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Microgeographic genetic variation of populations of *Idotea chelipes* (Crustacea: Isopoda) in lagoons of the southern English coast.

Marc T. JOLLY[1,2*], Alex D. ROGERS[1,3] and Martin SHEADER[1]
(1) *The University of Southampton, School of Earth and Ocean Sciences, Southampton Oceanography Centre, Empress Dock, Southampton SO14 3ZH, Hampshire, United Kingdom.*
(2) *Evolution et Génétique des Populations Marines, Station Biologique de Roscoff, CNRS-UPMC, UMR 7127, Roscoff, France*
(3) *British Antarctic Survey, High Cross, Madingley Road, Cambridge, CB3 0ET, United Kingdom*
**Corresponding author : E-mail: jolly@sb-roscoff.fr*

Abstract : Allozyme variability was examined using starch-gel electrophoresis between sub-populations of *Idotea chelipes* over a small geographic distance (approx. 104 km), in lagoons within the Solent area (Fort Gilkicker Moat; Ashlett Pond) and at one site in Dorset (the Fleet Lagoon). Genetic identity values indicate that all three populations are conspecific (I = 0.967 to 0.995), and mean heterozygosity per locus for all three populations was 8%. Significant levels of genetic differentiation (mean F_{ST} = 0.119***; p< 0.001) were detected over this small spatial scale, but this mainly resulted from the slow esterase locus (mean F_{ST} (no esterase) = 0.072***; p< 0.001). Genetic differentiation was moderate given the low geographic distances between populations with only one to two migrants per deme per generation ($N_em(F_{ST})$ = 1.85) from the Fleet to Gilkicker. There was no significant deviation from the genotypes expected under the Hardy-Weinberg equilibrium despite an overall slight excess of heterozygotes (mean F_{IS} = -0.01). Pairwise F_{ST} values indicated that there was limited genetically effective migration between the sampled lagoons. This genetic differentiation may have been promoted by the presence of the Southampton Water and Solent estuarine system that may act as a physical barrier to gene flow for this species. The previous use of the esterase (ET2) locus as a sub-specific biochemical marker for *Idotea chelipes* is placed in doubt by this study.

Résumé : *Différenciation génétique à petite échelle spatiale entre populations lagunaires d'*Idotea chelipes *(Crustacea : Isopoda) de la côte sud de l'Angleterre*. La variabilité enzymatique de trois populations lagunaires d'*Idotea chelipes* est examinée sur gel d'électrophorèse d'amidon à faible échelle spatiale (environ 104 km). Les trois populations font partie de la même espèce (I = 0,967 à 0,995) et l'hétérozygotie moyenne observée est de 8 %. Des valeurs de différenciation génétique significatives sont observées (F_{ST} = 0,119***; p< 0,001) bien que résultant principalement de l'estérase lente (F_{ST} (sans estérase) = 0,072*** ; p< 0,001). Etant donnée la faible distance géographique séparant les populations, le niveau de différenciation génétique reste modéré avec un à deux migrants par dème par génération ($Nem(F_{ST})$ = 1,85). En dépit d'un faible excès en hétérozygote (F_{IS} = -0,01), aucune déviation par rapport à l'équilibre de Hardy-Weinberg n'est observée. Les valeurs de différenciation génétique entre paires de populations indiquent que les échanges entre lagunes sont limités et que cette différenciation aurait pu être accentuée par la présence du système estuarien "Solent" qui jouerait le rôle de barrière au flux génique pour cette espèce. D'autre part, l'utilisation préalable de l'estérase lente (ET2) comme marqueur subspécifique du complexe *Idotea chelipes* est placée en doute dans la présente étude.

Keywords: *Idotea chelipes*, Allozymes, UK Coastal lagoons, Solent, Gene flow

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Introduction

Isopods of the genus *Idotea* are widespread in coastal marine habitats (Salemaa, 1985). First observed and described by Pallas (1766), under the name of *Oniscus chelipes*, *Idotea chelipes* is a brackish water species and a lagoon specialist (Barnes, 1980) that exhibits an island-like distribution around England (Bamber, 1997). It inhabits clusters of lagoons on the southern English coast and is also known to be present in the normal intertidal zone. It has been shown to occur mainly among submerged macrophytes, especially *Zostera marina* and *Ruppia spp.* and among three species of Chlorophycea (*Enteromorpha intestinalis*, *Chaetomorpha aerea*, *Cladophora rupestris*) (Naylor, 1955a-b). This isopod usually occurs in the most sheltered areas, although Salemaa (1979a) reported that populations of the Tvärmine archipelago (northern Baltic) prefer areas characterized by wave action and water movement. Unlike the littoral zone, lagoons are relatively closed areas and may be regarded as stable habitats with respect to tidal influence. However, they are highly dynamic and extreme habitats where catastrophic events may often take place. Environmental pressures that affect lagoonal populations of *I. chelipes* and other species include changes in the presence of substrates (macrophytes) and strong daily and seasonal variations in water temperature with variations in air temperature and direct irradiance by the sun. Variations in salinity, through evaporation, percolation and rainfall also occur with concomitant changes in water level in the lagoon. Human impacts such as the introduction of sewage, chemicals and/or freshwater effluents can also be a problem. Such harsh environmental conditions impose a high level of physiological stress on organisms, and may favour euryplastic species such as *I. chelipes* (or genotypes - see Pearson et al., 2002) whilst excluding potential competitor species.

Idotea chelipes is widely distributed (ranged between 35° and 60° North) and is characterized by a high tolerance to variation in salinity. The timing and duration of the breeding season and the number of generations are directly related to the geographical distribution of populations. In Gilkicker lagoon (Southern England), *I. chelipes* has a breeding season from April to November with two generations per year (Al-Suwailem, 1992), whereas populations in the Arcachon lagoon (France) and in the warm Mediterranean environment breed continuously, with up to four generations per year (Labourgh, 1971; Cloarec, 1983).

Genetic investigations carried out on *Idotea* species have mostly concentrated on *I. balthica* (Tinturier-Hamelin, 1963; Bulnheim & Fava, 1982; Kaim-Malka, 1983; Guarino et al., 1993). Only recently has the genetic structure of *I. chelipes* populations been studied. On the basis of studies of morphological differences (Charfi-cheikhrouha, 1996) and polymorphisms in haemocyanin (Charfi-cheikhrouha & Belhadj, 1992), and enzymes, estimated by electrophoresis (Charfi-cheikhrouha et al., 1998), it has been suggested that *I. chelipes* is a polytypic species comprising a complex of three subspecies: *Idotea chelipes bocqueti*, *Idotea chelipes mediterranea* and *Idotea chelipes chelipes*. Charfi-cheikhrouha & Belhadj (1992) used haemocyanin as a protein marker at the species level to confirm the identity of *I. chelipes* and *I. balthica*, but did not demonstrate the sub-speciation of *I. chelipes*. A further experiment (Charfi-cheikhrouha, 1994) concluded that the esterase locus constituted a sub-specific biochemical marker which discriminated each of the three sub-species, and that the amylase-2 locus was diagnostic and separated *I. c. bocqueti* from the two other sub-species.

Idotea chelipes is reported to occupy the Atlantic coasts of Morocco, Spain, France, as well as the North Sea, the Baltic and lagoons around the British Isles (Charfi-Cheikhrouha, 1996). Although the author suggests that the English *I. chelipes* corresponds to the subspecies, *I. c. chelipes*, no genetic studies have yet confirmed this.

Because *I. chelipes* exhibits an island-like distribution throughout Britain and broods its young, populations are prone to isolation and genetic differentiation. Although not reported previously, evidence of a small-scale geographic differentiation in lagoonal populations of *I. chelipes* may be interesting in the light of other studies. Microgeographic genetic differentiation has been revealed in species with restricted dispersal capacity (e.g. isopods, Piertney & Carvalho, 1994, 1995a; Carvalho & Piertney, 1997; amphipods; Kolding, 1985; De Matthaeis et al., 1994). It has also been identified in other lagoonal invertebrates, including the sea anemone, *Nematostella vectensis*, where significant genetic differentiation was detected between populations separated by 10s km on the south coast of Britain (Pearson et al., 2002).

Idotea chelipes is considered to be nationally scarce in the United Kingdom because the brackish/coastal lagoons that support this species are vulnerable to habitat loss from a variety of human impacts. However, limited knowledge is available on the population structure of the fauna inhabiting these lagoons, and the population genetics of *I. chelipes* around the United Kingdom has never been investigated. This study examines the degree of genetic structuring between subpopulations of *I. chelipes* on a small geographic scale, on the south coast of Britain, providing a basis for further studies on the genetic structure of this isopod. This study was also aimed at generating new data on the effective dispersal capabilities of lagoonal invertebrates with direct development on either side of the Solent estuarine system for comparison with studies on the clonal anemone *Nematostella vectensis* (Pearson et al., 2002). The conservation implications of the genetic structure of

I. chelipes populations (i.e. gene flow, genetic diversity and distinctiveness) in sites listed as Special Areas of Conservation (SAC), in the U.K., will also be assessed. The variation of the slow esterase locus, previously used as a sub-specific marker, was also specifically analysed in this study.

Materials and methods

Study area and collecting sites

Sampling was undertaken from May 1999 to July 1999. Samples of *Idotea chelipes* were obtained from two sites around the Solent estuary system (Fort Gilkicker Moat, Ashlett Pond) and at one site in Dorset (Fleet lagoon) (figure 1). The Fleet lagoon is relatively unpolluted, although there is some agricultural run-off and six treated sewage effluent pipes discharge into water-courses leading to the Fleet (Dyrinda & Farnham, 1985). The water of Ashlett Pond is used by the Esso Refinery as effluent and cooling water and there is no freshwater inflow. Gilkicker connects to the sea via a channel leading to a pipe through the sea wall and receives freshwater via storm drains. One hundred *I. chelipes* females were taken from each site, brought back to the laboratory alive, placed in labelled zip-log bags and stored at –70°C until electrophoresis.

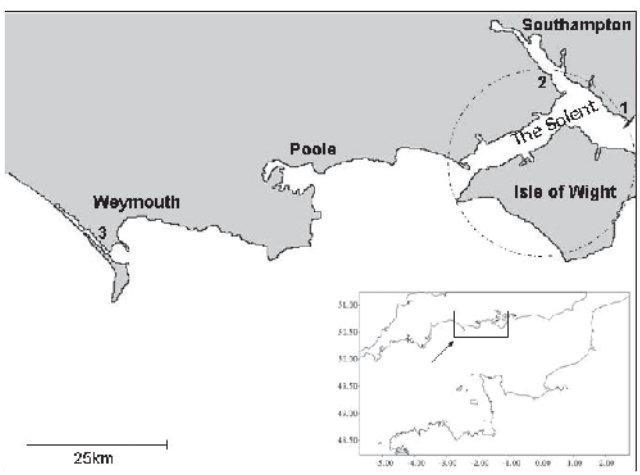

Figure 1. Map showing the positions of the coastal lagoons sampled. (1) Fort Gilkicker Moat, Gosport (4.3 Ha; sluiced ponds); (2) Ashlett Pond, Fawley (4.3 Ha; sluiced millpond); (3) The Fleet Lagoon, Weymouth (480 Ha, typical natural lagoon). [Ordnance Survey interactive atlas of Great Britain, 1998. Third edition. Oxford interative Ltd./ Ordnance Survey]

Figure 1. Carte montrant la position des lagunes échantillonnées. (1) Fort Gilkicker Moat, Gosport (4,3 Ha ; mare canalisée) ; (2) Ashlett Pond, Fawley (4,3 Ha ; bassin de retenue de moulin à eau) (3) Le Fleet Lagoon, Weymouth (480 Ha, lagune naturelle). [Ordnance Survey interactive atlas of Great Britain, 1998. Third edition. Oxford interative Ltd./ Ordnance Survey].

Electrophoresis

A 12.5% starch solution was prepared by heating 47.5g of potato starch (Sigma Chemical Co. Ltd., Poole, Dorset, U.K.) with 380ml of gel buffer (see below) in a 1000 ml side-arm pyrex flask over a Bunsen flame. The solution was heated until it had passed from a milky liquid to a viscous opaque fluid then to a clear liquid and finally had commenced boiling. The gel was then degassed by attaching a vacuum pump to the side arm of the flask using flexible tubing and placing a rubber bung over the flask. Following degassing the molten starch solution was poured into a 10 mm x 150 mm x 180 mm Perspex gel mould. This was sandwiched between two plates of glass (180 mm x 210 mm) and allowed to solidify and cool. Gels were prepared up to 12 hours before electrophoresis.

Whole samples were each placed in a labeled 2.0 ml centrifuge tube with 100 ml of grinding buffer [0.05M Tris/HCl, pH 8.0 (Redfield & Salini, 1980)]. Each animal was macerated with a glass rod prior to centrifugation at 14000 rpm in Eppendorf Model 5415C centrifuge for 4 min. The supernatant was then absorbed onto 4 mm x 12 mm filter-paper wicks (Whatman No. 1). The wicks were inserted along a straight slit cut approximately 2 cm from one end of the gel. This was then placed in a laboratory built electrophoresis tank (see Murphy et al., 1990 for similar design of tank). Electrophoresis tanks were run under the appropriate conditions for each of the buffer systems using a Bio-Rad Power-Pac 300.

Two types of buffer systems were employed: Buffer System I, Tris-borate-EDTA, continuous, pH 8.0 (Shaw & Prasad, 1970); Buffer System II, Tris-citric-boric-lithium hydroxide, discontinuous, electrode pH 8.29, gel pH 8.26 (Redfield & Salini, 1980). Buffer Systems I and II were run respectively at 300V, 50mA for 7h and 300V, 40mA for 7h.

Eight enzymes coding for a total of 10 loci were visualised using enzyme–specific stains given in Creasey (1998) and modified from Shaw & Prasad (1970), Harris & Hopkinson (1978) and Redfield & Salini (1980): alkaline phosphatase (ALP) E.C. 3.1.3.1 (buffer system II); esterase (EST) 3.1.1.1 (buffer system I); general protein (GP) (buffer system II); glutamate-oxaloacetate transaminase (GOT) 2.6.1.1 (buffer system I); malate dehydrogenase (MDH) 1.1.1.37 (buffer system I); malic enzyme (ME) 1.1.1.40 (buffer system II); phosphoglucomutase (PGM) 5.4.2.2 (buffer system I); phosphoglucose isomerase (PGI) 5.3.1.9 (buffer system II).

Statistical analysis

Allele frequencies were calculated for all samples of *Idotea chelipes* over all enzyme loci and for each of the three sites. Genetic variability, as summarised by the mean number of alleles per locus (N_O), the percentage polymorphic loci, the observed heterozygosity (i.e. the proportion of sampled individuals heterozygous under the 0.99 criterion), and the

expected heterozygosity (H_{NB}- Nei, 1987) based on the Hardy-Weinberg assumptions, was estimated using GENETIX 4.02 (Belkhir et al., 1996). Deviations from Hardy-Weinberg equilibrium were examined for each population, at each locus, by calculating Wright's fixation index F_{IS} as estimated by Weir & Cockerham's (1984) f which was then tested using Fisher's exact test with GENEPOP 3.3 software (Raymond & Rousset, 1995). Multilocus F_{IS} was calculated with Fstat 2.9 (Goudet, 1995).

The genetic structure over all the samples was first analysed by calculating Wright's F_{ST} statistics for each locus, estimated with GENEPOP 3.3 (Raymond & Rousset, 1995) following Weir & Cockerham's (1984) θ value. Exact tests for the null hypothesis of identity of allelic distribution across populations were performed with GENEPOP 3.3. Multilocus θ values were also estimated and tested between pair-wise combinations of populations using the same software. Overall genetic differentiation between all samples was also estimated using Nei's (1972) genetic identity (I) and genetic distance (D). These measures were used as a guide to the genetic similarity between populations of *I. chelipes*.

The number of migrants per deme per generation (N_em) was calculated from F_{ST} according to Wright's (1951) formula [$N_em(F_{ST}) = (1-F_{ST})/(4F_{ST})$].

Results

All enzyme loci investigated (except for GOT) produced good staining patterns. Glutamate-oxaloacetate transaminase (GOT) was not examined further because of poor resolution, although it appeared to be polymorphic. Four enzymes, accounting for a total of 4 loci, were found to exhibit polymorphism (Table 1): esterase (EST); malate dehydrogenase (MDH); phosphoglucose isomerase (PGI); phophoglucomutase (PGM).

Marked differences in allele frequencies were observed between the sampled populations across most of the polymorphic loci. For example, in esterase the *Est-b* allele was the most common among individuals from Ashlett (allele frequency. 0.69) and the Fleet population (allele frequency 0.86), whereas the frequency of *Est-a* (0.615) was highest in Gilkicker lagoon (Table 1). Differences in allele frequencies between the sampled populations were also considerable in PGI and PGM loci (see Table 1).

Genetic variation for all samples of *I. chelipes* is presented in Table 2. The average heterozygosity per locus for all population was 8%. The observed heterozygosity (H_O) did not show a significant degree of variation across the populations, ranging from 0.15 at the Gilkicker to 0.21 at Ashlett Pond. However, the percentage polymorphic loci were highest at the latter site, accounting for 11.1 % of the difference with the Fleet and Gilkicker lagoon populations.

Table 1. *Idotea chelipes*. Allele frequencies for all loci for all samples at each site (N: sample size). Relative mobilities of alleles in parentheses: the most common allele was attributed a mobility of 1.00 (* indicates a rare allele).

Tableau 1. *Idotea chelipes*. Fréquences alléliques aux locus enzymatiques pour chaque site (N: nombre d'individus testés). Mobilités relatives des allèles entre parenthèses : l'allèle le plus commun a une mobilité de 1,00 (* indique un allèle rare).

Locus Allele	Fort Gilkicker Moat N=100	Ashlett Pond N=100	The Fleet Lagoon N=100
Alp-A	1.00	1.00	1.00
Alp-B	1.00	1.00	1.00
Est-A (ET2)			
a (1.07)	0.615	0.31	0.14
b (1.00)	0.385	0.69	0.86
Gp-A	1.00	1.00	1.00
Me-A	1.00	1.00	1.00
Me-B	1.00	1.00	1.00
Mdh			
a (1.08)	0.03	0.025	-
b (1.00)	0.97	0.975	1.00
Pgi-A			
a (1.00)	0.995	0.935	0.88
b (0.65)	0.005	0.06	0.12
Pgm-A			
a (1.07)	-	0.11	0.19
b (1.00)	1.00	0.89	0.79
c* (0.92)	-	-	0.02

The mean number of alleles per locus was relatively similar throughout all populations.

Pairwise values of genetic differentiation (Table 3) indicated that populations inhabiting the Fleet Lagoon and Ashlett Pond are genetically more similar than with the Gilkicker population.

F_{ST} values were all significant ($p<0.05$) and ranged from 0.009* (MDH) to 0.229*** (EST) (Table 4), with an overall F_{ST} values of 0.164***, corresponding to a high level of genetic differentiation. This degree of divergence was halved and fell to moderate levels when the EST locus was excluded from the analysis but the overall figure still remained significant (mean $F_{ST} = 0.072$***). The number of migrants per deme per generation (N_em) estimated from F_{ST} values, with or without EST, indicated a moderate level of effective genetic migration between populations at the three sites (approximately 2 migrants per deme per generation).

F_{IS} values (Table 4 and 5), reflecting the within population structuring, were significantly different from zero at the PGM locus ($p<0.001$). Although this significant F_{IS} value for PGM, indicating a heterozygote deficiency,

Table 2. *Idotea chelipes*. Measures [means (standard error SE)] of genetic variability for all samples. The locus is considered polymorphic if the frequency of the most common allele does not exceed 0.99. (N_O: average number of alleles per locus; H_O = observed heterozygosity per polymorphic loci; H_{NB} = Hardy-Weinberg expected heterozygosity).

Tableau 2. *Idotea chelipes*. Valeurs [moyennes (erreur standard ES)] de diversité génétique pour tous les échantillons. Le locus est considéré comme polymorphe si la fréquence de l'allèle le plus commun n'excède pas 0.99. (N_O : nombre d'allèle moyen par locus ; H_O = hétérozygotie observée par locus polymorphes ; H_{NB} = hétérozygotie attendue selon Hardy-Weinberg).

Population	Sample size per locus	% polymorphic loci	N_O	H_O	H_{NB}
Fort Gilkicker Moat	100	33.3 [0.06]	1.75	0.15 [0.26]	0.136 [0.23]
Ashlett Pond	100	44.4 [0.05]	2	0.21 [0.19]	0.199 [0.17]
The Fleet Lagoon	100	33.3 [0.04]	2	0.18 [0.12]	0.198 [0.14]

Table 3. *Idotea chelipes*. Pairwise F_{ST} values based on data for 4 polymorphic loci for all samples. Tests of significance were performed with Genepop 3.3 (Raymond & Rousset, 1995) ([without *Est-A*]).

Tableau 3. *Idotea chelipes*. Valeurs des F_{ST} par paires de populations basées sur 4 locus polymorphes pour tous les échantillons. Les tests de significativité ont été exécutés à l'aide du logiciel Genepop 3,3 (Raymond & Rousset, 1995) ([sans *Est-A*]).

Population	Fort Gilkicker Moat	Ashlett Pond	The Fleet Lagoon
Fort Gilkicker Moat	- [0.063***]	0.167*** [0.145***]	0.292***
Ashlett Pond	-	0.044*** [0.02**]	

**= p< 0.01; ***= p< 0.001

Table 4. *Idotea chelipes*. Estimates of variance of allele frequencies between populations (F_{ST}) [without *Est-A*] and of the correlation between homologous alleles between individuals within population (F_{IS}) for all variable loci and for all samples of *I. chelipes*. The number of migrants per deme per generation was estimated according to Wright (1951) (N_em (F_{ST})).

Tableau 4. *Idotea chelipes*. Estimations de la variance des fréquences alléliques entre populations (F_{ST}) [sans *Est-A*] et de la corrélation entre allèles homologues entre individus à l'intérieur d'une population (F_{IS}) pour tous les locus polymorphes et pour tous les échantillons. Le nombre de migrant par dème par génération a été estimé selon Wright (1951) (N_em (F_{ST})).

Locus	F_{ST}	F_{IS}	Number of migrants
Est-A	0.229***	-0.125	
Mdh-A	0.009*	-0.024	
Pgm-A	0.096***	0.257**	
Pgi-A	0.05***	-0.05	
Mean	0.164*** [0.072***]	-0.01	
N_em (F_{ST})			1.27
N_em (F_{ST}) [no Est-A]			3.22

*= p< 0.05; **= p< 0.01; ***= p< 0.001

Table 5. *Idotea chelipes*. Inbreeding index (F_{IS}) for all variable loci for each population. Negative values represent heterozygote excess.

Tableau 5. *Idotea chelipes*. Indice de consanguinité (F_{IS}) pour tous les locus polymorphes pour chaque population. Les valeurs négatives représentent un excès d'hétérozygote.

Locus	Fort Gilkicker	Moat Ashlett Pond	The Fleet lagoon
Est-A	-0.114	-0.117	-0.158
Mdh	-0.026	-0.021	-
Pgm-A	-0.086	0.356***	
Pgi-A	0.000	-0.065	-0.037
ALL	-0.103	-0.053	0.095**

**= p< 0.01; ***= p< 0.001

may have been influenced by the presence of the rare *Pgm-c* allele in the Fleet Lagoon (F_{IS} = 0.356***), the removal of this allele from the analysis produced similar results. This inflated F_{IS} could reflect local genetic differentiation in the Fleet as samples from different localities (within a 4 km stretch) throughout the lagoon basin were pooled together. F_{IS} values indicated a slight but non-significant heterozygote excess at all other enzyme loci and overall there was no deviation from genotype frequencies expected under the Hardy-Weinberg equilibrium.

Nei's (1972) genetic identity (I) and genetic distance (D) indicated that all three populations of *I. chelipes* were closely related. All genetic identity values fell within the range given by Thorpe (1982) for conspecific populations (values of I are all above 0.95). The corresponding genetic distance values (D = 0.005- 0.034) agree with previous work on the amphipod *Talitrius saltator*, where genetic distances lower than 0.1 were recorded between populations within the same geographic group (De Matthaeis et al., 1994).

Discussion

Analysis of the genetic structure of endangered and rare species provides valuable data for conservation

management. Genetically differentiated populations may be considered as candidates for special management consideration to prevent the loss of unique and uncommon allelic variants (Leberg, 1996). In this study, sampling error was relatively low as sample size was high at each of the three sites (N =100). However, the low number of loci examined may have affected estimates of genetic identity and distance, and allozyme heterozygosity may have been biased (Archie, 1985).

Within population diversity and differentiation

The levels of genetic variation found in each sub-population were comparable to the variation found in other crustaceans (average heterozygosity per locus for all populations was 8%). Siegismund et al. (1985) found an average heterozygosity per locus of 6.5% for six species of *Gammarus*, and Nelson & Hedgecock (1980) found an average heterozygosity per locus of 5 to 6% in a study of 44 species of decapods. The genetic diversity, as revealed by the expected heterozygosity per polymorphic locus ($14\% < H_{NB} < 20\%$), was low and did not show any significant variation between lagoons, as did the allelic richness reflected by the average number of alleles per locus.

While inbreeding or local microgeographic variation may have contributed to the overall deficiency of heterozygotes at the PGM locus in the Fleet lagoon, F_{IS} values for EST, MDH and PGI indicated slight heterozygote excesses but no significant effects of inbreeding overall. This is surprising given the isolated nature of these populations apparent from F_{ST} analysis and may reflect relatively high local population sizes combined with the success of the brooding life-history strategy in these marginal habitats. Even though no significant levels of inbreeding were detected, local extinctions may occur in lagoonal populations leading to bottleneck effects. Tests carried out with the Bottleneck software (Cornuet & Luikart, 1996) did not suggest the occurrence of any recent bottleneck events. This suggests that the *I. chelipes* populations are relatively stable in the sampled lagoons at least over recent times. If extinction events were to occur regularly, this would prevent the re-accumulation of genetic variability in the population through mutation or immigration.

Between population gene flow

The degree of genetic variability between populations of *Idotea chelipes* has never been reported previously in the United Kingdom. An overall high level of genetic differentiation (average multilocus F_{ST} = 0.164***; p<0.001) was detected between three populations of *I. chelipes* from the southern English coast (Fleet Lagoon; Ashlett Pond; Fort Gilkicker Moat). The closer genetic similarity between the Fleet and Ashlett is quite surprising given the greater geographic distance separating these two populations. Possibly, the two populations exchange more genes than with Gilkicker, through occasional passive transport by wading birds or by rafting on surface drift-weeds and colonisation of habitats in a stepwise manner. Another explanation could lie in the adaptation to environmental conditions within the lagoons and particularly physico-chemical stresses that may act to induce modifications in the genetic structure of marine invertebrates through selection at enzyme loci (Battaglia & Bisol, 1988; Nevo, 1991). Although lagoons experiencing effluent discharges (the Fleet and Ashlett) seem to be genetically more closely related, no clear cut relationship can be established to correlate allele frequencies with environmental factors. Furthermore, morphometric differences (Jolly, unpublished data) are opposed to genetic data and seem to have resulted from ecophenotypic adaptive morphology in response to differing habitats. Significant differences in the shape of the telson were observed from the most heterogenous and ancient habitat (the Fleet) to the least heterogenous and most recently established (Ashlett Pond). Individuals of the Fleet are bigger and experience smaller variance in the distal extremities of the telson than do those inhabiting Gilkicker and Ashlett, most of which appear to exhibit *I. balthica*-like telsons.

Both F_{ST} and genetic identity values (I = 0.967- 0.995) suggest that all these populations are conspecific, but relatively isolated from each other even when the EST locus was excluded from the analysis (average multilocus F_{ST} [NO ESTERASE] = 0.072***; p<0.001). This level of genetic differentiation between populations was very surprising given the relatively small geographic distance separating these populations. However, such data fits with findings for other coastal invertebrates that indicate that levels of dispersal between local populations can be extremely low as a result of habitat fragmentation and direct development (i.e. for isopods, Piertney & Carvalho, 1994, 1995a; Carvalho & Piertney, 1997). As for previous studies on lagoonal invertebrates (e.g. Pearson et al., 2002) it would appear that these habitats act to isolate populations of *I. chelipes*. In the anemone *N. vectensis*, the significant variance observed among populations of the Solent was associated with clonal reproduction and limited dispersal between lagoons (Pearson et al., 2002). The presence of Southampton Water and the Solent estuary system between our sample sites may possibly act as a further barrier to gene flow for *I. chelipes*. The nature of tidal movements within the Southampton Water (double high water; strong ebb tide and high current velocities) may make it difficult to migrate across for small invertebrates with a limited ability to swim. Other explanations may also be possible for genetic differentiation over small geographic distances. Rather than genetic differentiation being governed by passive mechanisms, *I. chelipes* may actually behave to prevent

dispersal out of lagoon habitats. This is because these habitats are favourable to its survival and individuals passively dispersing away from the natal lagoon by rafting on surface drifting macro-algae are more likely to be washed out than introduced into small lagoonal systems by currents. Such behaviour would increase chances of mating and would help prevent the species being outcompeted by other idoteid species (i.e. *I. balthica*, *I. emarginata*, *I. metallica*). Lagoons are marginal habitats and populations may become locally adapted to the sets of environmental conditions present at a particular locality. Such local adaptation of populations may be accompanied by epistatic interactions that act to reduce the fitness of hybrids resulting from matings of individuals from different populations (e.g. see Rawson & Burton, 2002). Another alternative is that the populations analysed occur at a zone where there is a major phylogeographic break for *I. chelipes* (e.g. see for *Tigriopus californicus* along the coast of California; Burton & Lee, 1994). Interestingly, a study of mtDNA variation in *Littorina saxatilis* (Wilding et al., 2000) has revealed an east-west separation of populations along the southern English coast, with one haplotype having arisen via mutation on the south coast and which remains restricted to south-western Britain because of low gene flow. It is possible that one or several such mechanisms are acting to maintain significant levels of genetic differentiation between local populations of *I. chelipes*. However, if *I. chelipes* follows a metapopulation structure then dispersal would be necessary to avoid regional extinction of the species, especially as lagoons are prone to catastrophic environmental change or even destruction. It is possible that individuals may disperse via drifting seaweed and observations have indicated that there may be marked reductions in macrophyte biomass in winter.

Selection at EST locus and use of the locus to identify sub-specific relationships

Variation in allele frequencies appeared to a much greater extent at the *Est-A* (ET2 in Charfi-cheikhrouha, 1994) locus, F_{ST} = 0.229*** compared to the other enzymes 0.009*<F_{ST} <0.096***. Such a result may indicate that the *Est-A* locus could be under selection, in which case previous diagnosis of subspecies in the *Idotea chelipes* complex (Charfi-cheikhrouha, 1994) may have been biased. This enzyme is known to be under strong selective pressure in other species. A hypothesis put forward by Gosling & McGrath (1990) to explain gradual changes in allele frequencies of mussel spat between different tidal levels at both the *Est-D* and *Odh* loci was that a strong selective pressure operated within the first month of benthic existence. In the polychaete *Pectinaria koreni*, an electrophoretic mobility shift in the esterase enzyme was found to be diagnostic of one particular population in the northern Irish Sea, although the other loci together with mtDNA data revealed no genetic differentiation with populations present in the English Channel (Jolly, unpub. data).

Finally, the animals used in the present study seemed to exhibit most of the variation of the three previously identified subspecies, at the EST locus (Charfi-cheikhrouha, 1998). In our study the *Est-A* locus was found polymorphic with two alleles in all three populations of the southern English coast whereas in the three "subspecies" it was fixed for one allele. In terms of genetic identity, values (Nei, 1972) between *I. chelipes chelipes* and *I. c. mediterranea* are all above 0.95, and fall between 0.87 and 0.90 when considering *I. c. bocqueti*. Discrimination at the subspecific level was only possible on the basis of minor morphological characteristics (coxal plates and pleotelson shape) combined with the EST locus. However, morphological differences could be the result of ecophenotypic adaptive morphology in response to differing habitats or the result of differential growth rates, at least in the shape of the telson. At the EST locus, the zymograms produced by Charfi-cheikhrouha (1994) are difficult to interpret and protein extraction was performed on a group of individuals from the different populations they used in their study. The relative mobility of the three different alleles representing each of the three subspecies is therefore not clear. *I. chelipes* is a highly variable species which changes its morphological, physiological and ecological characteristics readily to suit the environment it colonises. All the above would suggest that the EST locus is not in fact diagnostic of sub-specific level differences in *I. chelipes* but instead reflects the population structure exhibited by this species.

Conclusion

Subdivided populations of *Idotea chelipes* exhibit moderate levels of genetic differentiation at the local scale around the Solent. This is probably a result of the life history of the species that includes brooding and the direct development of juveniles. Other factors, however, may contribute to such a population structure including behaviour, selection for locally adapted populations and phylogeographic considerations. The conservation management and restoration of lagoonal populations of *I. chelipes* and other lagoonal species should take into account the population structure and likely dispersal behaviour of such organisms. Because of the slow mutation rate of allozymes, genetic markers with a higher rate of evolution should be used and more populations should be analysed to give an accurate description of the contemporary dispersal behaviour of *I. chelipes*.

I. chelipes is reported to belong to a complex including three subspecies, but genetic studies on this isopod are limited and the molecular status of this species (and other *Idotea* species) is unclear. Additional studies are needed in

order to elucidate further the amount of genetic divergence between *I. chelipes* populations within the UK but also between the English coasts and the southern Atlantic/ Mediterranean coasts of France. The fact that significant genetic differentiation may even exist within lagoon complexes is particularly interesting and maybe reminiscent of microgeographic genetic differentiation detected in other Crustacea.

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