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Does the genetic structure of *Pectinaria koreni* (Polychaeta: Pectinariidae) conform to a source–sink metapopulation model at the scale of the Baie de Seine?

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Abstract According to a recent demographic survey, the population structure of *Pectinaria koreni* might fit a source–sink metapopulation model at least at a regional scale. Spatial and temporal genetic structure of the populations present in the Baie de Seine (eastern Baie de Seine and Baie des Veys) was assessed using four highly polymorphic microsatellite loci which have revealed strong intra-locality genetic diversity. In the eastern Baie de Seine, both temporal (1994–1996) and spatial (1994) genetic differentiation were relatively low but significantly different from zero despite a 15-day dispersing larval stage. Such structures may be explained by the settlement of larvae from different gene pools and differing recruitment histories among sites within the eastern Baie de Seine. At a larger scale, similar levels of spatial differentiation were observed in 1999 between the eastern Baie de Seine and the Baie des Veys. The lack of any significant differences in gene diversity and allelic richness rules out a source–sink functioning at the scale of our study. The present paper provides further knowledge on the population dynamics of a univoltine species and its persistence in a highly dispersive environment via a shifting spatial mosaic.

Keywords *Pectinaria koreni* · Microsatellites · Metapopulation · Genetic heterogeneity · Effective dispersal

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Introduction

Species are typically divided into a number of populations more or less interconnected by migration. Such a dynamic feature has many evolutionary consequences on populations, especially in terms of genetic diversity and population structuring. Due to a finite population size, genetic drift would tend to decrease the total amount of genetic diversity in small populations unless it is counterbalanced by migration. Besides, populations have finite life expectancies, making local extinction/recolonisation processes likely to be a key factor for the persistence of the species at the level of a set of local populations. Although the implication of subdivision and persistence of local populations on the evolutionary dynamics of species has long been emphasised (Wright 1940; Andrewartha and Birch 1954; MacArthur and Wilson 1967), the term “metapopulation” was first introduced by Levins (1969) and originally referred to a spatially structured population which persists through re-colonisation events despite localised extinctions.

Pannell and Charlesworth (2000) have reviewed the implications of extinction/recolonisation dynamics on the amount of genetic differentiation between local populations. They consider two founding models (the <migrant pool and the propagule pool), each having different genetic consequences depending on the theoretical population model used, the rate of extinction, and the migration rate (Slatkin 1977; Whitlock and McCauley 1990). Under the *n*-island-model with migrant pool colonisation, extinction and recolonisation tend to reduce genetic differentiation and increase the genetic diversity of the newly established populations only when the number of founders colonising new vacant areas is twice the number of migrants exchanged between extant populations (Wade and McCauley 1988; McCauley 1991). Under a propagule pool model, extinction is expected to (1) increase the level of differentiation relative to the case of no local extinction (Wade and McCauley 1988; Whitlock and McCauley 1990), and (2) reduce the amount of genetic diversity in newly established popula-

tions (Pannell and Charlesworth 2000). However, these predictions are also affected by the metapopulation structure itself (Dias 1996; Harrison and Hastings 1996; Hanski and Gilpin 1997). The extinction/recolonisation process establishes an age structure among local populations, which may either enhance or reduce the amount of genetic differentiation among subpopulations, as a function of both the migration model and the relative importance of parameters such as the number and the origin of founders (Slatkin 1977; Whitlock and McCauley 1990; Pannell and Charlesworth 2000).

Given those theoretical studies, the marine environment offers considerable potential for metapopulation studies. First, it is a highly dispersive environment characterised by strong substrate heterogeneity. Secondly, most benthic invertebrates are characterised by a complex life-cycle involving a benthic adult breeding stage followed by a dispersive larval stage (Thorson 1946), the duration of which may vary from only a few hours up to several months. As a consequence, the persistence and stability of fragmented benthic populations is governed by two processes: (1) the rate of migration, which depends on hydrodynamic processes (i.e. advective currents, turbulent mixing and eddy diffusion), intrinsic biological properties of the larvae, the reproductive effort of the adults and larval interactions in the plankton, and (2) the rate of population extinction, which also relies on hydrodynamics (i.e. disturbance at the benthic boundary layer), larval recruitment processes (i.e. substrate choice, intra-/inter-specific competition) and predation on adults. According to the supply-side theory (Underwood and Fairweather 1989; Grosberg and Levitan 1992), the spatio-temporal dynamics of benthic-pelagic populations, and thus the extinction rate, is initially dependent on larval input which is, in turn, influenced either by larval dispersal and larval retention in the vicinity of adult populations or by larval homing towards the adult population after a short dispersal phase.

In this context, the tubicolous polychaete *Pectinaria koreni* (Malmgren) is characterised by interesting biological features which can be used to analyse the metapopulation processes governing the evolution of natural populations in the marine environment. Based on recent findings, populations of this univoltine species living for 15–18 months may be described as an assemblage of geographically isolated populations potentially linked together by a planktonic larval phase of approximately 15 days (Lagadeuc and Retière 1993; Ellien et al. 2000). Thus, populations of *P. koreni* might constitute a metapopulation along the French coast of the eastern English Channel. Indeed, at the scale of the English Channel, both a strong tidal regime and the action of wind-induced currents govern patterns and rates of larval dispersal during the reproductive season (Ellien et al. 2000). In addition, demographic monitoring of French populations has revealed that some are transient whereas others are not (Ellien et al. 2000; F. Gentil and E. Thiébaud, personal observation), which supports the adequacy of a source–sink metapopulation model (as defined by

Pulliam 1988, with growth rate (r) being >0 in sources and <0 in sinks in the absence of migration) at least at the regional scale of the Baie de Seine (eastern Baie de Seine; Baie des Veys). In this study, we use molecular tools to investigate the likelihood of a source–sink functioning between the eastern Baie de Seine (BS) and the Baie des Veys (BV).

The eastern Baie de Seine may correspond to a potential source because it is considered to be the most important population in the English Channel both in density and surface area (300–650 individuals per square metre on a 400 km² area), and it exhibits a relatively stable inter-annual recruitment (Thiébaud et al. 1997). On the other hand, the Baie des Veys population might be regarded as a sink: suitable habitats are significantly smaller (in the order of 100 km²) and the population is demographically unstable, fluctuating from extremely low densities which may eventually lead to extinction, up to relatively high abundances (Ellien et al. 2000). In addition, hydrodynamic modelling of larval dispersal reveals that potential exchanges between both bays take place mainly from the eastern Baie de Seine towards the Baie des Veys (Ellien et al. 2000). Although there is no direct evidence of extinction events in the latter site between 1973 and 2000, the lack of annual demographic data does not allow definite conclusions (i.e. demographic data were collected at irregular time intervals, thereby disregarding the univoltine character of the species). Molecular ecology may provide powerful tools to describe and determine the relationships between populations in terms of recruitment, dispersal and origin of founders.

The present study aims to address the following questions: (1) Considering that *P. koreni* is univoltine, is there any temporal genetic variation in a population at one particular patch that could indicate an interannual settlement of genetically different larval cohorts? (2) Is the potential source population a mosaic of several genetic entities or are the patches relatively well interconnected? (3) Does the Baie des Veys represent a sink population? (i.e. can extinction events take place without larval supply from other geographic origins?), in which case lower levels of genetic diversity are expected relative to the source because of genetic drift associated with founding (Gaggiotti and Smouse 1996). Finally, are the results provided by hydrodynamic modelling (Ellien et al. 2000) representative of the effective larval dispersal?

Methods

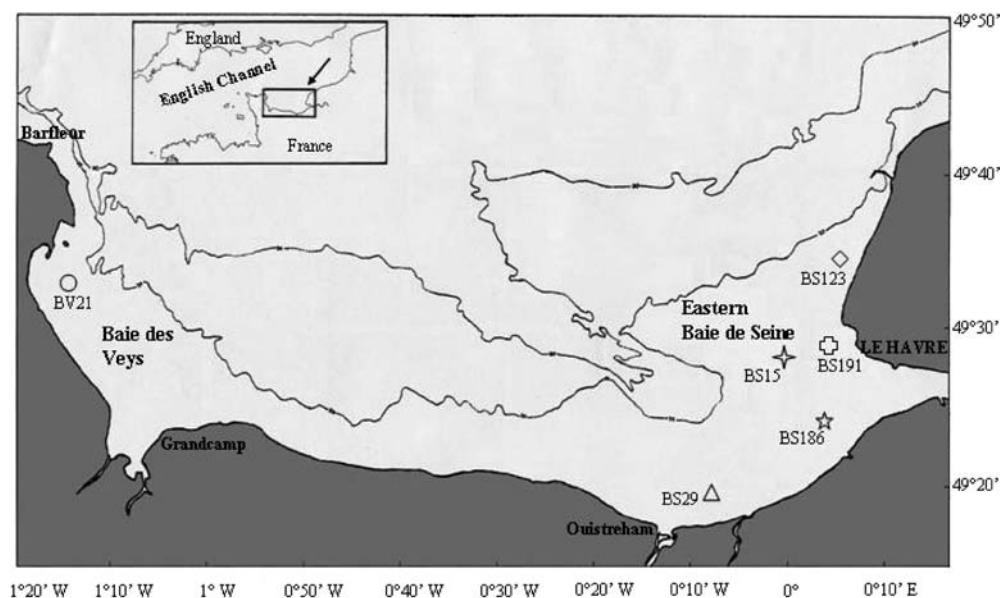
Biological model and sampling procedure

Populations of *P. koreni* (Malmgren) exhibit a highly fragmented distribution throughout the English Channel, associated with the distribution of muddy fine sands confined to bays and estuaries. Similarly, at the scale of a population, individuals form aggregates of highly variable densities (Lambert 1991). *P. koreni* is characterised by a benthic-pelagic life cycle with a main breeding period occurring between March and July, with the release of 20,000–430,000 oocytes per female (Elkaim and Irlinger 1987;

Table 1 Population names, location, sampling year and numbers of *Pectinaria koreni* (*N*) screened for microsatellite analysis for each sampling year

| | Latitude | Longitude | Date | <i>N</i> |
|-----------------------|-------------|------------|----------------|----------|
| Eastern Baie de Seine | | | | |
| BS15 | 49°27.50' N | 0°02.30' E | March 1999 | 33 |
| BS29 | 49°18.50' N | 0°91.98' W | March 1999 | 54 |
| BS191 | 49°28.30' N | 0°02.28' E | May 1994/95/96 | 24/24/24 |
| BS123 | 49°36.10' N | 0°04.50' E | May 1994 | 23 |
| BS186 | 49°23.76' N | 0°03.45' E | May 1994/96 | 25/19 |
| Baie des Veys | | | | |
| BV21 | 49°32.00' N | 1°15.50' W | March 1999 | 40 |

Fig. 1 Study area and location of sampling stations in the Baie de Seine [eastern Baie de Seine (BS); Baie des Veys (BV)] for 1994 and 1999 (details are given in Table 1). Labels represent sampling locations



Irlinger et al. 1991). Larvae may be transported up to 30–40 nautical miles from their site of release, depending on the hydrodynamic residual circulation and on the intensity of environmental forcing (Lagadeuc 1992; Thiébaud et al. 1998). In addition, Lambert (1991) showed that post-larval re-settlement may take place if the substrate where larvae first settle is not appropriate. While the density of settling post-larvae may reach 28,409 ind. m⁻², intraspecific competition may eliminate 40%–100% of young settlers (Lambert 1991).

Sampling was undertaken from May 1994 to March 1999 within the Baie de Seine. A preliminary survey of five sites was performed within the eastern Baie de Seine (1994–1999) and one additional site was sampled in the Baie des Veys (1999) (see Table 1 for details). Individuals of *P. koreni* were obtained by sampling the top 10 cm of the sediment with a 0.25 m⁻² Hamon grab. Samples were sorted directly on board, flash-frozen and kept in liquid nitrogen until DNA extraction. The study area is represented by Fig. 1.

DNA extraction and microsatellite genotyping

Gills were removed while individuals were still frozen and ground directly in 600 µl of a 60°C preheated 2% CTAB (cetyltrimethylammonium bromide) buffer solution, which differentially precipitates DNA from polysaccharides. The tissues were digested at 60°C with 0.1 mg ml⁻¹ proteinase K and the extraction was performed overnight. Proteins, lipids and carbohydrates were removed using a standard phenol/chloroform procedure and DNA

was precipitated with isopropanol after 1 h incubation at 37°C in the presence of 2 µl RNase (500 µg/ml) for RNA removal. The tubes were finally centrifuged at 15,000 rpm for 10 min. DNA pellets were washed with 70% ethanol and resuspended in 20–50 µl 1×TE (10 mM Tris- 20 mM EDTA pH 8) buffer prior to electrophoresis on 0.8% agarose gels to check the quality of the extracted DNA.

A total of 218 individuals were screened over four highly polymorphic microsatellite loci (PKGT1; PKAT/GT1; PKAT/GT2 and PKAT/GT4) isolated by Weinmayr et al. (1999).

DNA amplifications were performed on an MJResearch thermal cycler (PTC-200) with the following conditions: first an initial denaturation step at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing for 40 s (PKAT/GT1–2–4 at 58°C and PKGT1 at 51°C) and elongation at 72°C for 35 s and a final elongation at 72°C for 10 min. PCR reactions were performed into a 20 µl reaction volume consisting of 1×PCR buffer (supplied with polymerase enzyme); MgCl₂ at a concentration of 1.5 mM (PKGT1) or 2 mM (PKAT/GT1–2–4); 0.12 mM dNTP; 0.8 µM of forward and reverse primers; 0.01 µg µl⁻¹ T4gene32 protein; 0.5 U of high fidelity *Taq* polymerase (ABgene) (PKAT/GT1–2–4) or 0.5 U Thermoprime plus *Taq* polymerase (ABgene) (PKGT1); 25–100 ng CTAB-extracted genomic DNA. For each locus, one of the primers was labelled with Texas Red fluorochrome for genotyping. PCR products were run on a 6% polyacrylamide/8 M urea sequencing gel, using a Vistra DNA sequencer (Amersham Pharmacia Biotech).

Statistical analysis

Genetic diversity as summarised by (1) the observed heterozygosity (H_o), (2) the expected heterozygosity (H_{NB} ; Nei 1987), (3) the number of alleles per locus per population (N_o) and the allelic richness (R_s), was estimated using FSTAT 2.9 software (Goudet 1995). Standard errors of gene diversity values were estimated using GENETIX 4.02 (Belkhir et al. 1996) and tested using a simple Student's *t*-test. Tests of genotypic disequilibrium between each pair of loci in each population and over all populations were performed by using Fstat 2.9 (Goudet 1995). 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 and Cockerham's (1984) *f* and using Fisher's exact test on GENEPOP 3.3 software (Raymond and Rousset 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 and Rousset 1995) following Weir and Cockerham's (1984) θ value. Exact tests for the null hypothesis of identity of allelic distribution across populations were performed with GENEPOP 3.3. Multiloci θ values were also estimated and tested between pairwise combinations of populations by using the same software. To illustrate the extent of both spatial and temporal genetic variation among samples, a principal components analysis (PCA) was performed on allelic frequency data using PCAGEN 1.2 software (Goudet; <http://www.unil.ch/izea/research.html#softs>).

Results

Genetic diversity

Testing for linkage disequilibrium did not reveal any association of alleles between any of the four diploid loci examined, demonstrating the independence of the information provided by the four loci used.

Overall, the fixation index (F_{IS}) is high across all loci, with significant departure from Hardy-Weinberg proportions (multilocus average $F_{IS}=0.341$; $P<0.001$). Such heterozygote deficiencies are not explained by specific reproductive traits, but may result from the presence of genetically differentiated entities (Whalund effect). However, our data also suggests that null alleles may occur (Weinmayr et al. 1999; M.T. Jolly, personal observation). Nevertheless, the lack of correlation between F_{IS} and the number of individuals per locus which were not amplified suggests that null alleles are only partly responsible for the inflated F_{IS} values. Moreover, the F_{IS}

values are almost identical whatever populations or sampling dates. All together, this indicates that our conclusions on the genetic structure should not be affected. However, new studies are under progress to determine the origin of these large F_{IS} values.

High and similar levels of genetic diversity (N_o ; H_{NB} ; R_s) were observed throughout the eastern Baie de Seine in both 1994 and 1999 (Table 2), and for all loci. With the exception of PKAT/GT4, for which a lower number of alleles was sampled in 1994, levels of gene diversity (H_{NB}) were similar for both PKAT/GT1 and PKAT/GT2 ($H_{NB(\text{mean})}=96\%$). The number of alleles increases with sample size, indicating that sample sizes are probably too small to reflect the total diversity of *P. koreni* for the four loci. Comparable levels of genetic diversity have been found between the eastern Baie de Seine and the Baie des Veys (1999) (Table 2). This supports the idea that genetic drift is limited due to the potentially huge effective population size of *P. koreni*. However, significant differences in H_{NB} were found between BS29/BV21 at both PKGT1 and PKAT/GT4 ($t=2.2108$; $P<0.05$, and $t=3.336$; $P<0.01$, respectively) (Fig. 2).

Spatial heterogeneity

Within the eastern Baie de Seine (1994 and 1999)

The locus PKGT1 was excluded from the 1994 analysis as data were missing for two sampling sites. In 1994, low F_{ST} values were observed throughout ($0.001<$ pairwise $F_{ST}<0.012$) (Table 3), but both pairwise tests of differentiation ($0.0001<$ *P* value <0.004) and multi-loci F_{ST} ($F_{ST}=0.006$; $P=0.0001$) (Table 4) reveal low but significant genetic substructuring within the eastern Baie de Seine.

Pairwise tests for 1999 data (Table 5) reveal a small but significant level of genetic differentiation ($F_{ST}=0.001$; $P<0.001$) between BS15 and BS29, which is consistent with the levels reported for the 1994 samples between BS186 and BS191 (geographically closest to the 1999 sampling stations). Similarly to 1994, differentiation was mainly due to PKAT/GT2 ($F_{ST}=0.006$; $P=0.0001$).

Table 2 Genetic diversity and heterozygosity for each sampling site for all loci and for 2 years (total number of alleles) N numbers of individuals, N_o average number of alleles, H_{NB} non-biased ex-

pected heterozygosity, R_s allelic richness based on a minimum sample size of 10 individuals except for PKGT1; eastern Baie de Seine (BS); Baie des Veys (BV)

| Sampling sites | PKGT1[34] ^a | | | | PKAT/GT1 [48] | | | | PKAT/GT2 [59] | | | | PKAT/GT4 [26] | | | |
|----------------|-------------------------|-------|----------|-------|---------------|-------|----------|-------|----------------|-------|----------|-------|----------------|-------|----------|-------|
| | N | N_o | H_{NB} | R_s | N | N_o | H_{NB} | R_s | N | N_o | H_{NB} | R_s | N | N_o | H_{NB} | R_s |
| BS191-94 | – | – | – | – | 22 | 24 | 0.946 | 14.2 | 23 | 22 | 0.963 | 14.3 | 23 | 19 | 0.908 | 11.8 |
| BS186-94 | – | – | – | – | 22 | 24 | 0.972 | 15.2 | 23 | 21 | 0.949 | 13.2 | 22 | 14 | 0.866 | 9.0 |
| BS123-94 | – | – | – | – | 20 | 21 | 0.956 | 13.8 | 20 | 19 | 0.962 | 14.0 | 21 | 18 | 0.923 | 11.9 |
| BS15-99 | 23 | 17 | 0.946 | 15.7 | 28 | 24 | 0.955 | 13.7 | 26 | 28 | 0.968 | 15.1 | 28 | 20 | 0.935 | 12.1 |
| BS29-99 | 39 | 23 | 0.952 | 17.5 | 19 | 18 | 0.937 | 12.9 | 32 | 27 | 0.964 | 14.6 | 17 | 17 | 0.959 | 13.4 |
| BV21-99 | 29 | 18 | 0.939 | 15.6 | 31 | 22 | 0.949 | 13.1 | 27 | 28 | 0.971 | 15.4 | 26 | 17 | 0.935 | 11.7 |

^a R_s based on a minimum sample size of 17 individuals

Fig. 2 Graph showing the fluctuations of the gene diversity H_{NB} (Nei 1987) as represented by lines with standard error bars (obtained from 1,000 bootstraps), and the allelic richness (R_S) as represented by histograms, for all 1999 samples and for all loci. R_S (PKAT/GT1–2–4) are based on a minimum sample size of 10 diploid individuals whereas R_S (PKG1) is based on a minimum sample size of 17 diploid individuals

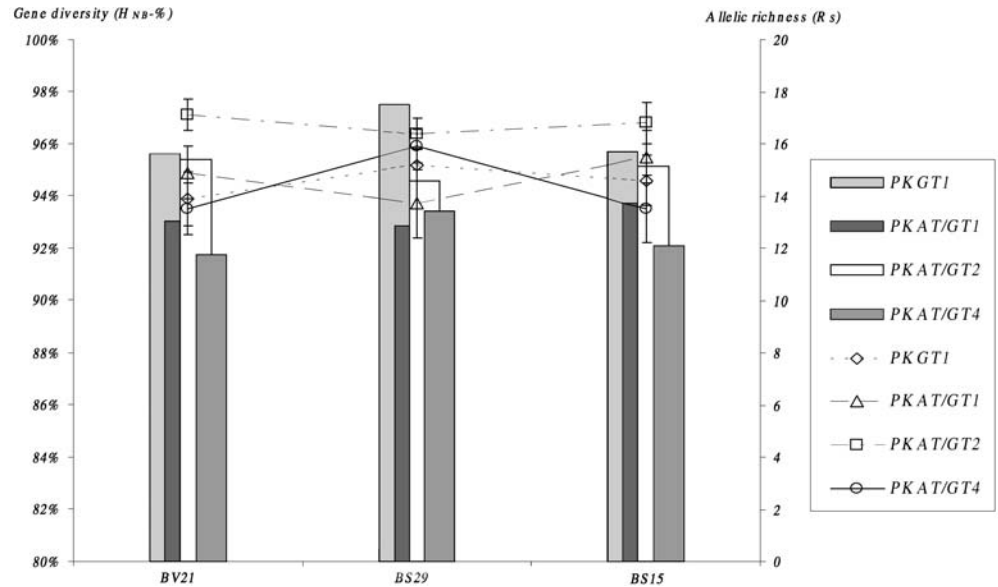


Table 3 Genetic differentiation within eastern Baie de Seine (BS) in 1994; Pairwise F_{ST} values at three diploid microsatellite loci (missing data for PKGT1)

| | BS191-94 | BS186-94 | BS123-94 |
|----------|----------|----------|----------|
| BS191-94 | – | 0.001** | 0.005*** |
| BS186-94 | | – | 0.012*** |
| BS123-94 | | | – |

** $P < 0.01$, *** $P < 0.001$

Table 4 Total F_{ST} values for all loci and multilocus average (P value in parentheses) for 1994 and 1999 data [eastern Baie de Seine (BS); Baie des Veys (BV)]. Tests were performed using Genepop (Raymond and Rousset 1995)

| Locus | F_{ST} (BS)-1994 | F_{ST} (BS/BV)-1999 |
|--------------------|----------------------|-----------------------|
| PKG1 | – | 0.013*** |
| PKAT/GT1 | 0.005** | –0.001 |
| PKAT/GT2 | 0.004*** | –0.0004 |
| PKAT/GT4 | 0.008* | 0.005** |
| Multilocus average | 0.006*** (0.0001) | 0.004*** (0.001) |

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 5 Genetic differentiation within the Baie de Seine in 1999 [eastern Baie de Seine (BS); Baie des Veys (BV)]. Pairwise F_{ST} values at four diploid microsatellite loci

| | BS15-99 | BS29-99 | BV21-99 |
|---------|---------|----------|----------|
| BS15-99 | – | 0.001*** | 0.008*** |
| BS29-99 | | – | 0.002*** |
| BV21-99 | | | – |

*** $P < 0.001$ after sequential Bonferroni correction

Between eastern Baie de Seine and Baie des Veys (1999)

Small but significant genetic differentiation across all 1999 sites (Table 4) (multiloci $F_{ST}=0.004$; $P=0.001$) was caused by two loci (PKG1 and PKAT/GT4). Pairwise F_{ST} values (Table 5) of genetic differentiation are four times higher between BS15 and the Baie des Veys ($F_{ST}=0.008$; $P < 0.001$) than between the latter site and BS29 ($F_{ST}=0.002$), suggesting that migration between the two bays would proceed preferentially along the coast via BS29. Elsewhere, the level of differentiation between the eastern Baie de Seine and the Baie des Veys was comparable to that obtained in 1994 (Table 4) and 1999 (Table 5) within the first site.

Overall differentiation

Results from principal components analysis (Fig. 3) using all our genetic data show both a strong temporal and spatial genetic heterogeneity within the eastern Baie de Seine, and between this site and the Baie des Veys. The first axis represents 32.5% of total inertia (i.e. corresponding to a significant $F_{ST}=0.01$), whereas the second represents only 14.5% of the genetic differentiation.

Temporal variation

Allele frequencies of *P. koreni* were estimated in samples from 1994/1995/1996 and from 1994–1996 at BS191 and BS186, respectively (Fig. 1), in order to trace potential temporal genetic heterogeneity in the eastern Baie de Seine. Table 6 indicates the estimated P values for temporal allelic differentiation for each locus and over the four diploid loci. At both stations a significant differentiation is observed for the most polymorphic locus

Fig. 3 Principal components analysis (PCA) performed using the allele frequency table available for all years and for all sampling stations [eastern Baie de Seine (BS); Baie des Veys (BV)]. Sampling locations are represented as in Fig. 1

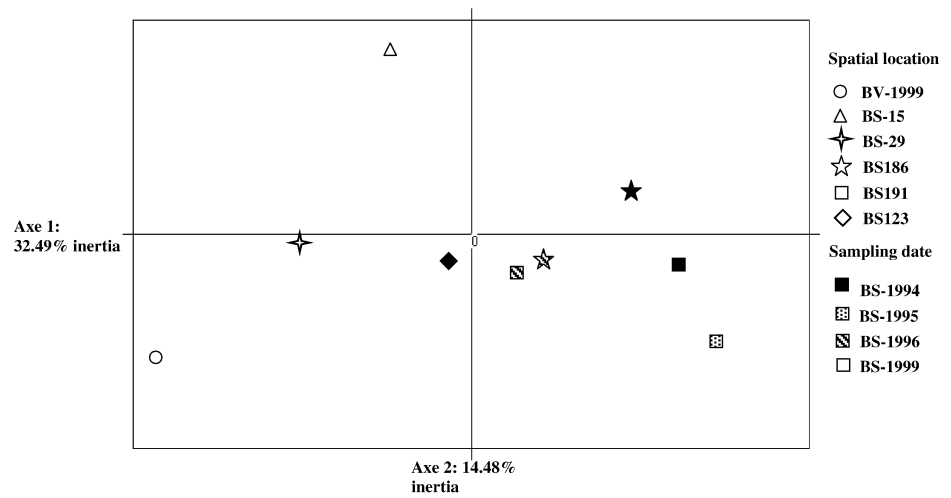


Table 6 Temporal variation P values for exact tests of allelic distribution (Weir and Cockerham 1984) for all loci between 1994 and 1996 at two sampling stations within the eastern Baie de Seine (BS)

| Locus | PKGT1 | PKAT/GT1 | PKAT/GT2 | PKAT/GT4 | All loci |
|-------------------------------|-------|----------|----------|----------|----------|
| BS191-1994/95/96 P value | 0.075 | 0.611 | 0.002 | 0.413 | 0.008 |
| BS186-1994/96 P value | – | 0.475 | 0.0001 | 0.027 | 0.0002 |

PKAT/GT2 and to a lesser extent PKAT/GT4 at station BS186. Three loci did not present any differentiation at BS191 (PKGT1, PKAT/GT1–4; $0.075 < P < 0.611$), and one at BS186 ($P_{(PKAT/GT1)} = 0.48$).

Overall, both sampling stations exhibit marked temporal genetic differentiation (multiloci P value < 0.01 and 0.001 at BS191 and BS186, respectively). Such a structure seems to indicate that pulses of larvae settling between 1994 and 1996 were genetically different.

Discussion

In this study we used four highly polymorphic microsatellite loci to demonstrate (1) a large genetic diversity throughout the Baie de Seine and (2) a small but significant genetic differentiation over time and space. We discuss these results in relation to the relative importance of larval dispersal and retention for the maintenance of both the eastern Baie de Seine and the Baie des Veys populations.

Genetic heterogeneity in the eastern Baie de Seine

Despite the idea that species with long planktonic dispersal are more or less genetically homogeneous over relatively large distances, statistical comparisons of allele frequencies among spatial and temporal samples of *P. koreni* within the eastern Baie de Seine both reveal significant genetic heterogeneity which may be categori-

sed as “chaotic” in a way similar to what Johnson and Black (1982) found for *Siphonaria jeanae*. Indeed, this heterogeneity does not follow a consistent pattern but forms a shifting spatial mosaic (with abundances of *P. koreni* and genetic composition changing on every patch in each generation) without any microspatial directionality in gene flow. It is probable that such a structure is correlated with the variability of wind-induced currents which may, given changes in wind speed and direction, bring about a strong variability in larval dispersal and recruitment patterns (Lagadeuc 1992).

Temporal and spatial genetic change may be explained by (1) a large variance in reproductive success between patches (Hedgecock 1994), and (2) the settlement of differentiated larval cohorts and thus the occurrence of several genetic entities (David et al. 1997). Both hypotheses will be discussed.

The fact that adults are distributed in patches of highly variable densities offers considerable potential for variance in reproductive success between adult aggregates. External fertilisation success has also been shown to be reduced drastically by the dilution of sperm and gametes when spawning adults occur at low densities (Levitan 1991). This strongly suggests that only disjunct massive aggregates of *P. koreni* are likely to reproduce effectively, possibly leading to the occurrence of different larval pools within the eastern Baie de Seine. However, such a variance in reproductive success is unlikely to be the only explanation for such levels of both temporal and spatial differentiation, although the contribution to a given cohort by a limited number of adults may re-

sult in significant drift effects between cohorts (David et al. 1997). Such a variance linked to the loss of larvae during the pelagic phase and/or the failure to reproduce (variable among patches), would tend to decrease total effective population size (N_e) and therefore result in demographic instability and increased genetic drift. In contrast, the eastern Baie de Seine population is characterised by a sex ratio close to 1:1 (Irlinger et al. 1991), and is larger (up to 650 ind. m^{-2} ; Lambert 1991) and demographically more stable than the Baie des Veys population, presenting a high level of genetic diversity among all patches. Because high genetic diversity may only be explained by either a large effective population size or a high mutation rate, the effective size of the population is expected to be considerable, although probably lower than the census size, as a function of variance in reproductive success. One way to estimate the effective population size is to use the method developed by Waples (1989), based on the analyses of the distribution in allelic frequencies over time. However, this method requires the assumption that the eastern Baie de Seine is a closed population, which does not seem to be true in the light of hydrodynamic modelling of potential larval dispersal (Ellien et al. 2000).

The hypothesis that the temporal heterogeneity of recruits is responsible for spatial genetic discontinuities at a local scale has been proposed from allozyme studies on several species characterised by a long planktonic dispersal phase (e.g. limpets, Johnson and Black 1984; sea urchins, Watts et al. 1990; crabs, Kordos and Burton 1993; McMillen-Jackson et al. 1994; and bivalves, David et al. 1997).

P. koreni is a univoltine species for which two recruitment events (May and July) have previously been observed within the eastern Baie de Seine (Irlinger et al. 1991), the first one being much more important than the second. During the spawning season, several reproductive events may take place at different sites within the eastern Baie de Seine, thereby producing different larval cohorts from different local geographical origins. Depending on the prevailing hydrodynamic conditions at each site of release, dispersal patterns will be affected differently, resulting in variable larval retention rates and geographic shifts in post-larval settlement patterns (Lagadeuc 1992; Ellien et al. 2002).

Given that the retention rates in the eastern Baie de Seine are greater than larval export rates (Ellien et al. 2000), pools of larvae from genetically different local entities within the eastern Baie de Seine may evolve separately according to their respective points of egg release, and subsequently settle at any site of the bay, replacing the old generation and forming a "shifting spatial mosaic" of genetic entities. Temporal variation may thus result from a reproductive asynchrony between genetically different adult aggregates within the eastern Baie de Seine or among nearby populations (Baie des Veys, Penly), depending on local disturbance and habitat quality. Asynchronous spawning events and variance in reproductive success could produce aggregates of differ-

ing genetic composition which would show as much genetic heterogeneity within a single patch as between different patches. In contrast, repeated extinction/recolonisation events are the main processes involved in producing similar amounts of both spatial and temporal genetic differentiation in the hydrothermal vent populations of *Alvinella pompejana* (Jollivet et al. 1998).

Spatial differentiation at the scale of the Baie de Seine

A small but highly significant differentiation is observed between the eastern Baie de Seine and the Baie des Veys in 1999. In fact, a similar level of differentiation is detected between sampling sites (i.e. patches or demes) within the eastern Baie de Seine in 1994 as between both distant populations in 1999, but no true extinction events have yet been evidenced. As explained by Balloux and Lugon-Moulin (2002), such small F_{ST} values do not necessarily mean that there is a weak genetic differentiation. Those values are deflated because of the high genetic diversity exhibited by our microsatellite markers.

The assumption of a source-sink metapopulation at such a regional scale (i.e. the Baie de Seine), in which the eastern Baie de Seine would be the source of migrants could explain differentiation by drift as long as the number of colonists arriving from the source is less than the number of migrants exchanged within the source populations. Although both populations are genetically different, there are no overall significant differences in the allelic richness and gene diversity. Therefore, a source-sink functioning at the scale of our study is unlikely.

Elsewhere, under a classical metapopulation model with extinction/recolonisation, we would expect to find greater variation in the genetic diversity between demes than in a subdivided population without recurrent extinction (Pannell and Charlesworth 1999, 2000). Our results therefore tend to indicate that the eastern Baie de Seine and the Baie des Veys belong to one single subdivided population made up of several breeding subpopulations or demes. Alternatively, as genetic differentiation values are close to significance thresholds, both populations may constitute one open population close to subdivision.

Under a migrant pool type of colonisation without extinction and with an unequal recruitment between demes (in both the eastern Baie de Seine and the Baie des Veys), genetic differentiation would be minimised, thereby resulting in no significant differences in genetic diversity. Thus, we may consider all patches as potential sources or sinks, depending on the prevailing hydrodynamic and stochastic environmental processes, where the rate of exchange depends on both the size of the patches and their spatial configuration. Patches are ephemeral and the yearly replenishment of the population is accompanied by a shift in the location of aggregates due to changes in hydrodynamic conditions during the spawning season. The Baie des Veys population probably persists chaotically, as no consistent pattern of colonisa-

tion/migration is observed, but in a shifting balance between reproduction, larval supply and the environmental stochasticity, thereby producing a mosaic of genetic entities throughout the whole Baie de Seine.

Fitting population genetic data to larval dispersal modelling

The Baie des Veys population appears to be genetically distinct from the eastern Baie de Seine. We can therefore assume that migration is substantially reduced between both populations, although both have similar levels of gene diversity and allelic richness. This result agrees relatively well with the 2-D larval dispersal model (Ellien et al. 2000, 2002), in that migration between both bays is very limited, with the Baie des Veys populations persisting at low densities. Migration mainly (if not only) occurs from the eastern Baie de Seine to the Baie des Veys with north eastern winds of 7 m s^{-1} (Ellien et al. 2002) (the reverse is only possible for strong south western winds of 15 m s^{-1}), with migration events occurring preferentially via sites which are at the range limit of both populations (e.g. BS29), which agrees with our genetic data. According to Ellien et al. (2000), larval retention rates in the Baie des Veys are more stable relative to the eastern Baie de Seine area, whatever the wind speed. This is mainly because tidal advection is stronger in the former site than in the latter, where diffusion is the dominant process. Larvae released in the Baie des Veys are therefore trapped in the Barfleur gyre, resulting in a more stable self-recruitment than in the eastern Baie de Seine. As a result, populations in the Baie des Veys may still persist locally in the absence of continuous migration events from the eastern Baie de Seine. Both modelling and genetic data therefore tend to discard the possibility of a source–sink metapopulation at the scale of the Baie de Seine.

Although colonisation of larvae from neighbouring populations into the eastern Baie de Seine is potentially weak (Ellien et al. 2000, 2002), potential larval fluxes may also occur from Penly (situated further north along the French coast) to the eastern Baie de Seine only when north eastern winds of 9 m s^{-1} are blowing (the reverse is possible only for south western winds of 12 m s^{-1}). The eastern Baie de Seine could therefore recruit stochastically from Penly, which in turn may be connected, under certain hydrodynamic conditions, to populations living around the Straits of Dover. Although the relative importance of delayed larval metamorphosis and post-resettlement behaviour on migration rates has not yet been quantified, migration may possibly be enhanced by such processes.

The present paper provides evidence for temporal and spatial genetic heterogeneity in a univoltine marine species with planktonic dispersal. The genetic structure of *P. koreni* populations in the Baie de Seine is that of a shifting mosaic, where genetic patchiness is renewed in each generation. Contrary to previous expectations from

demographic monitoring, there is no source–sink process (*sensu* Pulliam 1988; Gaggiotti 1996) between the eastern Baie de Seine and the Baie des Veys. Rather, both populations seem to constitute one single subdivided population, where migration is oriented from the Seine estuary to the Baie des Veys, with the latter site able to sustain itself at least in the short term.

There is an obvious need for a wider study at the scale of the English Channel in order to determine whether populations on both French and English coasts constitute a metapopulation and to what model they conform. Elsewhere, a more thorough sampling programme needs to be applied in both the eastern Baie de Seine and the Baie des Veys, in order to accurately estimate the effective population size and the total genetic diversity. A longer temporal genetic study of adults in these two areas would enable us to monitor patterns of genetic changes over time and to see whether there is synchrony/asynchrony in the genetic composition of the two areas. In addition, the analysis of larval clouds and/or newly settled recruits in the Baie de Seine and along the French coastline would provide information on their respective origins (within the bay or from other populations), in an attempt to determine the source of temporal variation in the genetic composition of recruits.

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