Multiple introductions promote range expansion of the mollusc Cyclope neritea (Nassariidae) in France: evidence from mitochondrial sequence data.

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Mitochondrial gene genealogies provide evidence for multiple origins of recently recorded *Cyclope neritea* (Nassariidae) populations in France

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ABSTRACT

Since the 1970’s, the nassariid gastropod *Cyclope neritea* has extended its previously recognized natural range by colonizing the French Atlantic coasts. Since French populations have gradually spread northwards at the edge of its native range, a natural spread due to recent warming of the North-Eastern Atlantic was hypothesized. However, given the low dispersal ability of the species and its location in the vicinity of shellfish culture sites, human-mediated introductions appeared as an alternative vector. In order to address the relevancy of both hypotheses (i.e. natural spread or human-mediated introductions), a comprehensive study of the six recently colonized French bays along with two populations located in the recognized native range of the species was carried out. Based on mitochondrial gene sequences (Cytochrome Oxidase I, 262 individuals), phylogenetic reconstruction revealed 22 haplotypes split into several evolutionary clades. The appearance of dense and sustainable French populations appeared to be mainly due to recurrent human-mediated introductions from various genetically differentiated sites in the natural range. The large within-population genetic variation due to genetic admixture appears to have contributed to the successful settlement of *C. neritea* in France. Nevertheless, a process of isolation by distance suggested that *C. neritea* may have been present but unnoticed for a long time on the French Atlantic coasts. Altogether, since the natural or introduced status for French Atlantic populations is not straightforward, *C. neritea* can be classified as a cryptogenic species. Such a category is of particular interest when dealing with species close to their recognized native range.
INTRODUCTION

In marine habitats and especially in coastal areas, the rate of biological invasions has significantly increased in the last few years (Williamson, 1996), inducing economical and social risks (Gouletquer, 1998; Grosholz, 2002). Moreover, introduction of non-indigenous species (NIS) is identified as the second most important cause (after habitat destruction by human activities) of the loss of biological diversity (Carlton, 1996, Coleman & Williams, 2002; Mooney & Cleland, 2001; Palumbi, 2001). However, the study of biological introductions in coastal systems has only really begun a little more than twenty years ago (Carlton, 1996; Grosholz, 2002) and the processes that underlie biological invasions in these habitats are still not precisely identified. Marine biological invasions are known to be highly related to the increase of human-mediated transport of non-native species in ship ballast waters as well as to intentional or accidental releases for aquaculture and fisheries (Carlton & Geller, 1993; Cohen & Carlton, 1998; Zibrowius, 1983). However, fast environmental changes, by altering native communities, may also favour settlement of NIS (Carlton, 2000; Dukes & Mooney, 1999). Habitat alterations or climate changes may weaken competitive ability of indigenous species, consequently facilitating the settlement of exotic species (Sax & Brown, 2000). For instance, the temperature is known to be a critical factor influencing the natural distribution of both animal and plant species: a wide array of species has been shown to have colonized western and northern Europe from the Iberian Peninsula after the ice ages (see Comes & Kadereit 1998; Taberlet et al., 1998 or Gomez & Lunt, in press for a detailed review) and Stachowicz et al. (2002) recently showed that changes in the maximum/minimum temperatures may have promoted the establishment of two invasive ascidians at native species’ expense. Species located on both sides of a biogeographic boundary are also able to
extent or restrict their natural range in response to changes in environmental conditions (Gaylord & Gaines, 2000).

In this context, the Bay of Biscay (North Eastern Atlantic) is an interesting area since the mean temperature of this geographical area increased by 0.6 to 0.8 °C per decade during the last 30 years (IPCC, 2001). The rapid and recent warming of the Bay of Biscay has already been proved to promote displacement of mollusc species within this Bay (e.g. Macoma balthica, Hummel et al., 2000). The recent changes of the distribution area of the gastropod Cyclope neritea might also be related to this process. This nassariid gastropod is native to the Mediterranean and Black Seas, and to the Atlantic coasts of Morocco, southern Spain and Portugal. Up to the 1950’s only discontinuous and ephemeral populations were recorded in North of Spain and South of the French Basque coasts, the Basque country being defined as the northern edge of the natural range of the species (for a detailed description, see Sauriau, 1991). Since 1976, new populations have been recorded along the French coasts, gradually from South to North: first in Arcachon Bay, then in 1983-84, in the Marennes-Oléron Bay, the Isle of Ré, and the Gulf of Morbihan, and more recently, on English Channel coasts in 2000s (see references in Bachelet et al., 2004). On one hand, the gradual appearance of C. neritea populations from South to North favours the hypothesis of a natural spread towards North during the past thirty years in relation to environmental changes. On the other hand, a natural spread over such a short period of time seems unlikely given the limited dispersal ability of this species. As a direct developer, C. neritea has no planktonic larval stage (Gomoiu, 1964) and is subjugated to a very particular substrate (i.e. sheltered spots, characterized by sandy-muddy substrates) such that the numerous discontinuities (due to occurrence of rocky shores) along the southern French Atlantic coasts may slow down natural migrations (Sauriau, 1991). Therefore, the appearance of C. neritea along the French Atlantic and English Channel (FAEC) coasts may be due not to a natural process but to human-
mediated introductions via oyster exchanges between Mediterranean and Atlantic shellfish culture sites (Boulhic & Tardy, 1986a; Le Roux et al., 1988; Pigeot, 1988; Sauriau, 1991). Exchanges of commercial species between distant shell-farming sites have been proved to be an efficient way of primary and secondary introductions of alien species (including mollusks) in new biotas (Wolff & Reise, 2002) and all the newly established French populations of C. neritea were found in the vicinity of oyster beds. Indeed, focusing on Arcachon Bay (Bachelet et al., 2004), the first recorded site of introduction of C. neritea, a mitochondrial sequence pattern suggestive of recurrent introductions was revealed. However, the colonization history of C. neritea along the French coasts cannot be elucidated based on a single population.

The aim of the present study was thus to examine all the currently recorded populations along the FAEC coasts to determine (i) if the French populations share common mechanisms for their establishment (e.g. number of sources, number of founders etc.) and (ii) the likelihood of two non-exclusive hypotheses to explain the actual distribution of C. neritea in France, i.e. a natural spread towards North and accidental human-mediated introductions. To address these questions, we combined classical population genetics analyses and an intra-specific phylogenetic approach by using mitochondrial markers. Mitochondrial genealogies are indeed useful to assess the mechanisms on which rely the settlement of introduced species (Kolbe et al., 2004). Moreover, because population effective size at mitochondrial genes is two to four-times lower than those of nuclear markers, genetic drift effects are emphasized and isolation-by-distance patterns (IBD, populations in close geographical proximity are expected to be genetically closer than more distant populations, Slatkin & Maddison, 1990; Wright, 1943) are expected to be more easily detected (Diaz-Almela et al., 2004). Given the low dispersal ability of C. neritea, if the gradual appearance of this species along the FAEC coasts was due to natural spread, IBD pattern should be
observed. Conversely, in the case of random human-mediated introductions, a stochastic haplotype distribution should be observed. Moreover, strong losses of genetic polymorphism are generally revealed when the introduction is due to rare events from a limited number of sources (see for instance introductions of Argentina ants *Linepithema humile* (Tsutsui *et al.*, 2000), Bennett’s wallabies *Macropus rufogriseus rufogriseus* (Le Page *et al.*, 2000) or Mauritian macaques *Macaca fascicularis* (Lawler *et al.*, 1995)). Conversely, if several genetically differentiated source populations contributed to the settlement of NIS, the introduced population is expected to show a mixture of evolutionary divergent lineages (admixture), each of them resulting from distinct local evolution histories in the native range (Hamblin & Veuille, 1999). These expectations were analyzed, based on a comprehensive sampling of *C. neritea* along the FAEC coasts including the Arcachon Bay population as well as two populations from the native range (Bachelet *et al.* 2004) by analyzing the mitochondrial gene Cytochrome Oxidase I (COI, 533 base pairs) over 262 individuals.
MATERIALS AND METHODS

Study species and sampling. *C. neritea* is a small gastropod (about 15 mm in maximum shell width) which lives in shallow waters and/or intertidal habitats, especially in sheltered areas such as estuaries and lagoons (Morton, 1960). *C. neritea* is a direct developer, depositing encapsulated eggs (a single embryo per capsule) on hard substrata such as cockle shells. Eggs take 2-5 weeks to mature and then hatching as benthic juveniles (Boulhic & Tardy, 1986b; D'Asaro, 1993; Gomoiu, 1964; Kisch, 1950).

Sampling sites (see Table 1, Fig. 1) were chosen in each bay of the French Atlantic and Channel coasts (FAEC) where *C. neritea* has been recorded since the 1970’s so that, to our knowledge, this sampling fits exactly with the actual distribution of the species in its current FAEC range, beginning at the edge of its previous recognized natural range. In addition to the sample of Arcachon Bay (Arguin, Bachelet et al., 2004), five populations of *C. neritea* were sampled between March 2002 and June 2003: Hossegor (French Basque Country) where *C. neritea* was unnoticed until our sampling and located at 50 km north of Socoa where the species was recorded in 1950 (Kisch, 1950); Ronce les Bains in Marennes-Oléron Bay (Sauriau, 1989), Rivedoux in Isle of Ré (Tardy et al., 1985) and Séné in Gulf of Morbihan (Le Roux et al., 1988), three bays where *C. neritea* was first observed in 1983-84; and Stérec in the Bay of Morlaix (English Channel), the most northern population known on French coasts since the end of the 1990s. For each population, adult specimens were collected and stored in 95% ethanol before DNA extraction. Two populations included in the study by Bachelet et al. (2004), Thau lagoon (French Mediterranean Sea) and Faro (South Portugal), were used as representatives of populations from the native range.
**DNA extraction, amplification and sequencing.** We followed the protocol as detailed in Bachelet et al. (2004). To summarize, total DNA was extracted from less than 15 mg of foot muscle using Dneasy™ Tissue Kit according to the manufacturer’s protocol (Qiagen, Germany). Amplification of the COI gene were performed using specific primers (Bachelet et al., 2004) and PCR products were directly sequenced using ABI PRISM® BigDye™ Terminators v3.0 Cycle Sequencing Kit following the manufacturer’s protocol (Applied Biosystems). Both strands were sequenced for each individual using an ABI PRISM® 3100 Automated DNA Sequencer (Perkin-Elmer Applied Biosystems, Foster City, CA) and sequence data were aligned using ClustalW (Thompson et al., 1994).

**Sequence analysis.** Over the whole dataset and for each population, genetic diversity was examined by computing the number of mitochondrial haplotypes \( N_H \), the number of segregating sites \( S \), nucleotide diversity \( \pi \) (i.e. average number of nucleotide differences between pairs of sequences) and gene diversity \( H_e \) (Nei, 1987) by using the software DnaSP (Rozas & Rozas, 1999).

Genetic divergence between each pair of populations was investigated by calculating an estimate of the pairwise \( F_{ST} \) parameter (Wright, 1951) using Arlequin v.2.0. (Schneider et al., 2001). The statistical significance of \( \hat{F}_{ST} \) values under the null hypothesis of no differentiation between populations was assessed using the permutation procedure implemented in Arlequin v.2.0. Isolation-by-distance model was used to test the hypothesis of a step by step dispersal of *C. neritea* from South to North. In this model, a positive correlation is expected between genetic distances (expressed by Rousset (1997) in term of \( \frac{\hat{F}_{ST}}{1 - \hat{F}_{ST}} \)) and geographic distances. To picture IBD, the genetic distance between all FAEC population pairs was plotted against the geographic distance in kilometres. Correlation between matrices of
pairwise geographic and genetic distances was estimated using Mantel tests as implemented in the software IBD 1.5 (Bohonak, 2002).

Three complementary analyses were used to test for the hypothesis of human-mediated introductions of *C. neritea* along the FAEC coasts and to determine if several populations might have contributed to these introductions. First, the link between the geographical distribution of the haplotypes and their genealogical relationships was analysed by building a haplotypic network: compared to tree analyses, network reconstructions are particularly accurate for the study of within-species phylogeny (Posada & Crandall, 2001). A median-joining algorithm performed with the software Network 4.0.0.0. (Bandelt et al., 1999, Cassens et al. 2003) was used: a minimum-spanning network was constructed and a maximum parsimony algorithm was applied to simplify the complex branching pattern and represent all the most parsimonious intra-specific phylogenies. Second, for polymorphic populations, the distribution of the observed number of pairwise nucleotide site differences (i.e. mismatch distribution) was computed in DnaSP v.3.53 (Rozas & Rozas, 1999) to examine the mixing of evolutionary divergent lineages at the population level. Finally, given the low resolving power of Tajima’s D-test (Galtier et al., 2000), tests for departure from mutation-drift equilibrium under an infinite-site model were carried out following Depaulis & Veuille (1998). This test (*K*-test) is based on the comparison of the observed haplotype number (*N*<sub>H</sub>) with the expected number under equilibrium (*N*<sub>EH</sub>) given the size of the sample (*N*) and the observed number of segregating sites (*S*). It detects recent population admixture when testing a departure from equilibrium in the direction of too few haplotypes (see the case of *Drosophila simulans*, Hamblin & Veuille, 1999). In the case of a recent admixture, evolutionary divergent haplotypes are present within the same population and several intermediates haplotypes are expected to be missing: for a given set of parameters (*N*, *S*), *N*<sub>H</sub> should thus be significantly smaller than *N*<sub>EH</sub> (Hamblin & Veuille, 1999). A coalescent
simulation approach (program modified from a program developed and kindly provided by
M.-H. Muller) was used to compute confidence intervals and one-tailed $P$-values for $N_H$.  

RESULTS

Molecular diversity over the dataset. A 533 base pair fragment of the COI gene was analyzed in each population (from 29 to 39 individuals according to the population, Table 1). Only synonymous substitutions were observed (Appendix). Over the whole dataset (N=262 individuals), 27 polymorphic sites and 22 haplotypes (GenBank accession numbers: AY789970-AY789991) were identified (Table 1, Appendix). The distribution of the 22 haplotypes is pictured in Fig. 1. Over the whole dataset, three haplotypes (A, B and C, see Fig. 1) were found at very high frequency, \( f = 0.32, 0.25 \) and 0.19 respectively (sum = 0.76), compared to the others (0.004 < \( f < 0.06 \)). However, according to the population, large variations in gene and molecular diversity indices were observed (Table 1). In particular, the number of segregating sites (\( S \)) and the number of haplotypes (\( N_H \)) ranged from 0 (Thau and Faro populations) to 21 (Rivedoux) and from 1 (Thau and Faro) to 16 (Séné) respectively. Thau and Faro, located in the recognized natural range of the species, were the only monomorphic populations (Fig. 1). Hossegor showed low genetic diversity compared to the other French Atlantic and English Channel (FAEC) populations: only 2 haplotypes were observed and nucleotide diversity (\( \pi = 0.00050 \)) was 2 to 3-times lower than in other populations (\( \pi = 0.00102 \) to 0.00153). Genetic variability was comparable in the other five FAEC populations with 14 (Séné) to 21 (Rivedoux) segregating sites and haplotype diversity values ranging from 0.589 to 0.911 (mean value \( H_e = 0.743 \pm 0.120 \)). However, haplotype and molecular diversity estimates were not always agreeing. Séné showed the highest number of haplotypes and haplotype diversity (\( N_H = 16, H_e = 0.911 \)) while the number of segregating sites and nucleotide diversity (\( S = 14, \pi = 0.01024 \)) were the lowest of the FAEC populations. Hossegor and Séné, both different from Arguin, Ronce, Rivedoux and Stérec, may thus have
different histories and/or have been originated from different introduction processes in comparison to the other recently settled French populations.

**Haplotypic network and genetic structure at the population level.**

The network (Fig. 2) pictures the most likely genealogies between haplotypes. The three most frequent haplotypes (i.e. A, B and C) corresponded to three main clusters (cluster 1: A, J and H, cluster 2: B, D, E, F and R and cluster 3: C, U and V). The genetic distances between these clusters were large (13 to 15 mutational steps) compared to genetic distances within clusters (1 to 5 mutational steps). The haplotype G, present only in the population of Rivedoux (6 individuals out of 39; Fig. 1) was found to be very distant from the other clusters (14 to 18 mutational steps according to the cluster). The other 10 haplotypes (I, K-Q, S and T) found at very low frequencies (over the whole dataset, $f = 0.015$ and 0.008 for I and K respectively, and $f = 0.004$ for the others) were close to each other, constituting an unresolved torso in the middle of the network.

Interesting features were observed when comparing the geographical distribution of haplotypes (Fig. 1) and their location in the network (Fig. 2). In five out of the six FAEC populations, namely Arguin, Ronces, Rivedoux, Séné and Stérec, haplotypes belong to two or all three of the clusters identified through the network analysis. This co-occurrence of individuals characterized by evolutionary divergent haplotypes is corroborated by the mismatch distribution constructed for each polymorphic population (Fig. 3). Arguin, Ronces, Rivedoux and Stérec all showed at least two major peaks featuring two or more groups of genetically divergent haplotypes co-occurring at the population level. This is exemplified by the haplotypic tests as the observed number of haplotypes ($N_H$) was significantly lower than expected under mutation-drift equilibrium ($N_{EH}$) in each of the four populations (Fig. 3). A different situation was observed for Hossegor and Séné for which the observed distribution
appeared to fit the expected curve constructed under a demographic equilibrium hypothesis. In Hossegor, the individuals appeared to be evolutionary closely related to each other. The $K$-test was irrelevant in this population since only one segregating site was found ($N_H$ always equals to 2). In Séné, a more uniform and continuous mismatch distribution than in Hossegor was observed. This may be related to the occurrence of a larger number of haplotypes that differ from each other over a large range of nucleotide divergence (from 1 to 14). In this population, a larger number of haplotypes than expected was observed (Fig. 3) suggesting a recent population expansion or ancient balanced polymorphism (Depaulis & Veuille, 1998).

**Genetic divergence across populations and isolation by distance models.** Over the whole study, the genetic differentiation was highly significant ($P < 10^{-5}$) and $\hat{F}_{ST}$ was equal to 0.382. Obviously, this substantial genetic structure was partly due to Faro and Thau, monomorphic for two divergent haplotypes (A and B respectively). However, when removing these populations, $F_{ST}$ estimate decreased to 0.225 only and was still significant ($P < 10^{-5}$). Interestingly, not all populations were equally genetically differentiated with each other. Population pairwise $\hat{F}_{ST}$ values indeed ranged from 0.042 to 0.458 (Table 2). While not significant when all six FAEC populations were considered ($P = 0.162$), IBD was revealed after removing Hossegor from the analysis ($P = 0.016$) highlighting an increase of pairwise $F_{ST}$ estimates when more distant populations were considered (Table 2; Fig. 4). For instance, when the population of Arguin is compared to populations located more and more northerly, i.e. Ronce, Rivedoux, Séné and Stérec, $\hat{F}_{ST}$ values increased (0.053, 0.147, 0.176 and 0.276 respectively; Table 2).
DISCUSSION

ADMIXTURE PATTERNS AND HUMAN-MEDIATED INTRODUCTIONS

In this study, *C. neritea* populations sampled in the natural range of the species (Thau and Faro) were found to be monomorphic. On the contrary, five of the six FAEC populations (i.e. Arguin, Ronce, Rivedoux, Séné and Stérec) exhibited high levels of genetic diversity (e.g. mean $H_e = 0.743 \pm 0.120$ and $\pi = 0.01343 \pm 0.00210$). This result is in agreement with an increasing number of genetic study of terrestrial, freshwater and marine NIS indicating that the expected decrease of genetic polymorphism is lacking for numerous introduced species (poaceae (Novak et al., 1993), brown mussel (Holland, 2001), thiarid snail (Facon et al., 2003), slipper limpet (Dupont et al., 2003) or Cuban lizard (Kolbe et al., 2004)).

As underlined by Kolbe et al. (2004), when natural populations are monomorphic, the occurrence of multiple introductions could promote the settlement of NIS by transforming among-population variation in native ranges to within-population variation in introduced areas.

The level of polymorphism (27 segregating sites defining 22 haplotypes over 262 individuals) is similar with that observed in many studies carried out with COI at the infra-species level (e.g. Gopurenko et al., 1999; Kirkendale & Meyer, 2004; Wilding et al., 2000). The striking difference in genetic polymorphism between Thau and Faro and the other French populations suggests that (i) at the population level, low polymorphism is an attribute of *C. neritea* in its native range and (ii) recurrent human-mediated non-intentional introductions may be at the origin of the settlement of the majority of the French populations recorded since the 1970’s. In addition, divergent haplotypes were found within these five populations. For instance, haplotypes belonging to clusters 1, 2 and 3 were observed in Arguin, Ronce and Stérec. This particular pattern is unlikely for populations at mutation-drift equilibrium but is
characteristic of recent admixture events (i.e. mixing of individuals coming from populations that have evolved independently). For four populations (i.e. Arguin, Ronce, Rivedoux and Stérec), this is illustrated by: (i) multi-peak mismatch curves and (ii) highly significant $K$-tests. The first result showed that these populations were characterized by the co-occurrence of at least two groups of genetically divergent individuals. The $K$-test confirmed that populations are not under a demographic equilibrium and that recent admixture events have occurred. Admixture implies that introductions did not originate from one given source but from several genetically differentiated sources. Oyster exchanges, known to be responsible for numerous NIS introductions along the Atlantic coasts of Europe (Goulletquer et al., 2002; Wolff & Reise, 2002), may have played a major role in the colonization of the FAEC coasts by *C. nerita* by introducing, in the vicinity of oyster beds, large numbers of individuals coming from various genetically differentiated populations. Such mechanisms and vectors have been hypothesized for other coastal marine introductions (see for instance *Ocinebrellus inornatus*, Martel et al., 2004). Séné and Hossegor appeared to be in a different situation. In Séné, a larger haplotype number than expected under the equilibrium hypothesis was observed ($K$-test) and these haplotypes were differing from each other over a large range of nucleotide divergence (from 1 to 14). At the population level, a star-like genealogy of haplotypes was observed suggesting a recent demographic expansion either in Séné or in the source population of Séné. Except if the species had been present but unnoticed for a long time in Séné, local expansion after the settlement of *C. nerita* seems unlikely since the species was recorded for the first time in this site only twenty years ago (1984, Le Roux et al., 1988). The alternative hypothesis of a limited number of source populations for Séné, one of them having recently undergone a large demographic expansion in the native range therefore seems more likely. Because our study did not aim at identifying the sources but looking at mechanisms for the settlement of the species along the FAEC coasts, none of our analyses allow ascertaining
either the number or the origins of these introductions. Such an issue would have required a different sampling scheme focusing on potential sources from the recognized native range of the species.

**STATUS OF HOSSEGOR: NATURAL OR INTRODUCED POPULATION?**

The most peculiar genetic pattern was observed in Hossegor. This population showed genetic characteristics closer to that of populations from the natural range than to the other FAEC populations, suggesting a natural status for this population. Three pieces of evidence support this hypothesis: first, the genetic diversity in this population was quite low ($H_e = 0.265$, $\pi = 0.00050$) compared to other FAEC populations ($H_e = 0.743 \pm 0.120$ and $\pi = 0.01343 \pm 0.00210$ mean values and associated standard deviations) whereas low diversity was also observed in Thau and Faro. Second, of the two haplotypes found in Hossegor, the most frequent, namely A, ($f = 0.85$) was characteristic of Faro, and the second (haplotype H) was diverging from A by only one mutational step (Appendix). Finally, observed and expected distribution of pairwise differences in the population of Hossegor matched perfectly, indicating that the population was at demographic equilibrium (Li, 1977; Rogers & Harpending, 1992; Slatkin & Hudson, 1991). The fact that Hossegor is not the result of a recent introduction but a native population is consistent with the presumed history of *C. neritea* in this geographical area. The species has been episodically recorded until 1950 along French Basque coasts (Kisch, 1950; Morton, 1960) in particular in Socoa, only 50 km in the South of Hossegor. The North edge of this area is located just south of the very exposed and inhospitable for *C. neritea*, coasts of the Landes region. Moreover, as underlined by Sauriau (1991), the southern coasts of the Bay of Biscay are close to a biogeographic boundary between northern temperate species and southern sub-tropical species (Glémarec, 1979). Such natural barriers are characterized by very specific patterns in term of species distributions,
hybridization between species (e.g. Bierne et al., 2003) or genetic differentiation at the population level (Jolly et al., 2005; Luttikhuizen et al., 2003). Hossegor may thus be the historical northern range limit of *C. neritea* along the French Atlantic coasts and the existence of a strong geographical barrier could have limited its natural spread northwards.

**LINKING HUMAN-MEDIATED INTRODUCTIONS AND NATURAL SPREAD**

An isolation-by-distance pattern (IBD) was clearly revealed between FAEC populations when removing the “natural” population of Hossegor. The gradual appearance of new FAEC populations of *C. neritea* could first be viewed as a consequence of the recent warming of the Bay of Biscay (i.e. natural range expansion towards North) enhancing the colonization along the French coasts. Indeed, oyster exchanges between aquaculture sites are very unlikely to be responsible for such an IBD pattern since human-mediated exchanges between shellfish culture sites occur randomly between very distant locations without continuous progression. In particular, all oyster spats are produced in the bays of Arcachon and Marennes-Oléron and transplanted from those bays to all the other FAEC and Mediterranean oyster culture areas (Goulletquer & Heral, 1997). If random human-dispersal was the only process structuring *C. neritea* populations, a random haplotype distribution would thus have been observed.

TO summarize, the genetic pattern observed in the FAEC populations (excluding the “natural” population of Hossegor) was characteristic of human-mediated introductions from multiple and divergent source populations and at the same time, a pattern of IBD that cannot be related to human activities was also observed. Can we explain this discrepancy? One likely explanation is the presence of *C. neritea* at low densities, and consequently unnoticed, on the FAEC coasts, long before 1976. For instance, Hossegor is likely to be a natural *C. neritea* population. However, in spite of continuous monitoring activities in this area (the Arcachon

Marine Station was created in the 1860’s), official records of the population of Hossegor were missing before our sampling. Similarly, in the Bay of Morlaix, the presence of *C. neritea* is still officially unrecorded in spite of the observation of the first individuals in Stérec 8 years ago (Ann Andersen, pers. comm.). The patchy distribution of *C. neritea* populations, even in its natural range, makes it difficult to detect, even more when population densities are low. *C. neritea* may thus have been present on the FAEC coasts as a cryptic species for decades and could thus be classified as a cryptogenic species as defined by Carlton (1996). Such species “not demonstrably native or introduced” represent probably a large part of newly recorded species (Carlton, 1996). Such a category is of particular interest when dealing with species close to their recognised native range. In the case of *C. neritea*, the most northern natural population on the Atlantic coasts might have been a source for populations located further North through natural migrations and/or more probably ancient and not recognized accidental introductions. As an example, the oyster *Ostrea edulis* has been exploited in France since the early twentieth century with exchanges between the Thau lagoon and the Bay of Marennes-Oléron (Hinard & Lambert, 1928). However, the increase of accidental introductions probably started only during the 1970’s because of the intensification of oyster transfers between Mediterranean and Atlantic aquaculture sites as well as among Atlantic oysters farms. At the same time, ecological alterations (i.e. the rapid temperature increase in the Bay of Biscay) may have favoured (i) the settlement of introduced individuals by weakening native communities (Dukes & Mooney, 1999) and (ii) the increase of migrants coming from the South, responsible for a demographic reinforcement of *C. neritea* French Atlantic populations.

Whatever the processes that played in the past (i.e. natural migration and accidental introductions) they were apparently too rare the settle large and detectable populations of *C. neritea*. The need of recurrent and massive introductions for a species to increase the chance for a sustainable establishment a new environment is well known: Sax & Brown (2000)
explained that species that become abundant and widespread often do so only after having failed to establish following multiple earlier introductions and that the same phenomenon is also true for natural colonization. The particular combination of factors, i.e. increase of repeated accidental introductions associated with reinforcement by natural migrations, can be responsible of the sustainable settlement of large (and thus detectable, from 400 to >1600 ind.m$^{-2}$ in Rivedoux, Tardy et al., 1985) *C. neritea* populations along the Atlantic coasts of France. Nevertheless, the population of Stérec has probably been founded exclusively because of human-mediated introductions since several hundreds of kilometres of rocky shores are isolating this site from the other French Atlantic populations and it is noteworthy that the population is still at low density.

In conclusion, we showed that French populations shared common mechanisms for their establishment. Our study ascertained the introduced status of *C. neritea* along the FAEC coasts, showing that mixing of different evolutionary lineages is the rule and has been the starting point for a rapid demographic expansion in sites where the species was absent or unnoticed but present at low density. In a previous study, Bachelet et al. (2004) pointed out that (i) *C. neritea* could compete with the native necrophagous nassariid, *Nassarius reticulatus* and (ii) conversely to this native species, *C. neritea* did not show a heavy parasitic load. These observations showed that this competitive gastropod may become definitively integrated in the ecosystem where it has been introduced. The temperature increase may facilitate this process by reinforcing the potential for this species to be established on a longer term and even becoming a real invasive species. Altogether, since the natural or introduced status for French Atlantic populations is not straightforward, *C. neritea* can be classified as a cryptogenic species and a more comprehensive study of the species over its native range is now required to understand short and long-term history of the species in Europe. Given the
limited polymorphism found in Thau and Faro, nuclear microsatellite markers recently
developed for *C. neritea* (Simon-Bouhet *et al.*, 2005) appear as promising markers. As
underlined by Davies *et al.*, (1999), assignment analyses based on multi-locus genotypes are
powerful to identify the limits of the natural range of species and sources for NIS. Since
ecological consequences of NIS introductions may be different when new populations are
introduced close to their previous native range (where the community is expected to be more
or less the same than in the native range of the NIS) or introduced far from their natural range
(where the NIS will find a totally different community), long-term ecological surveys are
needed to follow with a special interest those NIS located at the edge of their previous natural
range.
REFERENCES


Li HW (1977) Distribution of nucleotide differences between two randomly chosen cistrons in a finite population. *Genetics* 85, 331-337.


ACKNOWLEDGEMENTS

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FIGURE LEGENDS

Fig. 1. Haplotype distribution among the eight study populations. Numbers of individuals analysed per population (N) are given.

Fig. 2. Haplotypic network picturing the phylogenetic relationships between haplotypes. Circle sizes are proportional to haplotype frequencies over the whole dataset. (|) Single mutational events. (●) Missing intermediate haplotypes between observed haplotypes.

Fig. 3. Mismatch distributions. For each polymorphic population, the observed distribution of pairwise nucleotide differences between sequences (continuous lines) is plotted. The expected distribution under the null hypothesis of demographic equilibrium is indicated by a dotted line. Results of the K-test (see Material & Methods) are indicated for each population except for Hossegor (since $S = 1$ and $N_H = 2$), 95% confidence intervals (CI) for the expected $N_{EH}$ value are given together with the observed value ($N_H$). $P$-values (probability that $N_{EH}$ is inferior to the observed value) were computed based on 5000 simulations. For Séné, $P$-value ($P^*$) is the probability that $N_{EH}$ is higher than the observed value.

Fig. 4. Isolation by distance. Genetic distance $\frac{\hat{F}_{ST}}{1 - \hat{F}_{ST}}$ calculated for each FAEC population pair is plotted against geographic distance (D) in kilometres. Black squares represent pairwise values with the population of Hossegor. Regression lines are pictured with a dotted line when all populations are taken into account and a continuous line when Hossegor is removed from the analysis. $P$-values, associated to a Mantel test for a positive correlation between genetic and geographic distances, equals 0.162 and 0.016 with and without Hossegor respectively.
Table 1. Geographical location of the eight study populations. Numbers of individuals analysed (N) and genetic diversity estimators are given (\(N_H\): number of haplotypes, \(S\): number of segregating sites, \(H_e\): haplotypic diversity, \(\pi\): nucleotidic diversity)

<table>
<thead>
<tr>
<th>Population</th>
<th>Latitude</th>
<th>Longitude</th>
<th>N</th>
<th>(N_H)</th>
<th>S</th>
<th>(H_e)</th>
<th>(\pi)</th>
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</thead>
<tbody>
<tr>
<td>Thau (coastal lagoon)</td>
<td>43° 27’ N</td>
<td>3° 38’ E</td>
<td>29</td>
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<td>0</td>
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<td>44°35’ N</td>
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Table 2. Pairwise $F_{ST}$ matrix. The pairwise $F_{ST}$ (below diagonal) and associated $P$-values (above diagonal) are given.

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Figure 1.
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Figure 4.
Appendix: Definition of haplotypes A to V (GenBank accession numbers: AY789970-AY789991) and number of occurrence over the whole dataset (N) are presented. Positions of mutated sites are given. “-” indicates that the same character is present in haplotype A.

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