

# Limits to gene flow in the slipper limpet *Crepidula fornicata* as revealed by microsatellite data and a larval dispersal model

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**ABSTRACT:** *Crepidula fornicata*, one of the most invasive marine sessile invertebrates in Europe, has traits commonly associated with a weak population genetic structure (high population connectivity): wide environmental tolerance, high fecundity, and a long planktonic larval stage (21 d). In order to determine the scale at which effective dispersal occurs in this potentially highly dispersive species, we designed a hierarchical sampling protocol and analyzed genetic variation at 4 microsatellite loci in 17 populations from 6 French bays situated in the English Channel and at the entrance of the English Channel (Iroise Sea). A strong regional pattern with populations clustered into 3 main groups (Iroise Sea, western and eastern English Channel) was revealed, as was significant isolation by distance along the Channel coasts. Conversely, a high degree of genetic connectivity was observed within the study bays. These estimates of effective dispersal were in agreement with outputs of a 2D larval-dispersal model based on local hydrodynamic conditions, suggesting that connectivity between populations of *C. fornicata* is influenced by hydrodynamic feature. This study suggests the existence of barriers to gene flow of *C. fornicata* over moderate distances (ca. 100 km).

**KEY WORDS:** Slipper limpet · Larval dispersal · Invasive species · Gene flow · Dispersal modelling

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## INTRODUCTION

Pelagic larvae of benthic marine invertebrates are transported by oceanic currents for weeks to months, allowing for potential long-distance dispersal (Kinlan & Gaines 2003). Dispersal drives species spread and population replenishment. Because the scale of dispersal has a strong influence at both ecological and evolutionary levels, it has been the focus of numerous empirical and theoretical studies (Levin 2006 and references therein). The rate and scale of successful exchanges (i.e. connectivity) among populations of marine organisms also have major implications in conservation biology—for instance, for the design of marine protected areas or for predicting the spread of invasive species (Kinlan & Gaines 2003, Cowen et al. 2006).

In marine sessile invertebrates with pelagic larvae, an extended planktonic period is expected to be correlated with a high rate of larval exchange among populations, thus enhancing population connectivity over large distances (Kinlan & Gaines 2003, Levin 2006). Populations of those species with a benthic-pelagic life cycle were thus first assumed to be demographically open, with recruitment originating mostly from distant sources (Caley et al. 1996), preventing the delineation of 'local populations' (as defined in Waples & Gaggiotti 2006). This is summarized as the 'connectivity paradigm' by Levin (2006). The simple expectation of a correlation between long-distance dispersal ability (often based on the duration of the planktonic period) and effective (realized) dispersal is nevertheless largely debated. Recent works suggest that retention of larvae near their parental population is more frequent than

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suspected, and thus, that populations may be less open than originally thought (Levin 2006). Population genetic studies have indeed revealed limits to gene flow among conspecific marine populations without any obvious barriers to dispersal (e.g. Taylor & Hellberg 2003). These limits were assumed to be promoted by biological factors (e.g. spawning season, settlement cues; Hohenlohe 2004) but also by physical processes such as ocean currents. Larvae may, for instance, be retained by nearshore circulation patterns, especially in estuaries or bays (McShane et al. 1988). Modeling of larval dispersal as a function of hydrodynamic processes has shown that, depending on water circulation conditions (e.g. tidal residual currents, wind-driven currents, eddies, and gyres), larvae can be either retained in the vicinity of adult populations, thus enabling local recruitment, or conversely be transported away from their parental population (e.g. Ellien et al. 2000, 2004, Hohenlohe 2004). Direct measurement of dispersal is a difficult task in the marine environment (Nathan 2001) and determining how far larvae tend to effectively disperse requires multiple approaches (Gilg & Hilbish 2003, Hohenlohe 2004, Viard et al. 2006). Although still scarce, the joint analysis of realized dispersal (as measured by gene flow) and potential larval dispersal (based on realistic hydrodynamic models) is an efficient way to tackle this issue by balancing the caveats of each of these approaches (Thorrold et al. 2002, Kinlan & Gaines 2003).

In the present study, we examined the connectivity paradigm in a potentially highly dispersive species, *Crepidula fornicata*, using complementary methods: (1) genetic analysis based on a hierarchical sampling design was carried out to estimate the effective dispersal (gene flow) at 2 spatial levels (within and among bays); and (2) a 2D hydrodynamic model was used to simulate the patterns of larval movement throughout the study area. The slipper limpet *Crepidula fornicata* provides an interesting case study, as the life-history traits of this gastropod are commonly associated with panmixia and weak genetic structure: it displays wide environmental tolerance (Blanchard 1997), efficient reproductive behavior (i.e. capacity of sex reversal and polyandry within mating groups; Dupont et al. 2006), high fecundity (Richard et al. 2006), and a long planktonic larval stage (3 to 4 wk; Coe 1949). Moreover, *C. fornicata* is a representative of one of the most invasive marine species introduced into Europe during the 20th century (Gouletquer et al. 2002). Native to western North Atlantic coasts, the slipper limpet has successfully colonized the coasts from Scandinavia to the Mediterranean Sea (Blanchard 1997). It was first recorded in Europe >130 yr ago (at the end of the 1870s in the UK; Blanchard 1997, Thielges 2005) and its first appearance along the French coasts of Brittany

and Normandy occurred in the 1940s (Blanchard 1997). This species is now well established and its presence has major consequences for the indigenous macro-benthic fauna and their habitat (for review, see Thielges 2005). Over *C. fornicata*'s native range in the USA, Collin (2001) identified 2 mitochondrial clades, but with only little geographic separation, as both clades were present in samples from the northernmost and southernmost populations, although at different frequencies. The lack of strong regional pattern in the native range prevented the identification of source populations using mtDNA (F. Viard, I. Kruse, D. McGlashan unpubl. data). Besides, previous genetic studies based on enzymatic loci (Hoagland 1985, Dupont et al. 2003) showed that the European populations of *C. fornicata* (including those located along the French coast of the English Channel) displayed genetic characteristics similar to those from populations in the native range of the species. Altogether, these genetic data and historical records (Blanchard 1997) support a scenario of introduction of the slipper limpet involving repeated introductions with no founder effects, a pattern commonly found in other gastropod species introduced in Europe (e.g. *Cyclope neritea*, Simon-Bouhet et al. 2006).

On finer scales, conflicting results have nevertheless been reported on the effective dispersal between populations of *Crepidula fornicata* along the French Atlantic and English Channel coasts. Based on 8 allozymic markers, Dupont et al. (2003) showed an unexpected significant genetic differentiation between Atlantic and English Channel populations. Conversely, only weak barriers to gene flow were observed on a fine scale, i.e. across a gulf located in the English Channel in which hydrodynamic processes like eddies may play a major role (Viard et al. 2006). Dupont et al. (2003) and Viard et al. (2006) exemplified the need to investigate different spatial ranges to determine the scale at which long-distance dispersal ability is effective. Limits to dispersal are indeed important determinants in biological invasion studies (Sakai et al. 2001).

In the present study, we investigated 17 samples of *C. fornicata*, hereafter referred to as localities, belonging to 5 French bays (2 to 4 localities per bay) located in the English Channel and 1 bay (Bay of Brest) at the entrance to the English Channel. Gene flow (effective dispersal) was estimated by performing a hierarchical analysis of the distribution of genetic diversity using 4 microsatellite loci. The high mutation rate of these markers allows us to trace back recent events and is much less influenced by historical features, compared to allozymes or mtDNA markers (Jarne & Lagoda 1996). Because of its status as an introduced species, slipper limpet populations might not fulfill equilibrium assumptions used in tra-

ditional population genetics model (e.g.  $F$ -statistics). We therefore used assignment methods and tests of equilibrium based on Bayesian and maximum likelihood approaches. These methods are useful to trace back and analyze contemporary events (Manel et al. 2005). The genetic structure that was revealed was then examined in light of the outputs of a 2D hydrodynamic model covering the study area (the English Channel) and simulating the patterns of larval transport according to local hydrodynamic conditions. The aims of this study were to determine: (1) whether a bay constitutes a spatial unit in which population connectivity is the rule (as previously observed in the St. Malo Gulf; Viard et al. 2006); (2) whether the disrupted distribution of *C. fornicata* in the English Channel (i.e. this species is mainly located in bays resulting in geographic discontinuity between dense populations) is strong enough to limit exchanges between bays despite the extended larval stage in this species; and (3) to what extent the limits to gene flow can be explained by hydrodynamic features.

## MATERIALS AND METHODS

**Sample collection and microsatellite genotyping.** In order to examine the scale at which dispersal occurs on a regional scale in the slipper limpet *Crepidula fornicata*, a hierarchical sampling design was carried out. Adult individuals were collected in 6 bays situated on each part of the Cotentin peninsula in the English Channel (Fig. 1): the bays of Seine (Bs), Veys (Bv), Mont St. Michel (Bdm), St. Brieuc (Stb), Morlaix (Mx), and Brest (Br). Two to 4 localities per bay were sampled. The sampling methods and locations are indicated in Table 1. For Bdm and Br, genotypes were obtained from Dupont et al. (2006), Viard et al. (2006), and Dupont et al. (2007). For all other localities, individuals were genotyped at 4 microsatellite loci: CfCA2, CfCA4, CfGT14, and CfH7, following protocols detailed in Dupont & Viard (2003) and Viard et al. (2006). PCR products were screened on 6.5% polyacrylamide gel using a Li-COR NEN Global IR2 DNA sequencer system.

**Statistical analyses of molecular data. Genetic diversity and test of equilibrium assumptions:** For each locality, genetic diversity was analyzed by computing allele frequencies, number of alleles ( $N_{\text{all}}$ ), and expected heterozygosity ( $H_e$ ) using GENETIX v. 4.05 (Belkhir et al. 2004). To take into account variation in sample size, allelic richness ( $A_r$ ; El Mousadik & Petit 1996) was estimated using FSTAT v. 2.9.3, an updated version of v. 2.8 (Goudet 1999; available at <http://www2.unil.ch/popgen/softwares/fstat.htm>). The null hypothesis of independence between loci was tested from statistical genotypic disequilibrium analysis using GENEPOP v. 3.4, an updated version of v. 1.2 (Raymond & Rousset 1995; available at <http://genepop>).

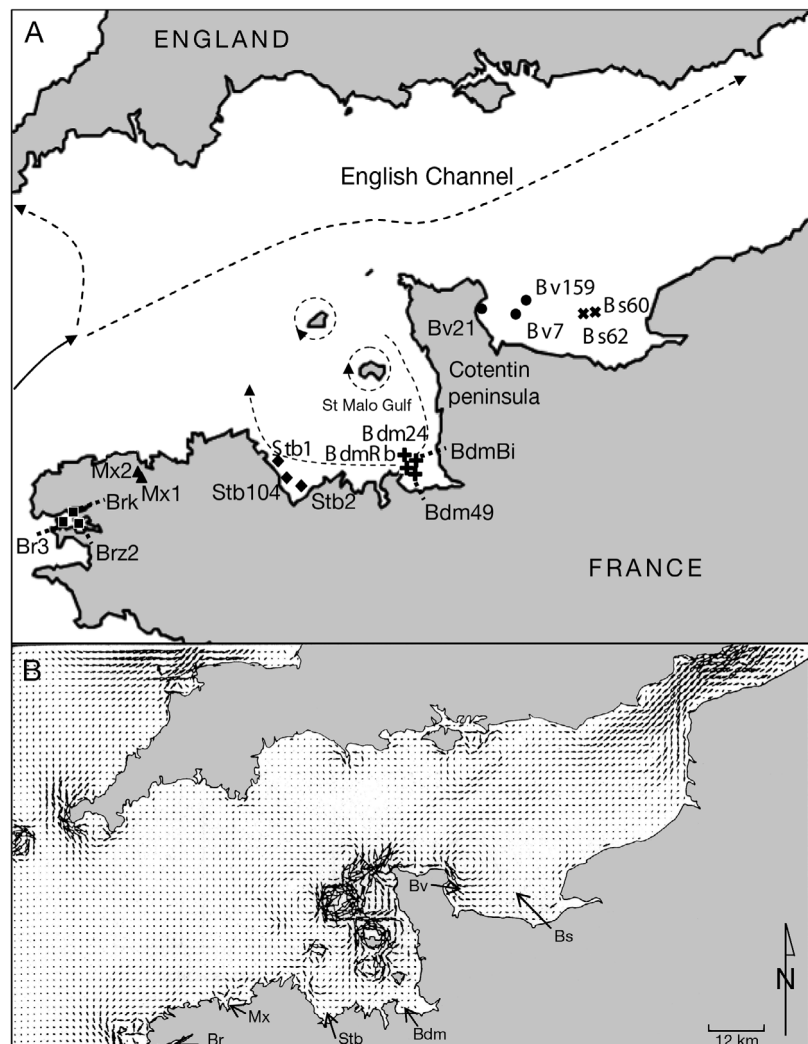


Fig. 1. Geographic and hydrodynamic characteristics of sampling locations. (A) Map of the 17 samples analyzed in the bays of Brest (Br, ■), Morlaix (Mx, ▲), St. Brieuc (Stb, ◆), Mont St. Michel (Bdm, +), Veys (Bv, ●), and Seine (Bs, ✕). Arrows show long-term, average current trajectories (based on Salomon & Breton 1993). (B) Residual Lagrangian velocity field for an average tide without wind, illustrating hydrodynamic currents (modified from Salomon & Breton 1991)

Table 1. *Crepidula fornicata*. Sampling characteristics and genetic diversity.  $N_{\text{ind}}$ , sampling size for genetic analysis;  $N_{\text{all}}$ , number of alleles;  $A_r$ , allelic richness;  $H_e$ , gene diversity ( $\pm$ SD);  $\hat{f}$ , heterozygote deficiency; H. grab, Hamon grab. Within bays, these indices were calculated considering the bay as a single population. Permutation test of deviation from Hardy-Weinberg proportions: \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$

Bay	Pop.	Location	Method	Date	$N_{\text{ind}}$	$N_{\text{all}}$	$A_r$	$H_e (\pm \text{SD})$	$\hat{f}$
Seine	Bs60	49° 31' 88" N 00° 14' 11" W	Dredging	2001	40	14.2	12.5	0.796 $\pm$ 0.204	0.066*
	Bs62	49° 31' 65" N 00° 16' 97" W	Dredging	2001	38	14.7	12.9	0.802 $\pm$ 0.180	0.092**
	Total				78	17.0	16.9	0.798 $\pm$ 0.191	0.078**
Veys	Bv159	49° 33' 71" N 00° 54' 90" W	Dredging	2001	26	11.7	11.6	0.804 $\pm$ 0.196	0.223***
	Bv7	49° 30' 99" N 00° 59' 64" W	Dredging	2001	39	14.7	12.7	0.804 $\pm$ 0.181	0.112**
	Bv21	49° 32' 00" N 01° 15' 50" W	Dredging	2003	41	13.7	12.0	0.756 $\pm$ 0.251	0.041
	Total				106	18.0	17.1	0.794 $\pm$ 0.208	0.122**
Mont St. Michel	Bdm24	48° 45' 95" N 01° 50' 10" W	H. grab	2002	50	14.7	12.1	0.770 $\pm$ 0.211	0.047*
	BdmBi	48° 45' 05" N 01° 50' 10" W	H. grab	2002	56	16.5	12.6	0.768 $\pm$ 0.242	0.110***
	BdmRb	48° 43' 25" N 01° 46' 70" W	H. grab	2002	52	17.5	13.1	0.761 $\pm$ 0.238	0.132***
	Bdm49	48° 40' 50" N 01° 45' 50" W	H. grab	2002	49	15.7	12.5	0.808 $\pm$ 0.187	0.138***
	Total				207	22.5	18.1	0.778 $\pm$ 0.221	0.109**
St. Brieuc	Stb2	48° 35' 00" N 02° 37' 80" W	H. grab	2002	31	14.5	13.3	0.806 $\pm$ 0.204	0.112**
	Stb104	48° 37' 37" N 02° 45' 41" W	H. grab	2002	32	12.5	11.5	0.759 $\pm$ 0.218	0.124**
	Stb1	48° 42' 62" N 02° 50' 90" W	H. grab	2002	31	13.0	12.0	0.780 $\pm$ 0.186	0.086*
	Total				94	19.2	18.5	0.784 $\pm$ 0.203	0.109**
Morlaix	Mx1	48° 40' 20" N 03° 53' 11" W	Diving	2002	92	17.7	12.4	0.788 $\pm$ 0.196	0.086***
	Mx2	48° 40' 22" N 03° 53' 17" W	Diving	2002	78	19.2	13.1	0.792 $\pm$ 0.199	0.134***
	Total				170	21.7	18.4	0.790 $\pm$ 0.197	0.107**
Brest	Br3	48° 19' 81" N 04° 30' 70" W	Dredging	2003	86	12.2	9.3	0.777 $\pm$ 0.134	0.091**
	Brz2	48° 19' 30" N 04° 31' 70" W	Dredging	2003	112	12.2	9.3	0.768 $\pm$ 0.135	0.076***
	Brk	48° 22' 33" N 04° 25' 69" W	Dredging	2003	87	11.7	9.2	0.773 $\pm$ 0.136	0.088***
	Total				287	14.7	12.0	0.772 $\pm$ 0.136	0.084**
TOTAL				940	26.5		0.790 $\pm$ 0.186	0.108***	

curtin.edu.au). Tests for deviation from genotypic proportions expected under Hardy-Weinberg equilibrium (HWE) were based on randomization procedures implemented in FSTAT v. 2.9.3 programs. To adjust for multiple comparisons, control of the false discovery rate (FDR) was applied: q-values were computed using the QVALUE R package (Storey 2002). The method of Brookfield (1996) as implemented in the program MICRO-CHECKER (Van Oosterhout et al. 2004) was used to determine the most probable cause of deviation from HWE expectations. Null alleles are a common problem with microsatellite loci and can lead to artefactual heterozygote deficiencies (e.g. Astane et

al. 2005). Such heterozygote deficiency may also be due to preferential amplification of small alleles (i.e. large allele dropout or short allele dominance) or by slippage during PCR (Van Oosterhout et al. 2004). MICRO-CHECKER estimates the null allele frequency and adjusts the observed allele and genotype frequencies appropriately.

The presence of *Crepidula fornicata* was first recorded along the French coast of the English Channel 70 yr ago, a short time scale relative to the time to reach population equilibrium. A previous study did not provide strong evidence for deviation from mutation-drift equilibrium in introduced slipper limpet popula-

tions (Dupont et al. 2003). However, as different markers were used in Dupont et al. (2003), we tested for the hypothesis of deviation to mutation-drift equilibrium in the study populations by using the approach proposed by Cornuet & Luikart (1996) and implemented in the software BOTTLENECK. Using a Wilcoxon test, the observed heterozygosity is compared with the heterozygosity expected under equilibrium considering a 2-phased mutational model (TPM) recommended for microsatellite data (Di Rienzo et al. 1994). Recently founded populations are expected to display a transient heterozygosity excess, whereas expanding populations (e.g. recovering from a bottleneck) should exhibit the opposite (Cornuet & Luikart 1996). Then, departure from a migration-drift equilibrium was tested using a Bayesian approach developed by Ciofi et al. (1999) and implemented in the program 2MOD by M. A. Beaumont (available at [www.rubic.rdg.ac.uk/~mab/software.html](http://www.rubic.rdg.ac.uk/~mab/software.html)). The likelihoods of 2 models are compared: a model in which allele frequencies within a population are determined by a balance between drift and gene flow, and a model where allele frequencies are evolving purely through genetic drift. The method uses a Markov chain Monte Carlo (MCMC) simulation approach with Metropolis-Hastings sampling to explore the set of possible ancestral gene frequencies, alternative models (isolation vs. gene flow), and the likelihood of producing the observed data. The MCMC search was carried out twice for  $10^5$  iterations, with the first 10% discarded to remove effects of initial starting parameters.

**Genetic differentiation between localities:** The genetic structure between localities or bays or group of bays was investigated by calculating  $\hat{\theta}$  (Weir & Cockerham 1984), an estimator of the fixation index  $F_{ST}$  with FSTAT v. 2.9.3. Because samples were not in HWE, tests for genetic differentiation were carried out using a G-test (as described by Goudet et al. 1996) and implemented in FSTAT. To adjust for multiple comparisons, the FDR was applied: q-values were computed using the QVALUE R package (Storey 2002).

To illustrate the extent of spatial genetic variation among samples, a principal component analysis (PCA) was performed on allelic frequency data using PCA-GEN 1.2 software (available at <http://www2.unil.ch/popgen/softwares/pcagen.htm>). An  $F_{ST}$ -based hierarchical analysis of molecular variance (AMOVA; Excoffier et al. 1992) was carried out to test for the regional structure of localities using Arlequin v. 3.1 (Excoffier et al. 2005): the genetic differentiation was investigated using a hierarchical analysis of the genetic variance by partitioning  $\hat{\theta}$  into  $\hat{\theta}_w$  and  $\hat{\theta}_b$ , indicating the genetic differentiation of samples within groups (e.g. within bays) and between groups (e.g. between bays), respectively. The level of significance

of  $\hat{\theta}_w$  and  $\hat{\theta}_b$  was computed by non-parametric permutational procedures of genotypes among populations within groups and among populations between groups, respectively.

Alternative approaches to  $F_{ST}$ -based methods, like assignment tests, have recently been proposed for gene flow inferences, especially for contemporary gene flow analyses and non-equilibrium situations (Manel et al. 2005). The overall low genetic structure and the limited number of loci used in our study prevented us from taking full advantage of these methods. However, for the sake of comparison with  $F_{ST}$ -based analyses and, more specifically, to examine the hypothesis of genetic homogeneity at the level of a given bay, we carried out an assignment analysis using a Bayesian approach as described by Rannala & Mountain (1997) and implemented in GeneClass v. 2.0 (Piry et al. 2004). We aimed to identify the relative proportion of individuals that were assigned to 1 locality external to the bay from where they were sampled. These individuals likely represent recent migrants, or individuals containing recently introgressed genomes.

**2D hydrodynamic Lagrangian model of larval dispersal.** We used a Lagrangian model to get a picture of the potential larval distribution (density and location of larvae) given hydrodynamic and climatic conditions following 1 episode of larval release from each of the study locations. Outputs of the model were summarized by building matrices of the number of larvae exchanged between each pair of populations. The hydrodynamic and larval transport models are detailed in Ellien et al. (2000, 2004) and the application to *Crepidula fornicata* is summarized in Viard et al. (2006). Briefly, this model, which simulates the transport of passive particles under the influence of a hydrodynamic regime dominated by tide and wind forces, is based on a hydrodynamic model for the English Channel, for which geographic limits are 48° 18' N and 51° 20' N in latitude, 6° 28' W and 3° 00' E in longitude, with a mesh size of 1 nautical mile (Salomon & Breton 1991; Fig. 1B). Fourteen of the 17 study samples are within those limits (localities of Bs, Bv, Bdm, Stb, and Mx), and the site of larval releases was chosen to fit exactly the location of each sample. The 2 localities in Mx were located in the same mesh and were therefore considered as a single site. In order to test the hypothesis of the isolation of the English Channel populations from the Atlantic, the model was also applied to a larval release at the entrance of the English Channel (Iroise Sea). *C. fornicata* is a perennial protandrous species with an extended breeding period from March to September (Richard et al. 2006). Biological inputs in the model were as follows. Larval dispersal was simulated over 21 d (i.e. larval lifespan) corresponding to the commonly accepted free-swimming period of the

pelagic veligers of *C. fornicata* (Coe 1949). As we were only interested in the pattern of maximal diffusion of larvae and its relative differences among locations, the mortality term was set to zero. The same nonrestrictive amount of larvae ( $10^7$  larvae) was released in each locality. This number, based on biological data available in the literature, is representative of the amount of larvae released in a theoretical population with a density of 3400 ind.  $m^{-2}$  (i.e. a nonrestrictive density, greater than the densities recorded in the St. Malo Gulf where the highest densities were observed; Viard et al. 2006), a female:male ratio of 0.67:1 (Dupont et al. 2006, 2007), and an average fecundity of 7500 oocytes per female (Richard et al. 2006). Larval dispersal was first studied considering an average tide and no wind influence. Then, further simulations were carried out with 2 types of wind-forcing, corresponding to the main wind directions influencing the study area: constant winds from SW (direction  $225^\circ$ ) and NE (direction  $45^\circ$ ) with a mean speed of  $6 \text{ m s}^{-1}$ . Matrices of larval exchanges were built for each wind condition. Larval exchanges between populations A and B were defined as:

$$\frac{L_{A \rightarrow B}}{\sum_i L_{i \rightarrow B} + L_B} + \frac{L_{B \rightarrow A}}{\sum_i L_{i \rightarrow A} + L_A}$$

where  $L_{i \rightarrow j}$  is the number of larvae transported from Population  $i$  to Population  $j$ , and  $L_i$  is the number of larvae retained in population  $i$ .

**Correlation between genetic distance, geographic distance, and larval exchanges.** To analyze the relationship between genetic distance ( $\hat{\theta}$ ), coastline geographic distance (km), and larval exchanges between localities (samples of the Bay of Brest are excluded, see the previous paragraph), Mantel tests were performed using GENEPOP v. 3.4. Mantel procedures can test for an association between 2 matrices using a randomization procedure. We tested for genetic isolation by geographic distance (Mantel 1967): coastline geographic distances (km), measured from a map, were plotted against  $\hat{\theta}/(1-\hat{\theta})$  to compute a linear relationship following the recommendations of Rousset (1997) to test for the null hypothesis of independence between genetic and geographic distances. Mantel tests were also used to test for the null hypothesis of independence between relative number of larvae exchanged among localities and geographic or genetic distances.

## RESULTS

### Genetic diversity, HWE, and null alleles

Exact tests for genotypic linkage disequilibrium between microsatellite loci within each locality showed only 2 significant  $p$ -values out of 102 pairwise

comparisons. Across the 17 samples, no pair of loci was found to be in linkage disequilibrium. All 4 loci were polymorphic in all samples; the number of alleles per locus varied from 4 (CfCA2) to 61 (CfGT14) over the whole data set. Summary statistics describing the genetic diversity across loci within the 17 study samples are given in Table 1. Whatever the locality and over the whole data set, a high genetic diversity was observed. In the localities of the English Channel, the mean number of alleles per locus ( $N_{\text{all}}$ ) was found to vary depending on the sampling locality (from 11.7 to 19.2) but this was mainly due to a sampling size effect as shown by the value of the allelic richness ( $A_r$ ), which was similar across sampling localities. The lowest values of  $N_{\text{all}}$  were found in the Bay of Brest, and this was not only due to a sampling size effect, as the estimates of allelic richness in these samples were also the lowest values observed in the data set. These estimates were nevertheless not significantly different from those in other populations ( $t$ -test;  $p > 0.05$ ).

Significant departures from HWE were observed in 21 of 68 single-locus permutation tests after FDR correction. Following the method of Brookfield (1996) as implemented in the program MICROCHECKER, null alleles are likely to occur at low frequency at locus CA4 in 3 samples (BdmRb, frequency of null alleles  $r = 0.072$ ; Bdm49,  $r = 0.078$ ; StB2,  $r = 0.062$ ), at locus CfH7 in 1 sample (Bv159,  $r = 0.140$ ), and at locus CfGT14 in all samples ( $0.050 < r < 0.247$ ). There was no evidence for null alleles at locus CfCA2. In addition, evidence of stuttering was found for the locus CfGT14 in 2 samples (BdmBi, Mx2). Correcting the data for null alleles did not change the number of significant departures from HWE. This result may be explained by an incomplete recovery of null alleles due to size homoplasy (i.e. loss or gain of a variable number of repeat units that generates alleles identical in state but not in descent; Chapuis & Estoup 2007). Another explanation is that departure from HWE is not due only to null alleles and technical artefacts. Apart from null alleles, heterozygote deficiencies at neutral loci can result from inbreeding or Wahlund effects. Paternity analyses (Dupont et al. 2006) carried out in the populations of the Bay of Brest (that are also investigated in the present study) did not reveal self-fertilization or assortative mating. In addition, heterozygote deficiencies are not congruent among loci, indicating that inbreeding is unlikely. Wahlund effects are also unlikely, as  $F_{\text{ST}}$  values are far lower than  $F_{\text{IS}}$  (i.e. correlation of genes within individuals with respect to genes between individuals within populations) values (David et al. 1997). Moreover, Dupont et al. (2007) demonstrated a genetic homogeneity between cohorts within 2 populations of the Bay of Mont St. Michel, suggesting that temporal Wahlund does not occur. Thus, none of the above factors is

clearly responsible alone for these departures from HWE explanations, but a combination of small additive effects of each of them might be the cause.

Outputs of the analyses carried out with the software BOTTLENECK did not provide any evidence of deviation from the mutation-drift equilibrium model in any of the samples tested under the TPM model, suggesting the absence of either recent expansion or recent bottleneck in the study localities. In addition, a Bayesian approach used to test for migration-drift equilibrium did not provide evidence of departure from this model: the pure-drift model was rejected in all simulations, and 100% of the simulated data points were consistent with the gene-flow-drift model.

**Genetic structure between localities within bays and over the whole data set**

Results for genetic structure between samples obtained with genotypic data both corrected and uncorrected for null alleles were generally similar. When different, results obtained with the corrected data set are presented in brackets. A significant genetic structure was revealed at the level of the whole study (17 samples,  $\hat{\theta} = 0.011$ ,  $p = 0.002$ ) as well as at the level of the Channel only (i.e. excluding the Bay of Brest from the analysis; 14 samples,  $\hat{\theta} = 0.004$ ,  $p = 0.002$ ). When considering each locality pair, 66 values of  $\hat{\theta}$  (76 with corrected data for null alleles) out of 136 (49% and 56%, respectively) were associated with a significant G-test (Appendix 1). Fifty-seven were still significant after the FDR correction. These genetic differences between samples were not randomly distributed across the data set: a genetic homogeneity was generally observed within bays, whereas most of the significant genetic differences involved comparisons between localities from different bays. In particular, 42 out of 57 (74%) of the significant  $\hat{\theta}$  values after FDR correction involved pairwise comparisons between the samples of the Bay of Brest with all other samples (Appendix 1). The genetic isolation of the Bay of Brest is exemplified by the results of the principal components analysis based on allelic frequencies (PCA; Fig. 2). Within bays, only 2 comparisons out of 17 (12%) revealed genetic differences between localities after FDR correction: significant values of  $\hat{\theta}$  were detected within Bv, between the localities Bv21 and Bv7 ( $\hat{\theta} = 0.022$ ,  $p < 0.001$ ) and within Stb, between the localities Stb1 and Stb104 ( $\hat{\theta} = 0.002$ ,

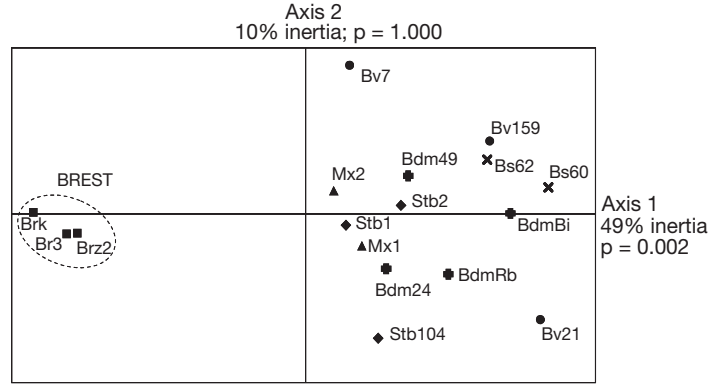


Fig. 2. Principal component analysis (PCA) performed using the allele frequencies of the 17 samples. Sampling locations as in Fig. 1

$p < 0.05$ ). Assignment tests revealed the same trend as  $F_{ST}$ -based analyses: individuals tended to be assigned preferentially to a locality of the bay where they were sampled (Fig. 3). In only 1 locality (Mx1), individuals were assigned in a higher proportion to another bay (22%; Br). In the other localities of the Channel, between 30 and 77% of individuals were assigned to a locality of the bay to which they belong. In the Bay of Brest, 72 to 77% of the individuals were assigned to their bay of origin.

To test the hypothesis of a regional structure, a hierarchical analysis of the distribution of the genetic diversity (AMOVA) was done (Table 2). First, the genetic differentiation between 5 groups featuring the 5 bays (Bs, Bv, Bdm, Stb, and Mx) was computed. No significant genetic heterogeneity was observed when the 5 bays were considered separately (genetic differentiation of populations between groups,  $\hat{\theta}_b = 0.001$ ,  $p = 0.225$ ), whereas the genetic differences between localities over the whole data set or within bays were significant. Second, regional patterns were tested by merging populations from neighboring bays within a

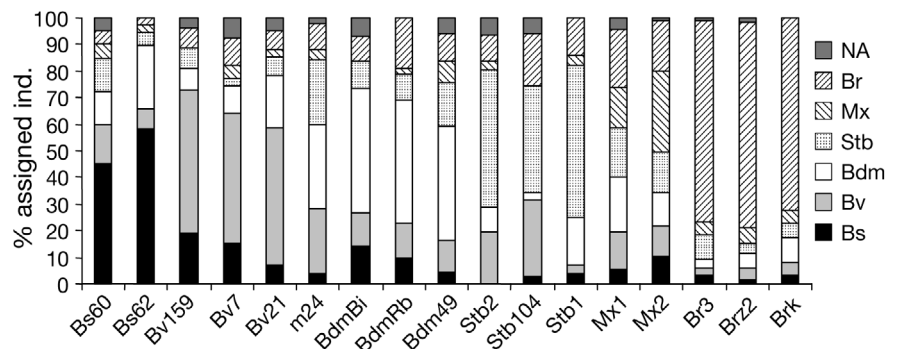


Fig. 3. Proportion of individuals assigned to each locality (assignment tests, GeneClass software). NA: individuals that could not be assigned ( $p < 0.05$ ). Sampling locations as in Fig. 1

Table 2. *Crepidula fornicata*. AMOVA.  $\hat{\theta}_b$ ,  $\hat{\theta}_w$ ,  $\hat{\theta}_t$ : genetic differentiation between groups, between localities within groups, and between localities over the whole data set (regardless of the grouping), respectively. **Bold**: significant. Parentheses: results obtained from the corrected dataset for null alleles

Grouping	Fixation index	p
5 groups: Bs, Bv, Bdm, Stb, and Mx		
$\hat{\theta}_b$	0.001	0.225 (0.272)
$\hat{\theta}_w$	<b>0.004 (0.005)</b>	0.021 (0.003)
$\hat{\theta}_t$	<b>0.005</b>	0.004 (0.001)
2 groups: (1) Mx + Stb and (2) Bs + Bv		
$\hat{\theta}_b$	<b>0.003 (0.002)</b>	0.010 (0.014)
$\hat{\theta}_w$	0.003 ( <b>0.005</b> )	0.116 (0.006)
$\hat{\theta}_t$	<b>0.006 (0.007)</b>	0.006 (0.001)
2 groups: (1) Mx + Stb + Bdm and (2) Bs + Bv		
$\hat{\theta}_b$	<b>0.002</b>	0.004 (0.014)
$\hat{\theta}_w$	<b>0.003 (0.004)</b>	0.026 (0.001)
$\hat{\theta}_t$	<b>0.005 (0.006)</b>	0.004 (0.001)
2 groups: (1) Mx + Stb and (2) Bs + Bv + Bdm		
$\hat{\theta}_b$	0.001	0.053 (0.051)
$\hat{\theta}_w$	<b>0.003 (0.004)</b>	0.012 (0.001)
$\hat{\theta}_t$	<b>0.005 (0.006)</b>	0.004 (0.001)

single group. The [Mx + StB] group differed significantly from the [Bs + Bv] group ( $\hat{\theta}_b = 0.003$ ,  $p = 0.010$ ), showing that the western and the eastern Channel groups are 2 entities that are genetically differentiated. In addition, the [Mx + StB + Bdm] group differed significantly from the [Bs + Bv] group ( $\hat{\theta}_b = 0.002$ ,  $p = 0.004$ ). The fixation index between the [Mx + StB] and [Bs + Bv + Bdm] groups was the lowest and was marginally significant ( $\hat{\theta}_b = 0.001$ ,  $p = 0.053$ ). The AMOVA results thus suggested that Bdm, although intermediate, is closer to the western Channel (Mx and StB) than to the eastern Channel (Bs and Bv) group.

#### Larval dispersal modeling, gene flow estimates, and geographic distances

The outputs of larval-dispersal modeling are summarized in Fig. 4. Whatever the wind conditions, after 21 d, we found no exchange of larvae between bays, except between Bs and Bv with a NE wind (Fig. 4A). In particular, no larva released from the Iroise Sea could enter the English Channel. Within bays, 2 different patterns were observed. Within Bdm, the larvae released for any given source locality were found to be roughly randomly dispersed over the other localities of Bdm, indicating that the different pools of larvae are well mixed within the bay, whatever the wind condi-

tion. A different picture was observed in the other bays where some localities received more larvae than others. For example, larvae released from Bv7 or Bv21 were mostly transported to Bv159. In addition, the locality Bv21 received very few larvae (in total <30%) from any locality of Bs or Bv and even no larva at all with a NE wind. Moreover, larvae released from Bv21 were not retained in Bv21. In Stb, the large majority of larvae released from Stb1 or Stb104 were retained in the source locality. Conversely, larvae released from Stb2, although partly retained in their source locality, were also dispersed towards Stb1 and Stb104.

We used Mantel tests to investigate the overall relationships between genetic distances, geographic distances, and rate of larvae exchanged between localities of the English Channel (i.e. 13 localities, Mx2 pooled with Mx1 since they belong to the same mesh). There was a significant correlation between genetic differentiation and geographic distance (i.e. isolation by distance pattern), where geographic distance explained 18.4% of the genetic difference ( $p = 0.005$ ). In addition, geographic distance was strongly negatively correlated with relative larval exchanges, whatever the wind condition ( $r = -0.762$ ,  $-0.773$ , and  $-0.740$ ,  $p < 0.001$ , with no wind, SW wind, and NE wind, respectively). A slight negative correlation was observed between larval exchange and genetic distance ( $r = -0.030$ ,  $-0.007$ , and  $-0.041$ , with no wind, SW wind, and NE wind, respectively). However, the correlation was only significant at the 10% threshold with a SW wind ( $p = 0.095$ ) and not significant with no wind ( $p = 0.283$ ) or a NE wind ( $p = 0.219$ ). Altogether, these Mantel tests showed that genetic data was in agreement with coastline geographic distances and larval movements.

## DISCUSSION

*Crepidula fornicata*'s characteristics (i.e. long-distance dispersal ability, wide environmental tolerance, and widespread distribution along the English Channel coasts) make it an ideal species for testing the relationship between long-distance dispersal ability and population connectivity (realized dispersal) and for examining multi-spatial scale genetic structure. Analyzing effective dispersal is a critical point for this exotic species. The spread of a non-native species in its introduction range is a key issue in invasion biology (Sakai et al. 2001).

#### Realized dispersal

The genetic study that we carried out on 17 populations of *Crepidula fornicata* located along the English



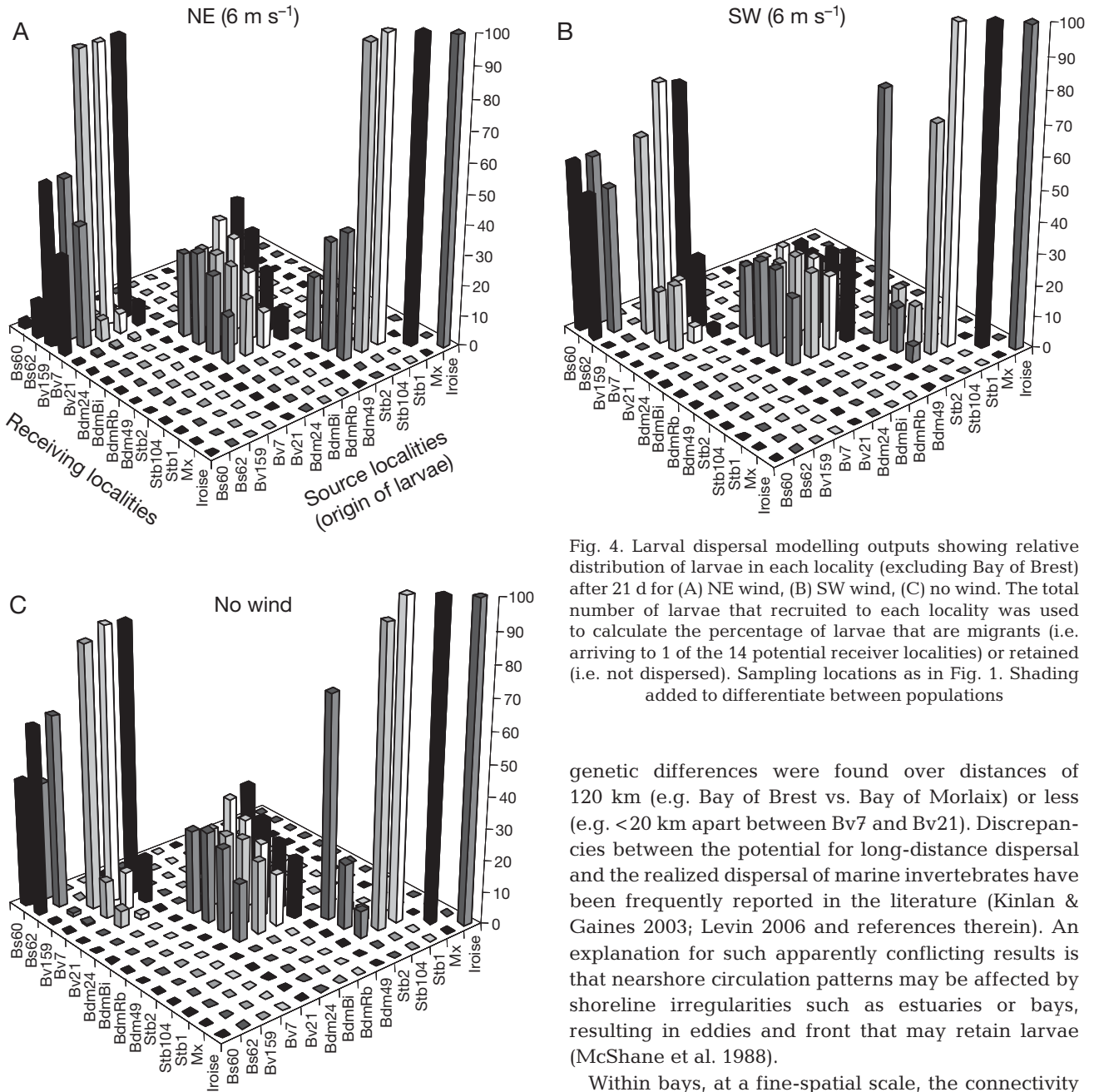


Fig. 4. Larval dispersal modelling outputs showing relative distribution of larvae in each locality (excluding Bay of Brest) after 21 d for (A) NE wind, (B) SW wind, (C) no wind. The total number of larvae that recruited to each locality was used to calculate the percentage of larvae that are migrants (i.e. arriving to 1 of the 14 potential receiver localities) or retained (i.e. not dispersed). Sampling locations as in Fig. 1. Shading added to differentiate between populations

genetic differences were found over distances of 120 km (e.g. Bay of Brest vs. Bay of Morlaix) or less (e.g. <20 km apart between Bv7 and Bv21). Discrepancies between the potential for long-distance dispersal and the realized dispersal of marine invertebrates have been frequently reported in the literature (Kinlan & Gaines 2003; Levin 2006 and references therein). An explanation for such apparently conflicting results is that nearshore circulation patterns may be affected by shoreline irregularities such as estuaries or bays, resulting in eddies and front that may retain larvae (McShane et al. 1988).

Within bays, at a fine-spatial scale, the connectivity between localities appeared to be the rule: high genetic similarity suggests a degree of gene flow between localities in all of the study bays. This pattern was confirmed by the output of the assignment test. This largely corroborates the results of Viard et al. (2006), who analyzed gene flow between 16 samples distributed in the Gulf of St. Malo, including the samples analyzed in the present study in the Bay of Mont St. Michel: only weak barriers to gene flow were found across the gulf despite a strong tidal regime and the action of wind-induced currents. Within the new study bays (Seine, Veys, St. Brieuc, Morlaix, and Brest), long-distance dispersal ability is thus also correlated with a

Channel coasts showed a relatively low level of differentiation among localities ( $-0.003 < F_{ST} < 0.039$ , Appendix 1). The present study's  $F_{ST}$ -estimate values are in accordance with those reported for other marine invertebrates with potentially high gene flow and found over similar geographic scales (e.g.  $0.001 < F_{ST} < 0.043$  in *Callichirus islagrande*, Bilodeau et al. 2005) or larger geographic scale (e.g.  $-0.0001 < F_{ST} < 0.061$  in *Placopecten magellicanus*, Kenchington et al. 2006). However, the overall genetic variation was not found to be randomly distributed across localities, and

large realized dispersal. Based on these results, a bay seems to be a relevant spatial unit for a population, defined in the ecological paradigm of Waples & Gaggiotti (2006) as: 'a group of individuals which co-occur in space and time and have an opportunity to interact with each other' (here through larval dispersal and recruitment). In 2 bays (Veys and St. Brieuc), we nevertheless observed more confused patterns, with local genetic differentiation, as discussed in the next section.

In contrast to the large genetic homogeneity found at the single-bay level, our study also demonstrated that significant genetic differentiation occurs between bays. In particular, a strong genetic isolation of the localities from the Bay of Brest was shown. This was not observed by Dupont et al. (2003), who used weakly polymorphic allozyme markers on samples collected from this bay. Indeed, although they reported an  $F_{ST}$  value between the localities of Brest and St. Brieuc ( $F_{ST} = 0.013$ ) similar to that in the present study ( $0.010 < F_{ST} < 0.018$ , 9 pairwise comparisons), a much lower  $F_{ST}$  was shown between Morlaix and Brest (their  $F_{ST} = 0.007$  vs. our  $0.011 < F_{ST} < 0.015$ , 6 pairwise comparisons). The relative isolation of the Bay of Brest is in agreement with the characteristics of the bay: a semi-enclosed marine ecosystem (Richard et al. 2006) favorable to the isolation of the larval pool and, in the long term, genetic isolation. Thus, in the absence of gene flow, populations might have diverged from populations of the English Channel as a result of genetic drift, a hypothesis supported by the lower number of alleles in the samples of this bay compared to the other study localities. Besides the specific case of Brest, a strong hierarchical structure was shown along the English Channel coasts, revealing 2 groups located on both sides of the Cotentin Peninsula: a western and an eastern group. That the Cotentin peninsula may limit gene flow between the eastern and western areas of the English Channel has already been suggested by Dupont et al. (2003), who found that populations from the western English Channel were genetically differentiated from populations located in more northeastern locations than Bay of Veys and Bay of Seine. However, we also showed that the Cotentin Peninsula is not an impenetrable barrier to gene flow, since the population of the Bay of Mont St. Michel holds an intermediate position, although genetically closer to the western than to the eastern group, and might act as a supply source of larvae for both sides of the Cotentin Peninsula.

Besides biotic factors (e.g. larval duration) or abiotic processes (e.g. hydrodynamic features), the invasion process might be an alternative explanation for the differentiation between bays. Invasive species may indeed represent a challenge for population genetics

studies, as introduced populations may retain genetic characteristics associated with the source population within the native range and can exhibit strong deviation from equilibrium assumptions, for instance because of strong founding events (Sakai et al. 2001). Multiple introductions of the slipper limpet along these study coasts might thus have originated from genetically differentiated sources (i.e. in relation to genetic structure in the native range) or major founder events may have occurred during each introduction process (i.e. successive founder effects enhancing the genetic differentiation between founding populations). However, several points make it unlikely that the invasion process alone could explain the observed genetic differentiation between bays. Firstly, a phylogenetic analysis of mitochondrial sequences from American samples of *Crepidula fornicata* revealed 2 distinct clades, indicating genetic differences among native US populations (Collin 2001). However, these genetic differences did not translate into a regional clustering of mitochondrial haplotypes of the 2 clades. Indeed, both clades were composed of northern and southern individuals, although at different frequencies. With a genetic structure not clearly related to the spatial distribution of the populations, different introduction processes from distinct spatial origins will be unlikely to translate into significant geographic genetic differences in the introduction area. Major founding effects are also unlikely. Indeed, Hoagland (1985) and Dupont et al. (2003) showed a similar level of genetic diversity at enzymatic loci between introduced and native populations. This was also confirmed with mtDNA data (F. Viard, I. Kruse, D. McClashan unpubl.): both native and introduced populations were characterized by a high and comparable genetic diversity. Moreover, in the present study, no departure to mutation-drift equilibrium was found in the introduced localities. Finally, the results of  $F_{ST}$ -based approaches were corroborated with outputs of the assignment tests that do not rely on equilibrium assumptions. This is in agreement with the temporal genetic stability observed in French populations of *C. fornicata* (Dupont et al. 2007). That equilibrium assumptions were not rejected and that outputs from different statistical methods (e.g. frequentist and Bayesian) were congruent suggests that the study populations might have reached equilibrium over a short time scale (ca. 100 to 200 generations with 1 to 2 generations  $\text{yr}^{-1}$ ), and thus that the effective population size is lower than suggested for many marine invertebrates (Palumbi 2003). Internal fertilization and biased sex ratio (due to protandry) are likely to decrease the effective size compared to an outbreeding and broadcast spawner (Turner et al. 2002). Estimation of effective size is a further step for understanding the contemporary dynamics of the slipper limpet. An alternative ex-

planation is that the introduction might have occurred for much longer than the 70 years reported for its introduction in France, a point that would deserve further investigation, for instance by the analysis of preserved specimens (McGlashan et al. 2007).

The multispatial scale genetic analysis revealed a contrast between the genetic homogeneity within bays and the reduced gene flow between bays which is exemplified by the isolation by distance pattern found in the present study. Isolation by distance (IBD) may occur in subdivided populations, when subpopulations exchange genes at a rate dependent upon distance, or within a continuously distributed population, when dispersal of gametes and/or zygotes is spatially restricted (Hardy & Vekemans 1999). IBD has been detected in coastal species with continuous distributions but usually over very large distances. For instance, a pattern of isolation by distance was reported in the coral *Balanophyllia elegans* (Hellberg 1994) and the abalone *Haliotis rubra* (Brown 1991) over geographic distances between 2500 and 3000 km. In *Crepidula fornicata*, the pattern of IBD revealed that the location of the *C. fornicata* populations (mainly within bays) might restrict gene flow over a much smaller distance (100 km).

### Effect of hydrodynamic features on genetic structure

Traditional population genetics provide information on the degree of gene flow between populations (Johnson & Black 1995), but include historical effects (Slatkin & Barton 1989) and generally (excluding assignment tests) do not precisely link 1 given source to 1 given receiving population for a given generation (Largier 2003). Thus, without data on larval exchange, an explanation of the observed genetic structure is incomplete. Because historical factors alone (i.e. invasion process) are unlikely to explain the observed genetic structure (see above), we here computed connectivity matrices and dispersal trajectories with a numerical model of larval dispersal to assess the extent to which effective (realized) dispersal is congruent with contemporary and potential larval diffusion. More specifically, we estimated the maximal distance of larval dispersal given the hydrodynamic conditions in order to test if genetic isolation might be explained by hydrodynamic features. The model predicted a high rate of larval retention within bays during 1 episode of larval discharge, which is consistent with the pattern of genetic isolation by geographic distance observed along the English Channel coasts: successive events of larval diffusion over a few years with a small number of migrants escaping their local natal populations is indeed compatible with such a pattern. Also, no larva

released from the Iroise Sea could enter the English Channel, a result in agreement with the strong genetic differentiation observed between the Br localities and all the other bays. Despite an extended pelagic duration (21 d), gene flow appeared to be constrained even at a small spatial scale (<50 km; within bays) in *Crepidula fornicata*. The mathematical modeling of larval dispersal suggested that this result may be largely explained by oceanographic mechanisms. Indeed, semi-enclosed bays are particularly effective at retaining water and plankton and reducing along-shore transport (Largier 2003). Moreover, larvae can be retained by eddies that may occur on different scales (from >100 km to  $\leq 1$  km, Largier 2003). Water recirculation in these eddies can retain larvae over time scales comparable with dispersal duration, and thus may account for enhanced local recruitment at specific locations. Our results corroborate the simulations of *Ophiothrix fragilis* larval dispersal by Lefebvre et al. (2003), who showed that the occurrence of several permanent clockwise and counterclockwise eddies in the St. Malo Gulf (cf. Salomon & Breton 1993) traps larvae for 3 wk and longer. In the same way, simulations of *Pectinaria koreni* larval dispersal carried out by Ellien et al. (2000) showed that larvae released from the Bay of Veys are retained in the eddy off Barfleur, situated northeast of the Cotentin Peninsula. The presence of eddies on each part of the Cotentin peninsula may explain the limited gene flow between the western and eastern English Channel (Fig. 2). In contrast to the St. Malo Gulf eddies, which persist whatever the meteorological conditions (Lefebvre et al. 2003), the eddy off Barfleur disappears when NE wind speed exceeds  $6 \text{ m s}^{-1}$  and larvae are advected westwards, north of the Cotentin (Ellien et al. 2000). The barrier to gene flow created by this eddy might thus be permeable depending on wind conditions.

Local genetic isolation was observed in 2 bays: Bay of Veys and Bay of St. Brieuc. These specific cases of genetic isolation at the level of a given bay were congruent with the outputs of the hydrodynamic model of larval dispersal. For example, larvae released from Bv7 or Bv21 were mostly recruited in Bv159. In addition, the population Bv21 received very few larvae (in total <30%) from any Bs or Bv population, and even no larva at all with a NE wind. This is congruent with the particular genetic structure detected within the Bay of Veys: Bv7 and Bv21 were genetically differentiated but each was similar to Bv159 (Appendix 1). In the same way, larvae released from Stb2, although partly retained in their parental locality, were also dispersed towards Stb1 and Stb104. This result is again in agreement with the genetic results that showed no difference between Stb2 and Stb1 or Stb104 but significant differentiation between Stb1 and Stb104.

Mathematical modeling of larval dispersal has been shown to explain patterns of genetic differentiation in other marine invertebrates, e.g. *Mytilus edulis* (Gilg & Hillbush 2003) and *Littorina* sp. (Hohenlohe 2004). In their reviews, Hellberg et al. (2002) and Palumbi (2003) concluded that fish and invertebrate larval transport exerts its greatest influence on genetic structure at intermediate scales (ca. 100 km). The congruence between the estimated genetic distances (i.e.  $\hat{\theta}/[1-\hat{\theta}]$ ) and the simulation outputs for *Crepidula fornicata* showed in the present study at a medium scale (100 to 200 km) fit well with these statements. Our results thus suggest that hydrodynamics might predict the general patterns of effective dispersal throughout the Channel in this species. However, we failed to obtain a significant correlation between the population pairwise matrices of genetic structure and larval exchanges. This might be due to the difference in the temporal scale of both analyses. Indeed, information from DNA passed through multiple generations, whereas simulations only showed events happening during the 21 d of dispersal. Extending this model to 3 dimensions and over multiple generations might more accurately test for the putative correlation between larval dispersal and gene flow in this species. In addition, despite the significant role of hydrodynamic mechanisms on larval retention, biological factors cannot be ignored. For instance, genetic differentiation within bays (e.g. Bv and Stb) might be explained by biological parameters that may limit gene flow, such as predation, selection on recently settled juveniles, demography, and fecundity (for review, see Hohenlohe 2004). A variety of larval behaviors may also be important. In particular, in sessile marine invertebrates, environmental stimuli that trigger larval settlement might also play a role in the retention of larvae near parental population. For instance, laboratory experiments of *C. fornicata* suggested that its aggregated distribution is the result of gregarious settlement in response to chemical cues released by conspecific adults (Pechenik & Gee 1993). Although the retention of larvae within bays seems important, we also must not ignore that not all larvae are retained. If even a small fraction of these successfully recruit elsewhere, their contribution to connectivity may be significant.

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