.00	1.00	0.78	0.97	0.92	0.98	1.00	0.99	S: n.s.
	b	0.00	0.00	0.00	0.22	0.03	0.08	0.02	0.00	0.01	
	He	0.00	0.00	0.00	0.34	0.06	0.15	0.04	0.00	0.02	
	Ho	0.00	0.00	0.00	0.39	0.06	0.16	0.04	0.00	0.02	S: n.s.
	D	0.00	0.00	0.00	0.15	0.03	0.09	0.02	0.00	0.01	S: n.s.
PGM	aa	31	27	31	42	35	23	33	23	16	
	ab	14	18	10	4*	11	20	13	17	24*	G: P < 0.001
	bb	1	1	2	0	0	3	0	0	0	
	a	0.83	0.78	0.84	0.96	0.88	0.72	0.86	0.79	0.70	S: n.s.
	b	0.17	0.22	0.16	0.04	0.12	0.28	0.14	0.21	0.30	
	He	0.28	0.34	0.27	0.08	0.21	0.40	0.24	0.33	0.42	
	Ho	0.30	0.39	0.23	0.08	0.23	0.43	0.28	0.42	0.60	S: P = 0.08
	D	0.06	0.15	-0.14	0.05	0.14	0.07	0.16	0.27	0.43	S: P = 0.02
Average	He	0.25	0.27	0.25	0.30	0.25	0.35	0.26	0.27	0.31	
	Ho	0.23	0.31	0.31	0.33	0.25	0.31	0.31	0.32	0.38	S: P = 0.04
	D	-0.04	0.09	0.09	0.09	-0.03	-0.04	0.14	0.13	0.16	S: P = 0.08

Table II.

Genetic distance (GD x 10^{-4}) between stations.

	S.1	S.2	W.1	W.2	T.1	T.2	B.1	B.2
S.2	67							
W.1	56	23						
W.2	8	103	102					
T.1	68	84	86	67				
T.1	82	118	148	61	15			
B.1	369	411	575	297	354	227		
B.1	71	95	114	60	4	3	276	
F.1	105	68	59	125	17	62	488	37

with the common hypothesis that heterozygotes are more able to cope with a heterogeneous and harsh environment (Zouros, *et al.*, 1980; Berger, 1983; Mitton & Grant, 1984). In conformity with this, the highest excess of heterozygotes was found at the most saline station (W.1). This agrees

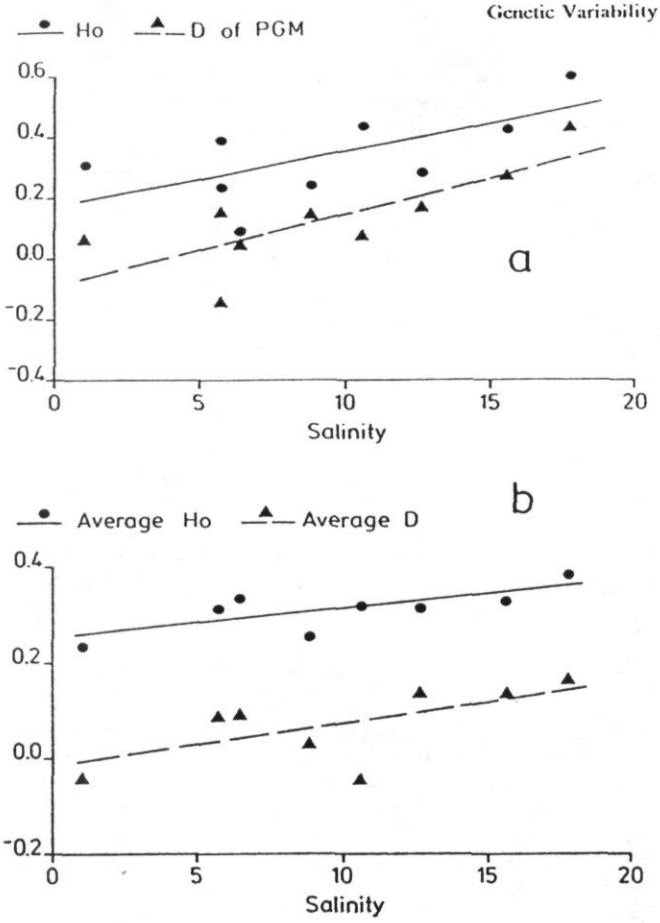

Figure 3. Observed heterozygosity (Ho) and heterozygote deficiency (D) for PGM (Figure 3a) and the average of all isozymes examined (Figure 3b) in relation to salinity. The lines of best fit are shown.

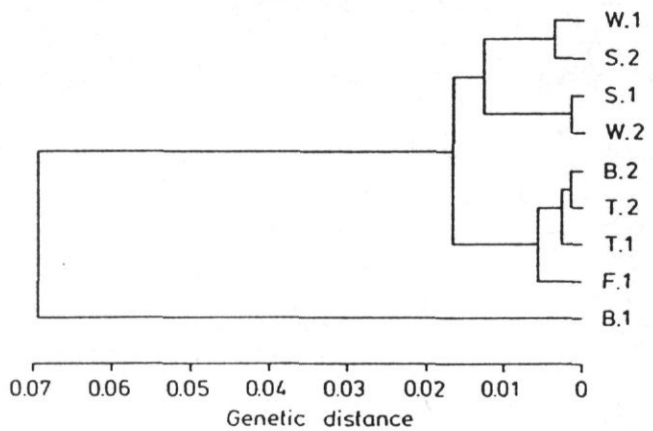

Figure 4. Dendrogram of the genetic relationships between prawns from the nine sampling stations as based on Nei's genetic distance. Dendrogram constructed by means of flexible sorting according to Legendre & Legendre (1979).

with the significant heterogeneous distribution of genotypes for PGM at this station.

The only other significant heterogeneous distribution of genotypes was found for station B.1 (Ellewoutsdijk). This station exhibited a relatively high genetic distance, of on average 0.04 to the other stations. Still, this distance is not on the level of subspecies; Avise (1974) mentioned an average genetic distance of 0.15 for subspecies. Yet, the higher genetic distance of Ellewoutsdijk is remarkable because the other stations showed no genetic differentiation from the French estuarine station which was thought to be much more distinct; distinct because of its distance and because it was tidal, whereas the other stations were non-tidal inland waters. Ellewoutsdijk was also the station showing an aberrant life cycle for the prawns (Bogaards, 1979; Gijswijt, 1987). Therefore, on the basis of the coincidence of a higher genetic distance and the differences in the life cycle, we conclude that *P. varians* at station B.1 has to be a different variety (ecotype). The isolation of this variety is probably not due to salinity, because the salinity at this station is in between that of other stations.

Nevertheless, we also conclude that at the other stations salinity may play a substantial role in determining the genetic constitution of *P. varians*, because quite clear relationships were found between salinity, allelic frequencies, and the number of heterozygotes.

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REFERENCES

Avise, J.C. (1974). Systematic value of electrophoretic data. *Systematic Zoology*, 23, 465-481.

Berger, E.M. (1983). Population genetics of marine gastropods and bivalves. pp. 563-596 *In:* W.D. Russel-Hunter, *The Mollusca*, Vol. 6, Ecology Academic Press, Orlando.

Berglund, A. & Lagercrantz, U. (1983). Genetic differentiation in populations of two *Palaemon* species at the Atlantic east-coast; does gene-flow prevent local adaptations. *Marine Biology*, 77, 49-57.

Bogaards, R.H. (1979). Autoecologie en levenshistorie van *Palaemonetes varians* (Leach, 1814) (Decapoda, Palaemonidae). *Rapporten en verslagen*, DIHO, Yerseke, 1979-10, 48pp.

Boyer, J.F. (1974). Clinal and size-dependent variation at the LAP locus in *Mytilus edulis*. *Physiological Zoology*, 56, 609-619.

Gijswijt, G. (1988). Autoecologisch en isoenzymenonderzoek aan de steurgarnaal *Palaemonetes varians* (Decapoda, Natantia). DIHO, Yerseke, Studentenverslagen, D2, 37pp.

Heerebout, G.R. (1974). Distribution and ecology of the Decapoda Natantia of the estuarine region of the rivers Rhine, Meuse and Scheldt. *Netherlands Journal of Sea Research*, 8, 73-93.

Hilbish, T.J., Deaton, L.E. & Koehn, R.K. (1982). Effect of an allozyme polymorphism on regulation of cell volume. *Nature*, 298, 5875, 688-689.

Jefferies, D.J. (1958). The ecology of *Palaemonetes varians* (Leach). PhD Thesis, University of Liverpool, 437 pp.

Koehn, R.K., Bayne, B.L., Moore, M.N. & Siebenaller, J.F. (1980). Salinity related physiological and genetic differences between populations of *Mytilus edulis*, *Biological Journal of the Linnaean Society*, 14, 319-334.

Koehn, R.K., Milkman, R. & Mitton, J.B. (1976). Population genetics of marine pelecypods. IV. Selection, migration and genetic differentiation in the blue mussel *Mytilus edulis*, *Evolution*, 30, 2-32.

Legendre, L. & Legendre, P. (1979). *Ecologie numerique. 2. La structure des donnes ecologiques*, Masson, Paris, 247pp.

Menken, S.B.J. (1982). Biochemical genetics and systematics of small ermine moths (Lepidoptera, Yponomeutidae). *Zeitschrift für zoologischen Systematik und Evolutionsforschung*, 20, 131-143.

Mitton, J.B. & Grant, M.C. (1984). Associations among protein heterozygosity, growth rate and developmental homeostasis. *Annual Reviews of Ecology and Systematics*, 15, 479-499.

Nei, M. (1972). Genetic distance between populations. *American Naturalist*, 106, 283-292.

Nei, M. (1975). *Molecular population genetics and evolution*, North Holland Publishing Co., Amsterdam.

Rose, R.L. (1984). Genetic variation in the oyster, *Crassostrea virginica* (Gmelin), in relation to environmental variation. *Estuaries*, 7, 128-132.

Sokal, R.R. & Rohlf, F.J. (1981). *Biometry*, W. H. Freeman and Co., Ney York, 859 pp.

Strickland, J.D.H. & Parsons, T.R. (1965). *A manual of seawater analysis*, *Fisheries Research Board of Canada Bulletin*, 15, 203 pp.

Theisen, B.F. (1978). Allozyme clines and evidence of strong selection in three loci in *Mytilus edulis* (L.)(Bivalvia) from Danish waters.*Ophelia*, **17**, 135-142.

Zouros, E., Singh, S.M. & Miles, H.E. (1980). Growth rate in oysters: an overdominant phenotype and its possible explanations. *Evolution*, **34**, 856-867.