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Spatial analysis of nuclear and cytoplasmic DNA diversity in wild sea beet (*Beta vulgaris* ssp. *maritima*) populations: do marine currents shape the genetic structure?

VIRGIL FIEVET,† PASCAL TOUZET, JEAN-FRANÇOIS ARNAUD and JOËL CUGUEN
UMR CNRS 8016, FR 1818, Laboratoire de Génétique et Évolution des Populations Végétales, Université de Lille 1, Bâtiment SN2, 59655 Villeneuve d'Ascq cedex, France

Abstract

Patterns of seed dispersal in the wild sea beet (*Beta vulgaris* ssp. *maritima*) are predicted to be influenced by marine currents because populations are widely distributed along the European Atlantic coast. We investigated the potential influence of marine currents on the pattern of spatial genetic structuring in natural populations of sea beet. Populations were located along the French coasts of the Anglo-Norman gulf that features peculiar marine currents in the Channel. Thirty-three populations were sampled, among which 23 were continental and 10 were insular populations located in Jersey, Guernsey and Chausey, for a total of 1224 plants genotyped. To validate the coastal topography influence and the possibility of marine current orientated gene flow on the genetic features of sea beet populations, we assessed patterns of genetic structuring of cytoplasmic and nuclear diversity by: (i) searching for an isolation-by-distance (IBD) pattern using spatial autocorrelation tools; (ii) using the Monmonier algorithm to identify genetic boundaries in the area studied; and (iii) performing assignment tests that are based on multilocus genotype information to ascertain population membership of individuals. Our results showed a highly contrasted cytoplasmic and nuclear genetic differentiation and highlighted the peculiar situation of island populations. Beyond a classical isolation-by-distance due to short-range dispersal, genetic barriers fitting the orientation of marine currents were clearly identified. This suggests the occurrence of long-distance seed dispersal events and an asymmetrical gene flow separating the eastern and western part of the Anglo-Norman gulf.

Keywords: assignment tests, landscape genetics, marine hydrochory, mitochondrial minisatellites, Monmonier's algorithm, nuclear microsatellites, spatial autocorrelation

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Introduction

Determining the genetic structure of subdivided populations provides insight on the fundamental evolutionary influences of selection, mutation, gene flow and drift in wild populations. Among these processes, the extent of gene flow, often considered as a constraining force in evolution, maintains species integrity because it constitutes a powerful homogenizing force that may increase the effective population size and prevent genetic divergence, which itself can lead to the evolution of reproductive isolation (Slatkin 1994). Gene flow depends not only on species' dispersal capabilities (Bohonak 1999), but also on natural and anthropogenic habitat heterogeneity that results in geographically subdivided populations. The influence of landscape features on the level of dispersal and migration pathways have therefore been increasingly investigated through genetic signatures left in spatial genetic structuring using molecular markers (e.g. Michels *et al*. 2001; Arnaud 2003; Castric & Bernatchez 2004; Coulon *et al*. 2004; Haag *et al*. 2005) but relatively few papers deal with plant population in a landscape genetic context (but see Palmé *et al*. 2003; Kitamoto *et al*. 2005; Honnay *et al*. 2006).

Correspondence: Jean-François Arnaud. †Present address: U.M.R. INRA-Agrocampus Rennes, UMR 1099 BiO3P, Laboratoire Écologie et Sciences Phytosanitaires, 65, rue de Saint-Brieuc CS 84215, 35042 Rennes Cedex, France. Fax: (0)320436979; E-mail: jean-francois.arnaud@univ-lille1.fr

Genetic markers are commonly used to determine the effects of genetic drift and gene flow on the structure of genetic diversity within and among populations (for review, see Ennos 2001; Raybould *et al*. 2002). In plants, genetic markers can be located in either of the nuclear, chloroplastic or mitochondrial genomes. In angiosperms, both cytoplasmic genomes are maternally inherited, and thus can only disperse in seeds, whereas the nuclear genome is biparentally inherited and dispersed through pollen and seeds. The use of organelle genome polymorphism to study gene flow in plants has proven useful over the past 20 years because gene flow can be separated into its pollen and seed components (Avise *et al*. 1987; McCauley 1995). Cytoplasmic and nuclear differentiation is expected to be contrasted because organelle effective population size is generally half the size of the nuclear one, depending on the sex ratio and on whether the species is hermaphroditic, dioecious or gynodioecious (meaning co-occurrence of females and hermaphrodites in populations; Birky-Jr *et al*. 1989; Laporte *et al*. 2000). This hypothesis is reinforced by the potential asymmetry in gene flow due to contrasted pollen and seed dispersal capacities (Hamilton & Miller 2002). Furthermore, in seed plants, genes generally move between existing populations by seed and/or pollen, but can only colonize new habitats when carried by seeds. Hence, spatial genetic structuring between populations may be enhanced when some populations are founded by a limited number of seeds, depending on whether colonists arise from a single or multiple sources (migrant vs. propagule pool model; see McCauley 1993; McCauley *et al*. 2001). Therefore the spatial distribution of cytoplasmic polymorphism, established by seed dispersal during the settlement of new populations via range expansion or multiple colonization events, might be better conserved and more marked than spatial patterns depicted with nuclear genetic markers that disperse both in the seed and pollen. As a consequence, the scarcity of seed gene flow among populations may result in only a few cytoplasmic haplotypes inherited from the original colonists emigrating from one or multiple sources (see Whitlock & McCauley 1990; Ennos 2001; Olson & McCauley 2002; McCauley *et al*. 2003).

This study deals with patterns of genetic variation in wild sea beet, *Beta vulgaris* ssp. *maritima*, a natural coastal species that grows along the shoreline. Preliminary investigations have revealed the ability of wild sea beet seed to float in sea water for a few days (unpublished results). As a consequence, seeds are expected to be dispersed over long distances by marine hydrochory. Many estuarine organisms resort to a larval export strategy. In this case, oceanographic features such as biogeographical boundaries, patterns of coastal or estuarine circulation are important processes that may influence both gene flow among populations and partitioning of genetic diversity within and among studied populations (e.g. Wares *et al*. 2001; Bilton *et al*. 2002; Billot *et al*. 2003; Perrin *et al*. 2004). In this study, we focused on wild sea beet populations located along the French coast of the English Channel within the Anglo-Norman gulf (comprising one French and two English Channel Islands). This site offers some interesting specific landscape features. Marine currents within the gulf are very peculiar and relatively well defined, as described by Salomon & Breton's (1993) hydrodynamic model. Particular dynamic features are revealed by long-term current modelling in the English Channel: the Anglo-Norman gulf is relatively isolated from the Channel and is excluded from the major eastern-orientated flow. Furthermore, islands are isolated from each other and from the coastline by tidal currents which form large gyres around them (Salomon & Breton 1993). Few studies have compared the genetic structure of plant populations on the mainland and on islands, especially at an intraspecific level (reviewed in Barrett 1996; Frankham 1997). As such, this situation is interesting as it may provide an opportunity to evaluate processes such as local founding events and genetic drift.

In this study we attempted to evaluate the influence of landscape features on the genetic structure of *B. vulgaris* ssp. *maritima* via current-mediated colonization. To this end, both biparentally (nuclear microsatellite loci) and uniparentally inherited markers (mitochondrial minisatellite loci) were used to determine the genetic structure of wild sea beet populations within the Anglo-Norman gulf. Our first step was to contrast patterns of genetic structuring of cytoplasmic and nuclear diversity to discriminate pollen- from seed-mediated gene flow. Second, we assessed the spatial arrangement of genetic variability by: (i) searching for an IBD pattern using spatial autocorrelation tools; and (ii) using the Monmonier algorithm to identify genetic boundaries in the study area. Next we performed assignment tests that use multilocus genotype information to ascertain population membership of individuals in order to validate the current-orientated gene flow on the genetic features observed in *B. vulgaris* ssp. *maritima* populations. Finally, we concluded whether the resulting patterns of genetic structuring could be explained by hydrodynamic predictions.

Materials and methods

Sampling and data collection

Species. B. vulgaris ssp. *maritima* is a short-lived perennial, wind-pollinated, gynodioecious and self-incompatible species. Populations are widely distributed along the western European coastline and in the Mediterranean basin. Fruits are aggregated in a glomerule (seed capsule) which can contain 1–7 seeds and glomerules are dispersed by gravity and potentially by marine currents for populations located along the coastline (Letschert 1993). As several

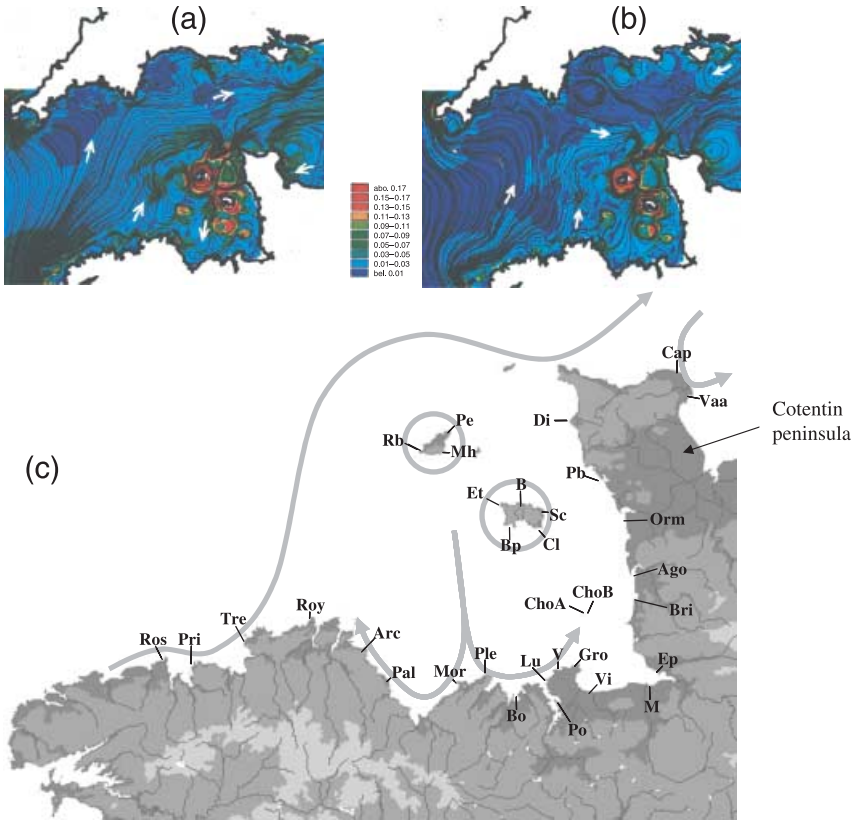

Fig. 1 (a) Long-term trajectories and velocity of water movement for a big tide and no wind; (b) Long-term trajectories and velocity of water movement for an average tide and no wind (according to Salomon & Breton 1993); (c) Spatial location of sampled populations; grey bold arrows summarize main current trajectories within the Anglo-Norman gulf.

seeds are contained in the same glomerule, the probability for populations to be founded by groups of sibs is greater. This colonization process by genetically related individuals is similar to a propagule pool model of colonization and can have tremendous consequences on the population structure in terms of level of inbreeding and population differentiation (Whitlock & McCauley 1990).

Study site. The dynamics of the English Channel have been intensively studied and marine currents are well defined and characterized. When reaching the western part of Brittany, the Gulf Stream is divided into two main trajectories. One of them enters the English Channel with an eastward flow. In north Brittany the water flow is deflected from the Anglo-Norman gulf and hits the north Cotentin coastline, isolating the gulf from the rest of the Channel (Fig. 1). Salomon & Breton's simulations (1993) revealed particular marine currents in the Anglo-Norman gulf that are characterized by tidally induced gyres. The weakest gyres are easily neutralized by the wind, but the strongest ones, especially around the Channel Islands, persist in a large range of weather conditions. The circular currents may promote the genetic isolation of insular populations once founded. Among the largest gyres, two gyres separate the gulf into western and eastern parts (see Fig. 1). Due to the foreshore location of the wild sea beet, a high average tide and no wind were considered as tidal current model parameters in Salomon & Breton (1993) simulations.

Sampling design. We focused on 33 populations within the gulf. Among these populations, 10 were insular, distributed in Jersey (five), Guernsey (three) and Chausey Islands (two), and 23 were continentally distributed along the coastline from St Vaast la Hougue (Vaa) in the East to Roscoff (Ros) in the West (Fig. 1, Table 1). For further molecular investigations, leaf tissues from 1224 individuals were collected and dried in silica gel following three successive samplings in 2000, 2002 and 2003. Leaves were randomly collected so as to minimize kinship. Number of individuals sampled ranged from 18 to 62 per population (mean = 40.51, SE = 1.51). Detailed sample sizes are reported in Table 1.

Molecular investigations

Extraction and purification of total DNA was performed using a DNeasy®96 Plant Kit following the standard protocol for isolation of DNA from plant leaf tissue outlined in the DNeasy®96 Plant protocol handbook (QIAGEN Inc.).

Mitochondrial minisatellites. Individuals were genotyped at four mitochondrial minisatellite loci named Tr1, Tr2, Tr3

Table 1 Names, acronyms, geographical coordinates and sample size of wild sea beet populations collected

Label	Sample location	Situation	*N*	Latitude N	Longitude W
Vaa	St Vaast la Hougue	Continental	50	49.587300	1.262366
Cap	Cap Lévi	Continental	50	49.688383	1.473283
Di	Port de Dielette	Continental	43	49.549938	1.864895
Pb	Portbail	Continental	42	49.330391	1.709271
Orm	Gué de l'Orme	Continental	62	49.218540	1.608931
Ago	Pointe d'Agon	Continental	18	49.001552	1.574942
Bri	Briqueville-sur-Mer	Continental	53	48.933679	1.543426
Ep	Gué de l'Epine	Continental	43	48.650683	1.392766
M	Mt St Michel	Continental	40	48.633171	1.509457
Vi	Le Vivier (Cancale)	Continental	40	48.633171	1.781600
Gro	Pointe du Groin	Continental	40	48.711166	1.844400
V	Plage du Verger	Continental	40	48.696383	1.879883
Lu	Iles Besnard	Continental	46	48.690816	1.950566
Po	Port St Jean	Continental	40	48.536508	1.961664
Bo	Beaussay	Continental	40	48.577038	2.175217
Ple	Phéhérel plage	Continental	39	48.654383	2.365216
Mor	Plage du Morvan	Continental	50	48.570250	2.579102
Pal	Plage du Palus	Continental	50	48.676033	2.883883
Arc	Pointe de l'Arcouest	Continental	50	48.820466	3.021600
Roy	Trévou, Tréguignec	Continental	50	48.824166	3.350100
Tre	Trébeurden	Continental	50	48.774033	3.582500
Pri	Primel Tregastel	Continental	50	48.713033	3.817850
Ros	Roscoff	Continental	50	48.720995	4.008234
ChoA	Grande Grève (Chausey)	Insular	50	48.876691	1.837569
ChoB	La Cale (Chausey)	Insular	47	48.872551	1.826068
Pe	Penbrook (Guernsey)	Insular	40	49.507483	2.532266
Rb	Lihou (Guernsey)	Insular	41	49.454966	2.653266
Mh	Moulin Huey (Guernsey)	Insular	34	49.426216	2.548950
Et	Etacq (Jersey)	Insular	40	48.240850	2.246383
B	Bonne Nuit Bay (Jersey)	Insular	40	49.251333	2.115350
Sc	St Catherine Bay (Jersey)	Insular	51	49.220550	2.025750
Cl	St Clement Bay (Jersey)	Insular	40	49.167116	2.061800
Bp	Port Bay (Jersey)	Insular	40	49.178600	2.208733

and Tr4 (Nishizawa *et al*. 2000). These four loci corresponded to mitochondrial variable number of tandem repeats (mtVNTR) of 32, 33, 66 and 30 bp for Tr1, Tr2, Tr3 and Tr4, respectively. We sequenced each locus to ensure that each new allele corresponded to a perfect number of tandem repeats. No cryptic polymorphism was found. Simple migration on an agarose gel was able to successfully separate the different alleles. PCR amplifications were performed in 15 μL volumes using both Perkin Elmer 9700® and DYAD Peltier® thermocyclers. Cycling conditions included an initial denaturation step of 5 min at 94 °C followed by 30 cycles of 30 s at 94 °C, 60 s at 51 °C for Tr1 and 62 °C for the other loci, 30 s at 72 °C. Final extension was conducted 10 min at 72 °C. Reactions contained 3 mM $MgCl_2$, 200 μM of each dNTP, 0.2 mg/mL of BSA, 120 μM of each forward and reverse primer, 0.625 U *Taq* polymerase (Perkin Elmer) and ~50 ng of template DNA. Polymorphism was revealed on 2% agarose gel electrophoresis and visualized after ethidium bromide staining under UV light using known individuals as internal size standards.

Nuclear microsatellites. All 1224 individuals were genotyped at seven microsatellite loci (CT4, GTT1, GCC1, Bvm3, CAA1, GAA1 and CA2) following protocols previously described in Mörchen *et al*. (1996), Viard *et al*. (2002) and Arnaud *et al*. (2003), with slight modifications. Briefly, two multiplex PCR amplifications were conducted on Bvm3, GAA1 and CA2, and GTT1 and GCC1. PCR amplifications were conducted in a total volume of 15 μL using both Perkin Elmer 9700® and DYAD Peltier® thermocyclers with one cycle of 3 min at 95 °C, 35 cycles of 40 s at 94 °C, 40 s at 55 °C for Bvm3/GAA1/CA2 multiplex and 54 °C for GTT1/GCC1 multiplex, 40 s at 72 °C and one final elongation step of 10 min at 72 °C. Reactions contained 1.5 mM $MgCl_2$, 200 μM of each dNTP, 0.2 mg/mL of BSA, 1.5 pmol of each forward and reverse Bvm3 or GTT1

primer and 2 pmol of each forward and reverse primer for the other loci, 0.5 unit of *Taq* polymerase (Perkin Elmer) and ~50 ng of template DNA. Electrophoresis and genotyping were performed on a LI-COR automated DNA sequencer model 4200 s (LI-COR Inc., Nebraska, USA). There was no evidence of multiple loci being amplified by a single pair of primers. Individuals were all scored for at least one allele on each locus so that the probability of null allele occurrence was very low.

Data analysis

Genetic diversity. Genetic diversity per locus and per population was assessed by: (i) the expected heterozygosity (H_E) for nuclear loci; (ii) the number of alleles observed (A_E); and (iii) the allelic richness (A_r). The expected number of alleles was calculated with an unbiased estimation following the rarefaction procedure of El Mousadik & Petit (1996b). These diversity parameters were calculated for both minisatellite and microsatellite markers using the FSTAT version 2.9.3 software (Goudet 1995). FSTAT was also used to calculate basic genetic data such as allelic frequencies for each population and over the whole data set. Using nuclear markers, within population departures from Hardy–Weinberg equilibrium (HWE) were assessed by exact probability tests following hypotheses of either excess or deficit in heterozygote (*U*-test), for each locus and over all loci, by population and for all populations, and were corrected for using Bonferroni adjustment (Rice 1989). The latter analyses and tests for genotypic linkage equilibrium were performed using GENEPOP v3.3 (Raymond & Rousset 1995).

Population differentiation. We first investigated population structure using *F*-statistics according to Weir & Cockerham (1984) to test for population differentiation across all populations using FSTAT version 2.9.3 (Goudet 1995). We used a permutation test (5000 runs of multilocus genotype randomization) to determine whether observed values of F_{IS} (f), F_{IT} (F) and F_{ST} (θ) were significantly different from zero for each locus and over all loci. For mitochondrial haploid data, only the F_{ST} (θ) values were computed. 95% confidence intervals (CI) were obtained by jackknifing over populations. Furthermore, to test whether there was significant isolation between continental populations and island populations, we performed a hierarchical *F*-statistics analyse according to Weir & Cockerham's (1984) nested ANOVA model. Genetic differentiation among groups (island vs. continental populations) will be referred to as F_{CT} and genetic differentiation within groups will be referred to as F_{SC}.

To test for significant difference in mean genetic diversity (H_E), mean level of inbreeding (F_{IS}) and mean genetic differentiation (F_{ST}) among groups of populations, the following statistics were calculated:

$$O_{S_x} = \sum_{i=1}^{\text{lngroups}} \quad \text{and} \quad \sum_{j=i+1}^{\text{lngroups}} (x_i - x_j)^2$$

where O_{S_x} refers to the mean difference in either H_E, A_r, F_{IS} or F_{ST}. To assess the significance of O_{S_x}, a permutation scheme was applied using 10 000 replicates. Whole samples were randomly allocated to the different groups (keeping the number of samples constant in each group), and S_x was calculated from the randomised data set. The *P*-value of the test was the proportion of randomised data sets resulting in a larger S_x than the observed O_{S_x}. All comparisons were carried out using FSTAT version 2.9.3 (Goudet 1995).

To illustrate genetic relationships among population samples, we quantified genetic divergence using the Cavalli-Sforza & Edwards's (1967) chord distance (D_{CE}), based on allelic frequencies. This genetic distance makes no assumptions about mutation rates among loci. Since there is no clear consensus on the microsatellite evolution mode (see Angers & Bernatchez 1998; Chambers & MacAvoy 2000), the D_{CE} genetic distance may be the most reliable to depict tree topology under either infinite allele model (IAM) or stepwise mutation model (SMM) assumptions (Takezaki & Nei 1996). Genetic distances and unrooted neighbour-joining trees of relationships were calculated and designed using cytoplasmic or nuclear data with the POPULATIONS software version 1.1.24 (available at http://www.pge.cnrs-gif.fr/bioinfo/). Trees were visualized thanks to TREEVIEW (available at http://taxonomy.zoology.gla.ac.uk/rod/treeview.html). Bootstrapped values on branches were determined using random replications over loci or populations (1000 replicates).

Pollen vs. seed mediated gene flow. Under the assumptions of the Wright's Island model (Wright 1965), Ennos (1994) has demonstrated that, assuming migration-drift equilibrium, a ratio (r) of the amount of pollen migration (m_p) over the amount of seed migration (m_s) can be inferred from *F*-statistics estimated by both nuclear biparentally inherited markers (F_{STN}) and maternally inherited markers (F_{STC}) from the following equation:

$$r = \frac{m_p}{m_s} = \frac{\left(\frac{1}{F_{STN}} - 1\right)(1 + F_{IS}) - 2\left(\frac{1}{F_{STC}} - 1\right)}{\left(\frac{1}{F_{STC}} - 1\right)}$$

In this equation, *F*-statistics were computed according to the Weir & Cockerham (1984) procedure and F_{IS} referred to the mean F_{IS} estimate over the seven microsatellite loci. Although first, some of our populations may not be at equilibrium and second, *B. vulgaris* is a gynodioecious species, the *r*-ratio should provide a reliable estimate of the relative strength of pollen vs. seed flow (see Bacles *et al.* 2004). Nevertheless, to test for the potential effect of the

breeding system, we calculated an *r*-ratio on a subsample of nongynodioecious populations.

Scale of spatial structuring. Spatial genetic structure was studied on the basis of three spatial scales. The largest one comprises the whole data set. As marine currents may directly affect the movement of seeds in this species, a map of marine currents in the region (Salomon & Breton 1993) provided the basis for additional hypotheses. First, marine currents whirling around a given island would imply that insular populations are potentially isolated from the continental ones. As a consequence, we focused on continental populations only, island populations being removed from the analyses. Second, some continental populations are suspected to be out of the general current dynamics of the Anglo-Norman gulf (see Fig. 1). Four populations were concerned: Vaa and Cap in the east, which are located outside of the Cotentin spit, and Pri and Ros in the west, which are located next to the current that bounces over the gulf (E. Thiebault, personal communication). Consequently, we expected the four populations not to be connected *via* marine currents with populations located within the Anglo-Norman gulf. According to these observations, we defined three sets of populations: (i) the first set pooled every population sampled, including continental and insular populations (level 1 in the text afterward, see Fig. 3); (ii) the second level included only the 23 continental populations (level 2); and (iii) in the third level, we excluded both insular populations and the four continental populations at the extreme eastern (Vaa and Cap) and western (Ros and Pri) locations (level 3).

Spatial analyses. To understand how the geographical network of populations was related to genetic variation, relationships between genetic divergence and physical isolation of populations were further investigated using different measures of geographical distance. We chose to use a standard Mantel test that compares distance matrices describing genetic and geographical relationships between populations. Genetic divergences were quantified using the D_{CE} chord distance (Cavalli-Sforza & Edwards 1967) as described above. The correlation value, named *rz* (normalized Mantel statistic) is computed from these two matrices (Smouse *et al*. 1986). Significance of each *rz* value was tested by randomly permuting rows and columns of one matrix while the other remained constant (10 000 permutations) (Smouse *et al*. 1986). Tests of association between genetic divergence and geographical variables were run using either 'overall' Mantel tests or unidirectional Mantel correlograms (Oden & Sokal 1986; Sokal *et al*. 1986; Arnaud *et al*. 1999; Arnaud 2003). The concept of genetic distance to measure dissimilarities among populations can be applied by constructing Mantel unidirectional correlograms where a set of binary connection matrices are analysed against the genetic distance matrix (Oden & Sokal 1986). The significance of each Mantel correlation was evaluated as described above. Mantel correlograms were designed independently using either the information of minisatellites or microsatellites data set, with the software PASSAGE (Rosenberg 2001), available at http://lsweb.la.asu.edu/rosenberg/Passage/.

Populations were first connected using pairwise linear distance. This connection criterion simply corresponds to the Euclidean distance between two points. A second criterion was based on a geographical distance strictly following the coastline. For this connection criterion, analyses were restricted to continental populations (i.e. levels 2 and 3). A third criterion was also considered according to Gabriel-connected graph (Gabriel & Sokal 1969). Two points are connected if no point is included inside the circle defined by their diameter. According to this criterion, connected localities correspond to immediate adjacent localities, mimicking a stepping-stone model when populations are linearly positioned.

Zones of sharp genetic change. A genetic boundary is a zone of sharp genetic variation. We used the Monmonier's maximum difference algorithm (Monmonier 1973) to depict them. We first connected populations using a Delaunay triangulation (Brassel & Reif 1979). Then, we used a Voronoï diagram, which implies that all possible points inside a polygon are closer to its centroid (the location of the sampled population) than to any other polygon. Once the network was obtained, each edge of the network was associated with its pairwise genetic distance (i.e. the D_{CE} chord distance) according to the distance matrix used. Then the barrier was initiated by crossing the edge associated with the highest distance measured and further extended across the next edge with the following highest genetic distance value (see Stenico *et al*. 1998; Palmé *et al*. 2003).

Assignment tests. Assignment methods are powerful tools to detect recent immigration events even when overall population differentiation is low (Rannala & Mountain 1997; Waser & Strobeck 1998; Castric & Bernatchez 2004). Indeed, assignment tests are particularly useful to trace contemporary dynamics of natural populations without requiring equilibrium assumptions based on long-term genetic processes (see Manel *et al*. 2005). As the pattern of misclassification of individuals within a population can be used to determine the direction of migration, assignment tests might be a pertinent method to investigate the influence of marine currents on gene flow. The probability that an individual sampled within a given population is a migrant originating from another population was determined by using a Bayesian method implemented in the GENECLASS2 software (Piry *et al*. 2004). This method is based on the Rannala & Mountain (1997) approach that determines the

likelihood of attributing a particular multilocus genotype from a resident and from a potential source population, considering that there is no genotypic disequilibrium among loci and HWE within populations.

The proportions and origins of immigrants within each population were used to test the following hypotheses: (i) within a given island, is gene flow more likely to occur between populations belonging to the same island rather than with any other population located outside this island? and (ii) do marine currents, as described in Fig. 1, drive the direction of gene flow? In other words, do local currents with complex bouncing patterns counteract the overall current of the Gulf that flows eastward? These hypotheses are based on the assumption that isolated islands, or sites previously defined by a genetic boundary, should exchange fewer migrants than other interconnected populations. If it is the case, proportions of individuals correctly assigned to their original site should be high. On the other hand, if insular populations behave as a sink, a number of immigrants coming from locations other than the island considered should be high. Differences between immigrant proportions were tested using logistic regression (following a binomial distribution, a log link function, PROC GENMOD, SAS) corrected for overdispersion (dscale option, PROC GENMOD SAS).

Results

Overall and within population polymorphism

Minisatellite loci varied greatly in their level of diversity. Tr1 exhibited the highest variability with a total of nine alleles, Tr3 and Tr4, four and three alleles, respectively, while no polymorphism was detected for Tr2. The number of sampled alleles (A_e) for Tr1 varied between one allele per population for Bp and six for Orm, M, Vi and Mor, with an average of 3.85 alleles per population (Appendix 1). A_e ranged between one and three alleles per population for Tr3 and Tr4, with an average of 2.27 and 1.51 alleles per population, respectively (Appendix 1). When focusing more specifically on insular populations, no significant difference in allelic richness was found between insular populations and either level 2 or 3 continental populations, or among insular populations (using permutation tests for comparison among groups). For subsequent analyses of cytoplasmic polymorphism, we considered a single haplotype determined by the different allele patterns generated by all three polymorphic loci, giving a total number of haplotypes of 27.

Microsatellite loci displayed much higher levels of polymorphism, with a total number of alleles per locus varying from four (GTT1 and GAA1) to 33 (CAA1), and a within-population gene diversity H_E ranging from 0.032 (GAA1) to 0.902 (CAA1) (see Appendix 1). Permutation tests did not identify any significant difference between insular and continental populations for the mean number of alleles sampled and for the unbiased expected heterozygosity. Similarly, no significant differences were found when Guernsey, Jersey and Chausey populations were compared for the mean number of alleles sampled or for their unbiased heterozygosity.

Overall, no significant difference was observed between continental and insular populations in mean allelic richness or gene diversity, whether on the cytoplasmic or nuclear loci.

Linkage disequilibrium

Among 21 possible pairs of microsatellite loci, two presented an overall significant genotypic linkage after Bonferroni correction, involving CT4/GCC1 and CT4/Bvm3 (both at $P < 0.001$). Within each population sample, exact tests for genotypic linkage disequilibrium only depicted two significant unbiased P-values out of 693 comparisons after Bonferroni corrections (35 expected from types I error at $\alpha = 0.05$). The two genotypic linkages were both found in the Bri population and involved CT4/GCC1 and CT4/Bvm3 pairs. Overall, these results suggested a lack of genotypic disequilibrium in populations.

Hardy–Weinberg disequilibrium

Using multilocus probability tests, we found that 21 populations out of 33 behaved as expected under HWE. Multiple probability tests across *all* populations revealed that five microsatellite loci out of seven deviated significantly from HWE expectations. As a result, combined probability tests over all loci and across all populations revealed a significant departure from HWE resulting from a general deficit in heterozygotes ($P < 0.001$, see Table 2 and Appendix 1). It must be kept in mind that using multiple probability tests across *all* populations results in an overall departure from HWE with only few significant single-locus departures within populations. As single-locus F_{IS} values corresponding to a significant heterozygote deficiency were not specific of one locus or one population (see the appendix for detailed within-population heterozygote deficits), heterozygote deficiency cannot be attributed to a null allele. Altogether, these results indicated a slight inbreeding effect within some populations, possibly due to substructuring (Wahlund effect) or to a kinship structure that results from the population being founded by groups of sibs.

A significant difference in mean F_{IS} estimates was noted when Guernsey and Jersey were compared (permutation tests, $P = 0.011$, see Table 2 and appendix 1). No significant difference in mean F_{IS} was found for any other comparison, whether among insular populations or between insular and continental populations.

Table 2 *r*-ratios following Ennos (1994) and Weir & Cockerham (1984) F_{ST}, F_{IS} and F_{IT} estimated over all loci for different levels of spatial scale (see text for explanations), using mitochondrial minisatellite loci and nuclear microsatellite loci. Numbers in parentheses (*N*) refer to the number of populations considered. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS: non significant

			Markers			
			Nuclear			Mitochondrial
	Populations (*N*)	*r*-ratio	F_{ST}	F_{IS}	F_{IT}	F_{ST}
Overall	Level 1 (33)	2.21	0.089***	0.068***	0.151***	0.278***
Continent	Level 2 (23)	1.42	0.086***	0.070***	0.149***	0.231***
	Level 3 (19)	1.27	0.079***	0.068***	0.141***	0.208***
Island	Guernsey-Jersey-Chausey (10)	1.44	0.095***	0.065***	0.154***	0.253***
	Guernsey-Jersey (8)	1.26	0.107***	0.063***	0.163***	0.269***
	Guernsey (3)	0.84	0.106***	−0.012NS	0.096***	0.254***
	Jersey (5)	0.60	0.119***	0.100***	0.207***	0.242***
	Chausey (2)	17.62	0.015NS	0.072**	0.086***	0.218***

Contrasting patterns of differentiation between cytoplasmic and nuclear markers

A strong spatial differentiation between populations was found using mitochondrial minisatellite markers (mean F_{ST} of 0.278; $P < 0.001$, 95% C.I. [0.201–0.354]; Table 2). The spatial genetic structuring was less pronounced for the seven nuclear microsatellites but remained highly significant ($F_{ST} = 0.089$; $P < 0.001$, 95% C.I. [0.070–0.109]; Table 2). Using nuclear data, we found no significant genetic differentiation among groups of island and continental populations ($F_{CT} = 0.00121$, $P = 0.25$) but a significant genetic differentiation within groups ($F_{SC} = 0.088$, $P < 0.001$). The same held for cytoplasmic markers with a $F_{CT} = -0.005$ ($P = 0.60$) and a $F_{SC} = 0.235$ ($P < 0.001$). This suggests an efficient spread of pollen flow across open waters and no significant structure between islands and continental populations for cytoplasmic variation. Note that no significant difference between insular and continental populations was found in mean F_{ST} estimates when using nuclear data (level 2 vs. insular populations $P = 0.692$ and level 3 vs. insular populations $P = 0.701$, permutation tests). The same holds using cytoplasmic data (level 2 vs. insular populations $P = 0.572$ and level 3 vs. insular populations $P = 0.581$, see Table 2). The only significant difference in mean F_{ST} was found between Jersey and Chausey populations using the nuclear data set ($P < 0.05$, permutation tests). No significant differences were found for any other comparisons involving Guernsey, Jersey and Chausey populations, whether with nuclear markers or mitochondrial markers. Based on these contrasted levels of genetic differentiation between cytoplasmic and nuclear markers, the *r*-value (relying on the pollen vs. seed gene flow ratio) was equal to 2.21 when all 33 populations were considered (Table 2). Recalculating a global *r*-ratio by removing gynodioecious populations from the analyses yielded a similar result (data not shown). Under the assumptions of a Wright's island model at equilibrium, this result may imply that pollen flow is more than twice as high as seed flow within the Anglo-Norman gulf. The *r*-values were less than two when continental or insular populations were considered alone (Table 2). When focusing on populations located within the same island, the *r*-values were nearly equal to one or less, indicating: (i) an equal ratio of pollen flow vs. seed flow between Jersey and Guernsey; and (ii) a more efficient spread of seed within Jersey and Guernsey. In contrast, *r* reached 17.62 for populations situated in Chausey (Table 2), suggesting a clear predominance of pollen flow over seed flow in this island.

Genetic relationships among populations

When visualizing population trees, no clear geographical clustering emerged from either cytoplasmic or nuclear markers. Bootstrap values rarely reached 50%, indicating a low degree of resolution of the dendrogram (Fig. 2a, b).

Based on information provided by the cytoplasmic loci (Fig. 2a), insular populations were not clustered by island but scattered throughout the tree, the most striking example being populations ChoA and ChoB situated in Chausey and separated by only 950 meters. Even for continental populations, there was no clear relation between geographical location and genetic affinities of populations. In a cluster where most populations were geographically close to each other, there was systematically at least one population that was located far away (e.g. Ple within the Cap-Vaa cluster).

When considering the microsatellite-based tree (Fig. 2b), all insular populations, with the exception of ChoA and ChoB, were also scattered among continental populations.

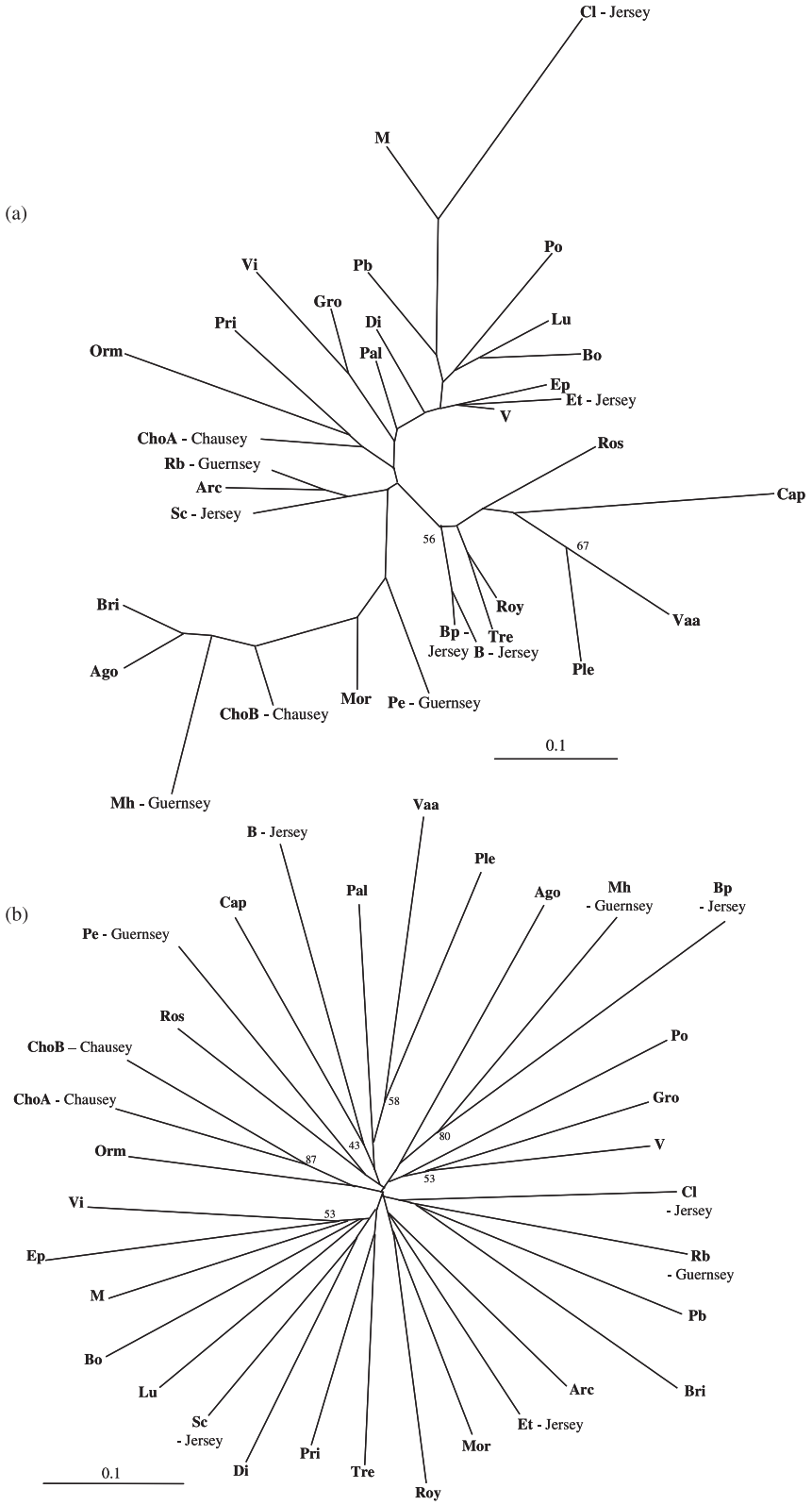

Fig. 2 Unrooted neighbour-joining tree based on the D_{CE} distance using (a) minisatellite and (b) microsatellite data. Only bootstrap values ≥ 50% are presented.

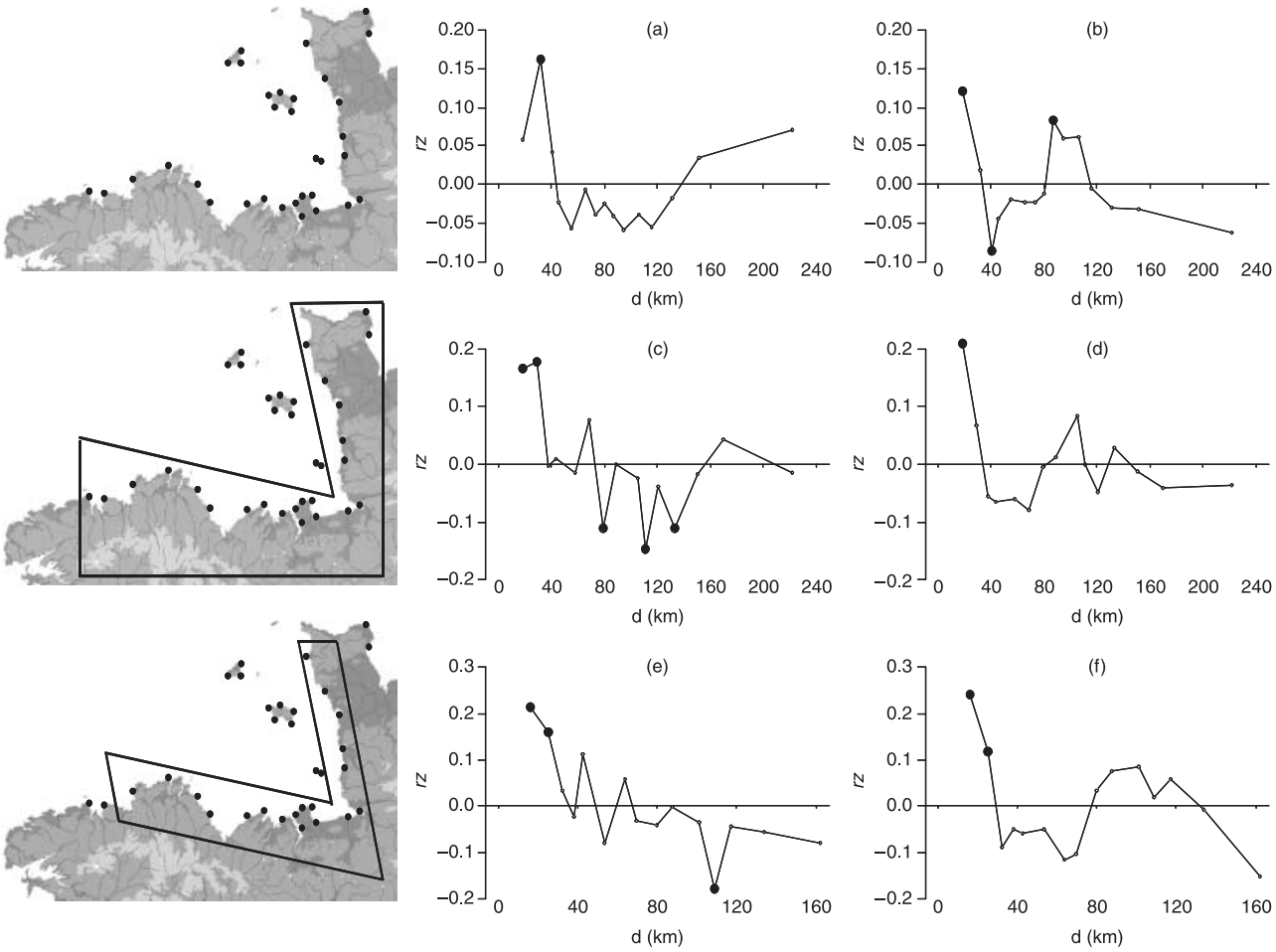

Fig. 3 Mantel correlograms based on nuclear microsatellite (a, c, e) and mitochondrial minisatellite (b, d, f) data. Three levels of spatial scale, referred as levels 1, 2 and 3 (see text for explanations) were considered. Black circles indicated a significant *rz* value at $P < 0.05$.

Nonetheless, microsatellite polymorphism led to a more consistent geographical clustering of continental populations with higher bootstrap values (e.g. Vi, Ep, M, Bo and Lu).

Testing for isolation by distance

With Euclidean distance as a measure of geographical distance, we first tested for IBD using the entire data set of all 33 sampled populations (level 1). Using microsatellites (Fig. 3a) and minisatellites (Fig. 3b), Mantel correlograms yielded significant positive *rz* values for the first distance class, followed by a rapid decline in genetic similarity, as would be expected under short-range dispersal. However, for larger distance classes, both correlograms exhibited large stochastic fluctuations that resulted in no clear continuous decrease of genetic similarity with increased geographical distance.

Second, we excluded island populations and focused on the remaining 23 continental populations (i.e. level 2; Fig. 3c, d). Mantel correlograms using nuclear data exhibited an *rz* value that decreased with geographical distance, i.e. a clearer pattern of IBD than the one previously observed at level 1. For cytoplasmic markers, only a short distance positive correlation was found, immediately followed by non-significant *rz* values close to zero for long-distance correlation (Fig. 3d).

Finally, we performed the analyses on the smallest spatial scale of the sampling scheme containing only 19 populations (level 3). Mantel correlogram based on microsatellite loci showed a consistent IBD correlogram outline (Fig. 3e). Using cytoplasmic minisatellites, a decrease in genetic similarity with increasing geographical distance was observed up to 40 km, stochastic fluctuation ruling the outline of the correlogram thereafter (Fig. 3f).

The use of coastal geographical distances rather than Euclidean distances for levels 2 and 3 roughly resulted in similar patterns of spatial genetic structure. Noteworthy, with coastal distance, the IBD patterns were clearer when

Table 3 Mantel test results for multiple comparisons between genetic divergence (estimated using the Chord distance of Cavalli-Sforza & Edwards (1967) and geographical descriptors between pairwise populations. Mantel tests have been carried out using separately the information provided by minisatellite and microsatellite loci for three levels of spatial scale (see text for explanation). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

	Minisatellites			Microsatellites		
	Level 1	Level 2	Level 3	Level 1	Level 2	Level 3
Coastal distance	—	0.049^{NS}	0.078^{NS}	—	0.168^{NS}	0.265*
Euclidian distance	0.060^{NS}	0.090^{NS}	0.103^{NS}	0.047^{NS}	0.172^{NS}	0.259*
Gabriel network	0.070*	0.149**	0.174**	0.039^{NS}	0.157***	0.193***

using nuclear data, but the stochastic fluctuations tended to be more pronounced when using cytoplasmic data (data not shown).

Results of global Mantel correlation for a combination of different geographical descriptors and genetic divergence confirmed our previous results (Table 3). For both cytoplasmic and nuclear markers and whether using coastline or Euclidean distances, rz values slightly increased when spatial scale was reduced from level 1 to level 3. This shows an increase in mean genetic similarity between neighbouring populations when only continental populations are considered. The same trend was observed with the Gabriel network ($rz = 0.070$, 0.149 and 0.174 for levels 1, 2 and 3, respectively, all at $P < 0.05$). The highest correlation values were obtained for nuclear microsatellites using the coastal distance when considering the smallest spatial scale (level 3; $rz = 0.265$; $P < 0.05$).

Depicting barriers to gene flow

The main genetic boundary defined by cytoplasmic markers (A) separated populations located within the Bay of the Mont Saint Michel (Populations M and Ep) from the others, extended within the Cotentin peninsula and stopped near the Pb population after isolating it (see Fig. 4a). We depicted another boundary (B) splitting the Anglo-Norman gulf into two regions. This result is in concordance with the main currents defined by Salomon & Breton's (1993) model of hydrology. The last boundary (C) isolated the Cl population, located on Jersey Island.

Genetic boundaries portrayed with microsatellite data are displayed in Fig. 4b. The first genetic boundary (A) separated the most eastern population Vaa from other populations. The second boundary (B) curved around Ple and reached Jersey Island, isolating population Bp in the process. Interestingly, this boundary divided the gulf into two regions along a line running from the north of Brittany to Jersey, as also suggested by cytoplasmic minisatellite data. Two others minors barriers (C and D) were observed: the first one encompassing Mh on Guernsey Island, the other one circling around population B on Jersey (Fig. 4b). This highlights the peculiar situation of island populations.

Assignments tests

The number of individuals assigned to a population that differed from the ones they were sampled in varied considerably between populations (mean = 56.56%, SE = 3.50%; min = 12%, max = 87% for Mh and ChoB, respectively) but continental and insular populations featured similar proportions of within-population misclassified individuals, Chausey excluded ($\chi^2_1 = 0.11$, $P = 0.733$). Indeed, both Chausey populations exhibited high levels of misclassified individuals (83% and 87% for ChoA and ChoB, respectively) whereas for Guernsey, Jersey or continental populations, more than 85% of individuals were well-assigned to the site they were sampled from. As a consequence, both Chausey's populations differed significantly from Jersey, Guernsey or Continental populations ($\chi^2_1 = 13.78$, $P < 0.001$; $\chi^2_1 = 11.86$, $P < 0.001$; $\chi^2_1 = 11.78$, $P = 0.001$, respectively).

By taking into account the B genetic boundary separating the gulf into an eastern and a western zone (see Fig. 4), we tested whether the rate of within-zone misclassified individuals differed between east and west parts. The proportion of individuals not assigned in the zone from which they were sampled considerably differed: 6.5% for the eastern zone and 35.6% for the western one. Populations located within the eastern part of the gulf tended to have fewer migrants whether or not we withdrew the islands from the analyses ($P < 0.01$ in both cases).

Considering the proportion of migrants at the population level, we explored whether a migrant sampled in a given population was preferentially assigned to a population located within the same zone (eastern or western). We found that the number of migrants assigned to the zone they were sampled in was significantly greater in the eastern zone ($\chi^2_2 = 136.13$, $P < 0.001$). Results of assignment tests thus pointed to an asymmetrical gene flow between eastern and western areas and a lower 'permeability' of the eastern zone.

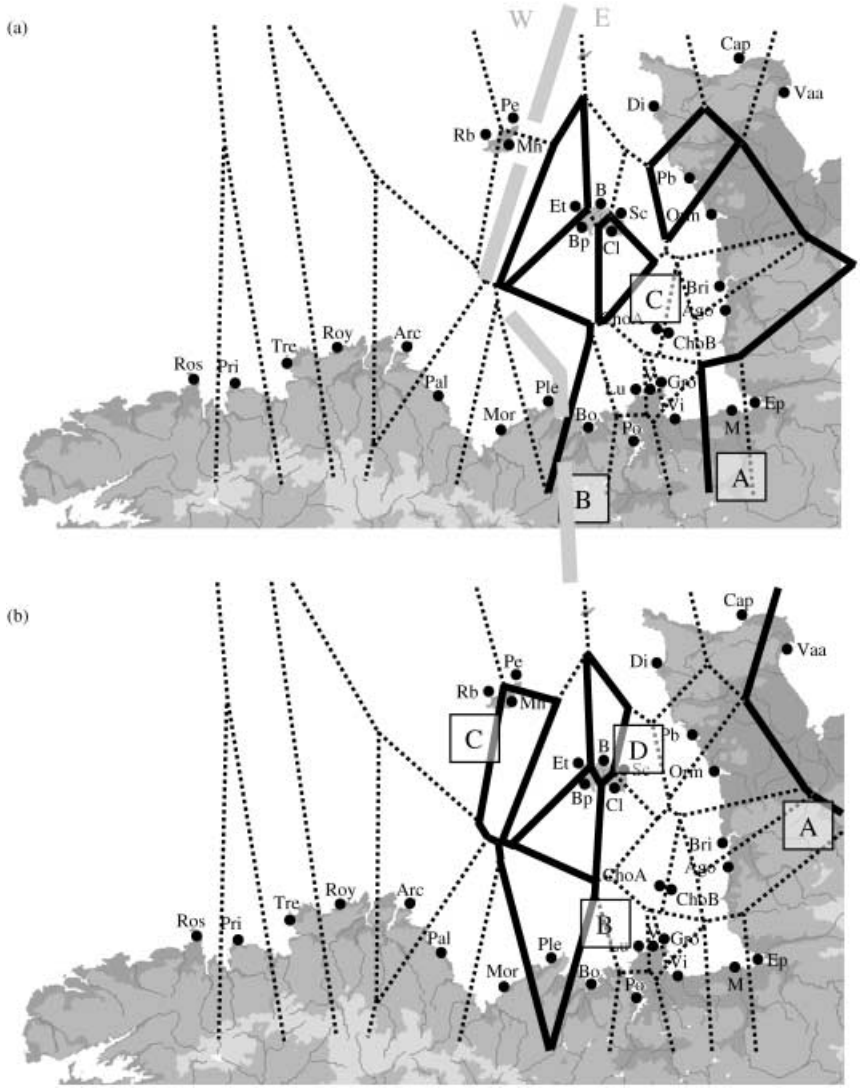

Fig. 4 Delaunay triangulation (black dotted lines) and genetic boundaries (bold lines) obtained with Monmonier's maximum difference algorithm. The first main boundaries are shown as A, B, C for minisatellite data (a) and A, B, C and D for microsatellite data (b). Populations are indicated according to their code given in Table 1. In Fig. 4a the west and east region defined for assignment tests can be visualized by a grey dotted large line.

Discussion

Cytoplasmic and nuclear genetic polymorphism

The polymorphism displayed by both microsatellite and minisatellite DNA markers at the regional scale demonstrated a significant geographical genetic structuring among the 33 populations of *B. vulgaris* ssp. *maritima* sampled in this study. The nuclear and mitochondrial DNA diversity we observed revealed great differences in population structure of nuclear and maternally inherited genes, as shown by F_{ST} values ($F_{STN} = 0.089$, $F_{STC} = 0.278$). These differences might be the simple consequence of an inheritance mode. Indeed, the effective population size of nuclear and mitochondrial genes differ about two-fold in hermaphroditic or gynodioecious species, since all individuals are functionally females (McCauley 1998; but see Laporte *et al.* 2000). However, both genomes might also differ in their dispersal ability: nuclear genes can disperse via seed and pollen flow whereas mitochondrial genes can only disperse in seeds. The resulting asymmetric gene flow greatly influences the level of differentiation of both genomes (Ennos 1994). Other comparative studies of cytoplasmic and nuclear variation concord, as maternally inherited cpDNA or mtDNA were more highly structured than nuclear DNA (see Ennos 1994; McCauley 1998; Laporte *et al*. 2001; Oddou-Muratorio *et al*. 2001; Cozzolino *et al*. 2003; Bacles *et al*. 2004).

Gene flow and long-distance migration

Wild sea beet populations live along the shoreline. As a consequence, the relative rate of pollen and seed migration from nuclear and cytoplasmic variability is difficult to unravel because, while pollen is dispersed by wind, seed movements can either follow a classical short-range

pattern of dispersal due to gravity, or follow occasional long-distance episodes of migration through hydrological currents during the greatest tides.

With respect to the genetic isolation by distance (IBD) pattern, several general observations can be made. First, there was evidence of a distance-dependant pattern of dispersal only for short-distance classes when considering the whole data set of populations, regardless of the markers (cytoplasmic vs. nuclear). Indeed, insular populations were very difficult to take into account in the detection of IBD, and as such, highlighted their peculiar situation within the Anglo-Norman Gulf, as also suggested by the different *r*-ratios (see below). This also suggests very localized dispersal. Second, no significant difference was found between Euclidean and coastal distances to detect an IBD pattern using cytoplasmic markers. This suggests that coastal topography may not be one of the ecological factors shaping seed dispersal. In contrast, the coastline distance worked better for depicting the IBD pattern when using nuclear microsatellite data. The occurrence of such a pattern of genetic variability is likely the result of a neighbourhood structure induced by short-range dispersal of pollen, strictly following the coastline owing to the direct barrier to inland pollen flow. This finding is supported by *r*-ratio results: the *r*-ratios were of only 1.42/1.27 for continental populations and 0.84/0.60 for Jersey and Guernsey, but increased when islands were compared (1.26) and when the whole data set was analysed (2.21). Overall, these results indicate more efficient spread of pollen flow than seed flow across open waters, but containment of pollen flow due to inland barriers within islands and along the continental coastline. Finally, the IBD pattern was better visualized when the spatial scale of study decreased (i.e. when islands and populations outside the gulf were removed from the analyses), demonstrating that the Anglo-Norman gulf forms a particular environment within the Channel.

Landscape context and influence of marine currents

In a landscape context, the dispersion of genes among populations is affected by both the spatial arrangement of populations and their degree of connectivity within the landscape (Michels *et al*. 2001; Wiens 2001; Arnaud 2003; Haag *et al*. 2005). In most cases, *Beta vulgaris* ssp. *maritima* populations are located far enough away from the sea and are not affected by tides, except for the highest tides. One of these high tides occurs in September, during the second bolting period of wild sea beet. Therefore, a significant proportion of seed could be transported via sea water and subsequently orientated by the direction of marine currents. Interestingly, when testing for a genetic boundary with both microsatellites and minisatellites, we found a zone of sharp genetic change that separates the Anglo-Norman gulf into an eastern and a western part. This barrier exactly fits the junction of two major currents that prevail during the high tide period (Fig. 4). In addition, results of assignment tests revealed that a population preferentially exchanged migrants with other populations located within the area defined by this boundary. We also found a higher proportion of misassigned individuals within the western part of the gulf, suggesting that the barrier is more frequently crossed by migrants from the eastern part. Indeed in the eastern part, some *B. vulgaris* ssp. *maritima* populations grew along the upper part of a cliff where seeds could directly drop into the sea without needing a high tide. In normal wind and tide conditions, the main current is a westward flow (Salomon & Breton 1993). This could explain the asymmetric exchange between the eastern and western parts. Altogether, these results confirmed the considerable impact of marine currents on gene exchange via seed dispersal between *B. vulgaris* ssp. *maritima* populations of the gulf.

Island colonization

Island populations, especially on Guernsey and Jersey, were expected to have peculiar characteristics due to their isolated location and the occurrence of circular currents. In sharp contrast with expectations, insular populations from a given island exhibited few genetic affinities in spite of their proximity and relative insular isolation. This observation was confirmed with several methods. The first piece of evidence was that insular populations often clustered with continental populations rather than with populations from the same island, according to both cytoplasmic and nuclear markers. This suggests that independent colonization events occurred from different continental populations. Second, the majority of populations singled out by a genetic boundary were located on islands — the minor barriers detected within an island were likely to be the consequence of a high genetic differentiation between nearby populations due to recent founding. This increase in genetic differentiation is probably accentuated when sib groups founded the populations, as several seeds could float together in the same glomerule. This particular colonization process seems to be similar to the propagule pool model of colonization described by Whitlock & McCauley (1990), McCauley (1993) and McCauley *et al*. (2001), and may also explain some of the within-population departures from HWE that we observed by inflating the mean level of inbreeding.

Moreover, insular populations were also very different in terms of relative rates of emigration/immigration events. On Jersey and Guernsey, 80% of individuals belonging to five populations out of eight (Pe, Mh, B, Bp and Sc) were correctly assigned to the population where they were collected. In contrast, Rb, Et and Cl populations were characterized by only ~45% of correctly classified individuals. Altogether, these results highlight the independent settlement of insular populations, with a mixture

of well-established and isolated populations and more recently established populations leaving nuclear and cytoplasmic signatures of founding events.

Additionally, despite some peculiar characteristics of insular populations, we did not find any clear evidence of the isolation of insular populations from the continent as shown by hierarchical analysis of *F*-statistics. Only the comparison between the global *r*-ratio and the *r*-ratio computed for islands suggested that gene flow might be contained within the different areas (i.e. continent, Guernsey and Jersey). Overall, insular populations probably have a recent continental origin but, in some cases, enough time has elapsed to erase the genetic effects of founding events. Populations in Chausey provide a good illustration: they exhibited close genetic affinities with *different* continental populations as revealed by cytoplasmic markers, but clustered together when nuclear markers were used. Moreover, the high value of the *r*-ratio (17.62) strongly suggests a high pollen-mediated gene flow as opposed to a seed-mediated flow. These results highlight the homogenizing effect of high pollen flow between insular populations of Chausey, keeping them from genetic drift. Hence, contrasting genetic patterns revealed by nuclear and cytoplasmic markers provides a good illustration of ancient and independent settlements of the Chausey populations.

Conclusion

The Anglo-Norman gulf features the highest tides in Europe (up to 15.5 m) and many *B. vulgaris* spp. *maritima* populations are regularly disturbed along the shorelines. In this study, we demonstrated the potential role of marine currents in shaping cytoplasmic genetic structuring, but also the greater efficiency of pollen flow over seed flow in open waters. Tides are probably the main cause of long-distance gene flow via seeds (promoting colonization process). In this context, some populations are most likely a source of genes, whereas others behave as a sink, as shown by asymmetry in gene flow between the eastern and western part of the Anglo-Norman gulf. It must be noted that *B. vulgaris* is a gynodioecious species. Gynodioecy dynamics is expected to be strongly determined by the relative rate of nuclear and cytoplasmic gene flows (McCauley 1998; Laporte *et al*. 2001). Therefore, our work sheds new light on factors and processes that might affect the evolution of sex ratios in structured populations.

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This work was a part of Virgil Fievet's Master in evolutionary ecology. He is currently doing a PhD on aphids' population dynamics and ecology. The remaining authors from the 'Laboratoire de Génétique et Évolution des Populations Végétales' (GEPV) are involved in population genetics studies of plant species, with a special attention given to the evolution of plant breeding systems. More information about the activities of GEPV can be found at the following web site: http://www.univ-lille1.fr/gepv/

Appendix

Summary of genetic diversity at four mitochondrial minisatellite loci and seven nuclear microsatellite loci among 33 wild populations in *Beta vulgaris*: total and corrected number of alleles ($A_t/\hat{A}$) at each locus, and expected heterozygosities (H_E) and F_{IS} estimates for each population sample at microsatellite loci. Mean number of alleles (A_t) and mean allelic richness per locus (mean $\hat{A}$) in each population sample as well as mean H_E and F_{IS} within each population; the total allele number per locus A_t and overall allelic size range at each locus (S) in base pairs are displayed in the last two rows of the table. $\hat{A}$ is the allelic richness corrected to $n = 17$ using the rarefaction method of El-Moussadik & Petit (1996b) using FSTAT version 2.9.3

	Minisatellite locus				Microsatellite locus							Microsatellite multilocus mean		
Populations	TR1	TR2	TR3	TR4	GTT1	CT4	GCC1	Bvm3	CA2	CAA1	GAA1	A_t	$\hat{A}$	H_E/F_{IS}
Vaa	2/1.99	1/1.00	1/1.00	1/1.00	3/2.06 0.08/−0.02	8/7.43 0.82/0.18	3/2.33 0.31/0.05	13/9.89 0.86/0.00	4/2.69 0.48/0.02	13/8.11 0.70/0.13	3/2.64 0.17/0.29	6.71	5.02	0.48/0.089
Cap	3/2.59	1/1.00	1/1.00	1/1.00	3/2.33 0.22/0.45	5/4.59 0.63/0.11	2/2.00 0.49/−0.14	10/7.84 0.78/0.12	2/1.99 0.28/0.11	13/8.61 0.78/0.18	3/2.99 0.51/0.10	5.42	4.34	0.52/0.112
Di	4/3.84	1/1.00	3/2.99	2/2.00	3/2.67 0.19/−0.08	8/6.71 0.80/0.04	2/2.00 0.51/0.17	8/6.63 0.68/0.42	4/3.38 0.26/0.10	11/10.31 0.90/0.03	3/2.98 0.45/0.12	5.57	4.95	0.54/0.132
Pb	6/5.75	1/1.00	3/2.99	2/2.00	3/2.53 0.37/0.16	8/7.24 0.76/−0.07	2/2.00 0.41/−0.38	11/7.87 0.75/0.37	4/3.79 0.47/0.24	14/11.35 0.87/0.21	2/1.99 0.20/−0.11	6.28	5.25	0.54/0.107
Orm	5/4.49	1/1.00	2/2.00	3/3.00	3/2.98 0.39/0.04	8/6.55 0.73/0.07	4/3.43 0.56/−0.18	12/7.43 0.72/−0.09	4/3.85 0.40/0.39	138.74 0.76/0.07	3/2.27 0.34/0.04	6.71	5.03	0.55/0.033
Ago	4/4.00	1/1.00	2/2.00	2/2.00	4/3.94 0.38/−0.16	6/5.89 0.64/−0.12	3/2.94 0.49/0.10	6/5.89 0.72/0.15	3/2.94 0.54/0.48	7/7.00 0.78/0.03	2/2.00 0.11/−0.03	4.42	4.37	0.52/0.082
Bri	3/2.97	1/1.00	2/2.00	1/1.00	4/3.32 0.46/−0.11	9/7.57 0.79/0.31	3/2.35 0.39/−0.10	7/6.16 0.79/0.10	4/3.35 0.61/−0.08	10/5.80 0.66/0.02	2/1.73 0.06/−0.02	5.57	4.32	0.53/0.052
Ep	6/5.80	1/1.00	3/3.00	2/1.89	3/3.00 0.64/−0.11	9/6.55 0.52/0.00	3/2.80 0.54/−0.10	9/7.77 0.70/0.25	4/3.65 0.51/−0.02	11/8.77 0.85/0.00	2/2.00 0.21/−0.12	5.85	4.93	0.56/0.004
M	6/5.53	1/1.00	3/3.00	2/2.00	3/3.00 0.51/−0.05	10/8.00 0.69/0.00	2/2.00 0.51/0.04	6/4.94 0.67/−0.16	4/4.00 0.71/0.68	10/8.57 0.85/−0.07	2/2.00 0.21/0.17	5.28	4.64	0.59/0.083
Vi	3/2.84	1/1.00	2/2.00	2/2.00	3/2.99 0.54/0.01	8/7.47 0.83/0.17	2/2.00 0.47/0.30	7/6.17 0.72/0.08	3/2.92 0.37/0.54	15/12.51 0.90/0.15	2/1.99 0.18/−0.09	5.71	5.15	0.54/0.164
Gro	4/3.98	1/1.00	2/2.00	1/1.00	3/2.91 0.27/−0.11	9/7.36 0.74/0.09	2/2.00 0.46/−0.17	3/3.00 0.66/−0.19	3/2.71 0.54/0.70	9/6.89 0.74/0.09	2/1.99 0.17/0.54	4.42	3.83	0.51/0.103
V	5/4.72	1/1.00	3/2.99	1/1.00	4/3.54 0.32/−0.14	7/5.73 0.67/−0.03	2/2.00 0.51/−0.08	6/5.03 0.70/−0.03	2/2.00 0.44/0.52	8/7.11 0.74/−0.03	2/1.99 0.17/−0.08	4.42	3.91	0.50/0.020
Lu	5/4.31	1/1.00	3/2.91	2/2.00	4/3.41 0.43/−0.05	13/9.43 0.76/0.25	3/2.42 0.51/−0.21	10/7.25 0.77/0.29	3/2.99 0.40/0.31	11/9.70 0.87/0.00	3/2.90 0.40/−0.19	6.71	5.44	0.59/0.079
Po	5/4.25	1/1.00	2/2.00	2/2.00	3/2.84 0.23/0.15	9/6.90 0.74/0.24	3/3.00 0.61/0.18	10/8.00 0.84/0.04	5/4.41 0.61/0.68	9/7.58 0.78/0.16	3/2.47 0.25/−0.13	6	5.02	0.58/0.215
Bo	3/3.00	1/1.00	3/3.00	2/2.00	3/3.00 0.51/−0.00	10/8.77 0.75/0.17	2/2.00 0.46/0.01	9/7.69 0.84/0.13	4/3.76 0.42/0.25	13/11.16 0.89/0.09	2/2.00 0.36/−0.12	6.14	5.48	0.60/0.092
Ple	2/2.00	1/1.00	1/1.00	1/1.00	2/1.96 0.12/−0.05	7/5.47 0.44/0.27	2/2.00 0.49/−0.33	5/4.43 0.66/−0.04	2/2.00 0.42/−0.11	7/6.08 0.72/0.09	2/1.53 0.03/0.00	3.85	3.35	0.41/−0.020
Mor	6/5.18	1/1.00	2/2.00	2/2.00	3/1.68 0.04/0.00	14/9.64 0.81/0.14	3/2.93 0.56/0.05	11/7.75 0.79/0.07	4/3.04 0.24/0.07	13/10.17 0.88/0.14	2/2.00 0.31/−0.09	7.14	5.31	0.51/0.083

Appendix *Continued*

	Minisatellite locus				Microsatellite locus							Microsatellite multilocus mean		
Populations	TR1	TR2	TR3	TR4	GTT1	CT4	GCC1	Bvm3	CA2	CAA1	GAA1	A_t	$\hat{A}$	H_E/F_{IS}
Pal	4/3.71	1/1.00	3/2.85	1/1.00	2/1.83 0.08/−0.03	13/8.29 0.60/0.27	3/2.96 0.56/0.11	11/7.24 0.76/0.07	3/2.58 0.39/−0.02	9/7.32 0.80/0.01	2/1.89 0.10/0.38	6.14	5.58	0.47/0.097
Arc	4/3.61	1/1.00	3/2.70	1/1.00	3/2.76 0.23/0.10	8/7.16 0.76/0.17	3/2.65 0.41/−0.27	6/5.34 0.74/0.02	3/2.87 0.30/0.00	13/10.26 0.86/0.04	2/2.00 0.27/−0.01	5.42	4.71	0.51/0.023
Roy	2/1.95	1/1.00	3/2.90	1/1.00	3/2.19 0.08/−0.02	12/8.06 0.75/0.11	3/2.90 0.48/0.03	9/6.84 0.72/−0.09	2/1.95 0.13/−0.06	12/9.20 0.86/0.06	2/2.00 0.49/0.06	6.14	4.73	0.50/0.030
Tre	4/3.60	1/1.00	3/2.26	2/2.00	3/2.58 0.26/−0.14	10/7.66 0.75/0.02	2/2.00 0.48/−0.30	12/8.44 0.82/−0.02	3/2.48 0.14/−0.05	12/8.69 0.79/0.05	2/1.89 0.10/−0.04	6.28	4.82	0.47/−0.046
Pri	2/1.99	1/1.00	2/2.00	2/2.00	3/2.36 0.17/−0.08	7/5.43 0.57/0.09	2/2.00 0.48/0.05	6/4.77 0.71/−0.05	4/3.68 0.29/0.21	9/7.42 0.78/0.02	2/2.00 0.38/0.04	4.71	3.95	0.48/0.034
Ros	4/3.71	1/1.00	2/1.73	2/2.00	3/2.66 0.19/0.33	6/5.43 0.65/0.02	3/2.97 0.57/0.02	8/6.12 0.69/0.14	2/2.00 0.28/0.00	14/10.53 0.87/0.00	2/1.95 0.12/0.37	5.42	4.52	0.48/0.068
ChoA	4/3.62	1/1.00	2/2.00	2/1.95	3/2.36 0.40/−0.23	10/8.02 0.81/0.02	3/2.96 0.56/0.28	6/4.33 0.62/−0.01	4/3.21 0.36/0.16	8/6.36 0.73/0.04	2/1.99 0.19/0.14	5.14	4.17	0.52/0.053
ChoB	3/2.96	1/1.00	2/1.95	1/1.00	3/2.61 0.32/−0.19	5/4.38 0.73/0.25	3/2.86 0.51/0.22	5/4.53 0.65/−0.08	3/2.80 0.49/0.21	11/8.19 0.76/0.09	2/2.00 0.21/−0.12	4.57	3.91	0.52/0.090
Pe	4/4.00	1/1.00	2/1.84	1/1.00	3/3.00 0.49/−0.17	8/6.63 0.77/−0.06	3/3.00 0.62/−0.18	6/5.51 0.75/0.10	2/2.00 0.33/0.11	11/8.92 0.82/−0.07	2/2.00 0.33/0.11	5	4.43	0.58/−0.037
Rb	4/3.99	1/1.00	3/2.99	1/1.00	3/2.72 0.17/−0.06	10/8.44 0.69/−0.01	3/2.95 0.43/−0.13	11/7.69 0.70/−0.06	3/2.89 0.29/0.38	9/8.36 0.86/−0.02	2/1.55 0.03/0.00	5.85	4.94	0.45/−0.007
Mh	2/2.00	1/1.00	1/1.00	1/1.00	2/1.99 0.16/−0.08	6/5.13 0.60/0.00	4/3.49 0.45/0.15	7/6.38 0.80/0.08	4/3.49 0.47/−0.14	11/8.71 0.77/−0.08	2/1.99 0.16/0.28	5.14	4.45	0.48/0.013
Et	5/4.71	1/1.00	2/2.00	2/2.00	2/1.97 0.14/−0.07	9/7.51 0.66/−0.13	3/2.44 0.52/0.11	11/7.89 0.76/−0.08	5/4.04 0.28/0.55	15/10.92 0.89/−0.03	2/2.00 0.36/0.15	6.74	5.25	0.51/0.021
B	3/2.73	1/1.00	2/2.00	1/1.00	3/2.44 0.23/0.10	7/6.04 0.62/0.11	2/2.00 0.21/0.63	8/6.40 0.75/−0.12	3/2.46 0.51/0.14	9/7.55 0.82/−0.02	3/2.95 0.46/0.20	5	4.26	0.51/0.078
Sc	4/3.94	1/1.00	2/2.00	1/1.00	4/3.29 0.35/0.12	10/7.46 0.81/0.15	2/2.00 0.51/0.18	10/7.57 0.68/0.17	4/3.56 0.54/0.42	11/9.44 0.87/0.12	2/2.00 0.25/0.01	6.14	5.04	0.57/0.175
Cl	4/3.70	1/1.00	3/2.94	1/1.00	3/2.99 0.39/0.08	8/6.38 0.66/0.03	2/2.00 0.48/0.24	8/6.87 0.83/−0.10	3/1.97 0.06/−0.01	10/7.31 0.69/0.16	2/2.00 0.26/−0.17	5.14	4.21	0.48/0.044
Bp	1/1.00	1/1.00	2/2.00	1/1.00	2/2.00 0.31/0.27	6/5.57 0.72/−0.07	3/2.67 0.48/0.69	11/7.94 0.77/−0.27	2/2.00 0.44/0.37	6/4.66 0.70/0.25	2/1.67 0.05/−0.01	4.57	3.78	0.49/0.144
A_T	9	1	5	4	4	18	4	29	6	33	4			
S					117/126	148/168	97/106	98/138	225/230	137/197	198/208			