marine ecology

Marine Ecology. ISSN 0173-9565

ORIGINAL ARTICLE

Evaluating the genetic effects of the invasive *Ocenebra inornata* on the native oyster drill *Ocenebra erinacea*

Eric Pante[1], Pierre-Yves Pascal[2], Vanessa Becquet[1], Amélia Viricel[1], Benoit Simon-Bouhet[1] & Pascale Garcia[1]

1 Littoral, Environnement et Sociétés (LIENSs), UMR 7266 CNRS – Université de La Rochelle, La Rochelle, France
2 Systématique-Adaptation-Evolution, Equipe Biologie de la Mangrove, Laboratoire de Biologie Marine, UMR 7138 CNRS-MNHN-UPMS, Université des Antilles et de la Guyane, Pointe-à-Pitre cedex, Guadeloupe, France

Keywords
Cryptic species; evolutionary ecology; evolutionary response; glacial refugium; haplotype diversity; invasive species; *Ocenebra erinaceus*; *Ocinebrellus inornatus*.

Correspondence
Littoral Eric Pante, Environnement et Sociétés (LIENSs) UMR 7266 CNRS – Université de La Rochelle, 2 rue Olympe de Gouges, 17000 La Rochelle, France.
E-mail: pante.eric@gmail.com

Accepted: 24 June 2014

doi: 10.1111/maec.12208

Abstract

Studies focusing on the effect of invasive species on the genetic diversity of native marine invertebrates remain scant. Here we report diversity among French populations of the intertidal gastropod *Ocenebra erinacea* (Linnaeus, 1758) sampled in the presence and absence of the invasive *Ocenebra inornata* (Recluz, 1851). Between 1999 and 2004, a total of 352 individuals of *O. erinacea* was collected from 15 sites (five of which had the invasive present) and was genotyped at the mitochondrial locus *Cytochrome Oxidase 1* (*cox1*). No statistical difference was observed between polymorphism levels recorded within native populations exposed to the invasive, compared with populations sampled in the absence of *O. inornata*. No sign of native population decline was detected in response to the invader. While significant shifts in native *O. erinacea* population sizes have previously been reported in the literature, genetic effects may take longer to accumulate, or may be undetectable without a larger panel of genetic markers. By contrast, large genetic distances and significant population differentiation were recorded between Atlantic and Mediterranean *O. erinacea* samples, suggesting that these populations have distinct evolutionary histories. Comparison of genetic divergence within the closely related genus *Nucella* suggests that the Atlantic populations of *O. erinacea* and those from Thau Lagoon in the Mediterranean may belong to different species or subspecies.

Introduction

Natural movements of species' ranges (expansions, regressions, displacements) play a considerable role in the evolution of species. Most of the time, these phenomena are progressive and marked by the tempo of geological processes (*e.g.* Hewitt 1996). However, during the last few decades a growing number of species has undergone changes in their natural range owing to both changes at a global scale (*e.g.* Parmesan & Yohe 2003) and artificial transfers as a result of human activities (*e.g.* Carlton 1989; Seebens *et al.* 2013).

The number of biological invasions has greatly increased during the last few decades (*e.g.* Mack *et al.* 2000; Ruiz *et al.* 2000; Mooney & Cleland 2001). In the marine environment, these phenomena are mainly the result of aquaculture, especially shellfish farming, which represents a major cause of introduction, intentional or not, of exogenous species (Elton 1958; Carlton 1992).

Apart from potentially important economic consequences, the arrival of these introduced species can also cause serious ecological impacts on local fauna. Introduced species are likely to decrease the abundance of indigenous species, excluding them from part of their distributional area, or even cause their extinction by modifying invaded habitats, hybridizing with native species, exchanging pathogens, preying upon them or competing with them (*e.g.* Lockwood *et al.* 2007 and references therein).

 © 2014 Blackwell Verlag GmbH

Moreover, when they exert strong selective pressures, introduced species can also reduce the genetic diversity of native populations (Kim *et al.* 2003; Wittmann *et al.* 2013).

Such impacts, although poorly known (*e.g.* Strauss *et al.* 2006), may have heavy ecological consequences as adaptive potential depends on the genetic diversity of a population. Reductions in genetic diversity are generally considered detrimental (*e.g.* Frankham 1995; Lande 1995; Strauss *et al.* 2006) and may contribute to extinction (Wittmann *et al.* 2013). Various authors have shown a link between the fitness of a species and its genetic diversity, particularly in mollusks (Mitton & Grant 1984; Garton & Haag 1991; Zouros 1993; Launey & Hedgecock 2001; Hedgecock *et al.* 2007). In addition, a reduction in genetic variability of an indigenous population could promote the range expansion of other species that are phylogenetically close. However, very few studies have addressed changes in genetic diversity of an indigenous species under the competitive pressure generated by a biological invader.

The oyster drills *Ocenebra erinacea* (Linnaeus, 1758) and *Ocenebra inornata* (Recluz, 1851) constitute a noteworthy model to study the genetic effects of indigenous–invasive interactions on indigenous populations. A native of the Northwestern Pacific (Choe & Park 1997; Amano & Vermeij 1998), *O. inornata* (previously known as *Ocinebrellus inornatus*; see Houart & Sirenko 2003; Bouchet & Houart 2014) has recently invaded European coasts, probably following massive imports of oysters (De Montaudouin & Sauriau 2000; Pigeot *et al.* 2000; see Lützen *et al.* 2012 for review). Genetic data suggest that French populations may come from Asia and the USA (Martel *et al.* 2004a). The introduction of *O. inornata* may have important economical consequences as it is a predator of cultivated mollusks (*e.g.* oysters, blue mussels, Goulletquer *et al.* 2002). It coexists at several French sites with an indigenous muricid, *O. erinacea* (Linnaeus, 1758), which ranges from the straits of Gibraltar to the Netherlands, and inhabits all British and Mediterranean coasts (Graham 1998). Although *O. erinacea* and *O. inornata* differ in some life-history traits (Martel *et al.* 2004c), these muricid gastropods fill similar ecological niches, and may compete for habitat (both species live on hard substrates and drill the shells of bivalves to feed on them; *e.g.* Lützen *et al.* 2012). Pigeot *et al.* (2000) recorded a decrease in population density of *O. erinacea* in parallel to an increase in numbers of *O. inornata* in Marennes-Oléron (Charente-Maritime, France) between 1997 and 1999 (two years after the invasive was first detected). While the introduction and expansion patterns of *O. inornata* have been investigated in previous studies (Martel *et al.* 2004a,b), its ecological impacts on the native *O. erinacea* are poorly known.

In a previous study (Martel 2003), seven allozyme loci were analysed in populations of *O. erinacea* and *O. inornata* collected from seven sites of the French Atlantic coast where the two species live in sympatry. These markers revealed that genetic diversity indices were systematically weaker within the native *O. erinacea* than within the invasive *O. inornata*. This finding was counter-intuitive, as (i) the founder effect linked to an introduction event should lead to low genetic diversity within the populations of the introduced species and (ii) this phenomenon should be all the more marked if the invasion is recent (see Sakai *et al.* 2001 for review). Consequently, lower genetic diversity within the populations of the exogenous species compared with the populations of the indigenous species was expected. It is thus of importance to test whether the genetic diversity of the indigenous species *O. erinacea* is correlated with the presence of the introduced species *O. inornata* in zones of sympatry. Indeed, *O. inornata* may induce a selective pressure on *O. erinacea*, leading to a decrease in levels of polymorphism in this local species.

Here, we tested this hypothesis by sampling *O. erinacea* from the Atlantic and Mediterranean French coasts, in the presence and absence of *O. inornata*, and by measuring genetic diversity of the native species using the mitochondrial marker *cox1*. While investigating the genetic effects that the presence of *O. inornata* may have on sympatric populations of *O. erinacea*, we came across a very strong genetic break between the Atlantic and Mediterranean populations. This break is detailed and potential biogeographic causes are discussed.

Material and Methods

Sampling

A total of 352 adult specimens of *Ocenebra erinacea* was collected between 1999 and 2004 at 15 sites on the French coast, along line transects (<200 m in length). At each site, specimens from different rocks were collected to reduce sampling bias in favor of a particular lineage. The sites were located within both oyster farming zones and unexploited areas (Table 1). In order to show a possible impact of the presence of *Ocenebra inornata* on the genetic diversity of *O. erinacea*, five locations where the two species live in sympatry and 10 sites free of *O. inornata* were sampled. The presence of *O. inornata* was assessed by direct observation. After collection, specimens were stored in 95% ethanol before DNA extraction.

DNA extraction, amplification and sequencing

Total DNA was extracted from <15 mg of foot muscle using a Dneasy™ Tissue Kit according to the manufacturer's protocol (Qiagen, Hilden, Germany). Part of the mitochondrial *cox1* gene was PCR-amplified with the HCO2198/

Table 1. *Ocenebra erinacea* collection sites (listed from north to south, following the coastline), site name abbreviations (ab., as used in Fig. 2), number of *Ocenebra erinacea* specimens collected and sequenced (n), geographic co-ordinates, year of collection and characteristics of the locations: presence (+) or absence (–) of oyster farms and of *Ocenebra inornata*.

Location	ab.	n	Latitude	Longitude	Year of collection	Shellfish area	Presence of *Ocenebra inornata*
Blainville	Bl	31	49°03′ N	1°36′ W	2003	+	–
Chaussey	Ch	25	48°52′ N	1°48′ W	2003	+	–
Saint Malo	SM	22	48°39′ N	2°01′ W	2004	–	–
Saint Quay	SQ	23	48°39′ N	2°50′ W	2004	–	–
Trébeurden	Tr	24	48°48′ N	3°35′ W	2004	–	–
Crozon	Cr	23	48°17′ N	4°27′ W	2004	–	–
Le Croisic	LC	22	47°18′ N	2°31′ W	2004	–	–
Morbihan	Mo	12	47°33′ N	2°51′ W	2004	–	–
Bourgneuf	Bo	24	47°01′ N	2°01′ W	2003	+	+
Loix	Lo	28	46°13′ N	1°24′ W	2003	+	+
Aytré	Ay	20	46°06′ N	1°07′ W	2003	+	+
Fouras	Fo	27	46°00′ N	1°07′ W	2004	+	+
Oléron	Ol	13	45°53′ N	1°10′ W	2004	+	+
Arcachon	Ar	21	44°40′ N	1°12′ W	1999	+	–
Thau	Th	37	43°24′ N	3°35′ W	1999	+	–

LCO1490 primers (Folmer *et al.* 1994), which have proved useful for neogastropod studies (*e.g.* Zou *et al.* 2011, 2012). Polymorphism at *cox1* is high in *Ocenebra inornata*, a phylogenetically close species (Martel *et al.* 2004a).

PCRs were carried out in 50 μl total volume, with 1× PCR buffer, 1.85 mM $MgCl_2$, 125 μM dNTPs, 0.25 μM of each primer, 1.6 U Red Hot DNA Polymerase (ABgene, Epsom, UK) and about 10 ng DNA template. The following cycling profile was performed using a MJResearch (St. Bruno, Canada) PTC 100 Thermal Cycler: initial 5-min denaturation step at 94 °C followed by 40 cycles of 30 s at 94 °C, 30 s at 50 °C and 1 min at 72 °C, and by a final 5-min extension period at 72 °C. PCR products were purified using MultiScreen-PCR MANU03010 plates (Millipore, Molsheim, France).

Sequencing was performed by GenoScreen (Lille, France) using an ABI PRISM 3730 XL Automated DNA Sequencer (Perkin-Elmer Applied Biosystems, Foster City, CA, USA). Sequences were aligned using CLUSTALX (Thompson *et al.* 1997).

Data analyses

Genetic analyses were aimed at (i) quantifying and comparing genetic diversity among populations, (ii) analysing the spatial distribution of polymorphism and genetic exchanges among populations and (iii) studying the evolutionary relationships among populations. Haplotype number (H), number of polymorphic sites (S), haplotype diversity (H_e) and average per site nucleotide diversity (π) (Nei 1987) were calculated for each population using the software DNASP 5.10.1 (Librado & Rozas 2009).

We tested the null hypothesis of the standard neutral model in ARLEQUIN v. 3.5 (Excoffier & Lischer 2010) by calculating the D and Fs statistics, as defined by Tajima (1989) and Fu (1997), respectively. When these statistics are significantly different from zero, populations may have undergone purifying selection, a selective sweep and/or expansion (<0), or balancing selection and/or a population decline (>0). Statistical significance was tested by generating 10,000 random samples under the hypothesis of selective neutrality and population equilibrium. These tests were performed for each sampling site separately, and also for pooled sites in the presence or absence of the invasive.

The differentiation index Φ_{ST} (Excoffier *et al.* 1992), an estimator of F_{ST} (Wright 1951) calculated from frequency values and distances between haplotypes, was computed with ARLEQUIN v. 3.5. The Kimura two-parameter (K2P) model of nucleotide substitution was used to estimate genetic distances, and 10,000 permutations were used to test statistical significance under the null hypothesis of no difference between populations (Excoffier *et al.* 1992).

Finally, a haplotype network was built using the median-joining algorithm implemented in NETWORK 4.6.1.1 (www.fluxus-engineering.com, Bandelt *et al.* 1999). This method is one of the most accurate for inferring intraspecific networks in the absence of recombination (Woolley *et al.* 2008).

To help interpret the large genetic divergence observed between Atlantic and Mediterranean specimens of *Ocenebra erinacea*, we looked for mitochondrial *cox1* data in the BOLD database (Ratnasingham & Hebert 2007).

Table 2. Molecular diversity of populations and results of the neutrality tests. The number of segregating sites (S), the number of haplotypes (h), the haplotype diversity ($H_e \pm 1$ SD) and the nucleotide diversity ($\pi \pm 1$ SD) are given for each sampling site, site groups in the presence and in the absence of the invasive (with and without the Mediterranean population of Thau), and for the entire data set. Sites where *Ocenebra inornata* was present are indicated by.[a] For the neutrality tests of Tajima and Fu, statistical significance after sequential Bonferroni correction is indicated by an asterisk.

Sampling sites	S	H	$H_e \pm$ SD	$\pi \pm$ SD ($\times 10^{-3}$)	Tajima's *D*	Fu's *Fs*
Blainville	1	2	0.065 ± 0.059	0.12 ± 0.28	−1.14	−1.24*
Chaussey	3	4	0.230 ± 0.110	0.44 ± 0.58	−1.73	−3.08*
Saint Malo	1	2	0.091 ± 0.081	0.17 ± 0.34	−1.16	−0.96
Saint Quay	0	1	0	0	0	0
Trébeurden	5	5	0.377 ± 0.122	0.90 ± 0.89	−1.83	−2.80*
Crozon	2	3	0.316 ± 0.118	0.60 ± 0.70	−0.86	−0.87
Le Croisic	4	4	0.333 ± 0.124	0.81 ± 0.84	−1.67	−1.74
Morbihan	0	1	0	0	0	0
Bourgneuf[a]	2	2	0.083 ± 0.075	0.30 ± 0.47	−1.51	−0.19
Loix[a]	5	5	0.270 ± 0.109	0.65 ± 0.72	−2.01*	−3.57*
Aytré[a]	4	4	0.363 ± 0.131	0.89 ± 0.89	−1.64	−1.61
Fouras[a]	1	2	0.074 ± 0.067	0.14 ± 0.30	−1.15	−1.12
Oléron[a]	2	3	0.564 ± 0.112	1.12 ± 1.06	−0.13	−0.17
Arcachon	1	2	0.095 ± 0.084	0.17 ± 0.35	−1.16	−0.92
Thau	22	6	0.342 ± 0.098	2.52 ± 1.76	−2.50*	−0.42
Sites in presence of invasive	11	9	0.247 ± 0.0535	0.57 ± 0.65	−2.10*	−9.42*
Sites in absence of invasive	31	22	0.399 ± 0.3976	10.04 ± 5.52	0.22	−0.06
Sites in absence of invasive (without Thau)	16	16	0.179 ± 0.0368	0.38 ± 0. 51	−2.38*	<−10*
All populations	37	29	0.356 ± 0.0325	7.63 ± 4.20	−0.81	−4.97

However, besides three other BOLD *cox1* sequences from Spanish specimens of *O. erinacea*, we produced the only available mitochondrial sequences for the genus *Ocenebra*. We therefore used *cox1* sequences from six species of the closely related genus *Nucella* Röding 1798 (*e.g.* Pascal 2004) to measure intra-specific and inter-specific genetic distances. *Nucella* and *Ocenebra* are both characterized by a non-planktonic larval development and lay egg capsules on hard substrates (Martel *et al.* 2004c; review by Krug 2011). We used the K2P model of nucleotide substitution (Kimura 1980), widely used in DNA barcoding (Hebert *et al.* 2003; Barrett & Hebert 2005), to measure genetic distances among *cox1* haplotypes.

Results

A 550-bp fragment of *cox1* was sequenced for 352 individuals, and 29 haplotypes were identified (GenBank accession numbers AY995771–AY995799; Popset 63109090). Sequences included 37 polymorphic sites, 20 of which were parsimony informative and one of which had three character states. No indels were observed (Table 2).

Population-level genetic diversity and demographic stability

Genetic diversity was comparable among the Atlantic populations of *Ocenebra erinacea* studied here, but values for the different diversity indices are low compared with other recently studied marine mollusks. The number of polymorphic sites between two different sequences varied between zero and six among the Atlantic populations. Two of these populations (Morbihan and St Quay) are each characterized by a single haplotype, and the 12 other Atlantic sites have no more than five haplotypes (sample sizes provided in Table 1). Consequently, haplotype diversity H_e is low, except for Oléron ($H_e = 0.564$; Table 2). In Fouras, where *Ocenebra inornata* and *O. erinacea* are sympatric, the haplotype and nucleotide diversities are respectively four- and sixfold higher in the invasive drill ($H_e = 0.348$ and $\pi \times 10^{-3} = 0.83$; data from Martel 2003) than in the local one ($H_e = 0.074$ and $\pi \times 10^{-3} = 0.14$).

A single Mediterranean population (Thau) was sampled. The haplotype diversity ($H_e = 0.342$) and the nucleotide diversity ($\pi \times 10^{-3} = 2.52$) are respectively two- and fivefold higher than all of the Atlantic populations combined (Table 2). Moreover, 22 polymorphic sites were found among 37 individuals sampled in Thau, a value considerably higher than the 24 polymorphic sites observed among the 315 Atlantic individuals.

Lastly, haplotype and nucleotide diversities of *O. erinacea* populations co-occurring with *O. inornata* were lower ($H_e = 0.247 \pm 0.0535$ and $\pi \times 10^{-3} = 0.57 \pm 0.65$) than for populations located in zones where *O. inornata* was not detected ($H_e = 0.399 \pm 0.3976$, $\pi \times 10^{-3} = 10.04 \pm 5.52$) (Table 2). However, this pattern is entirely the result of the higher diversity

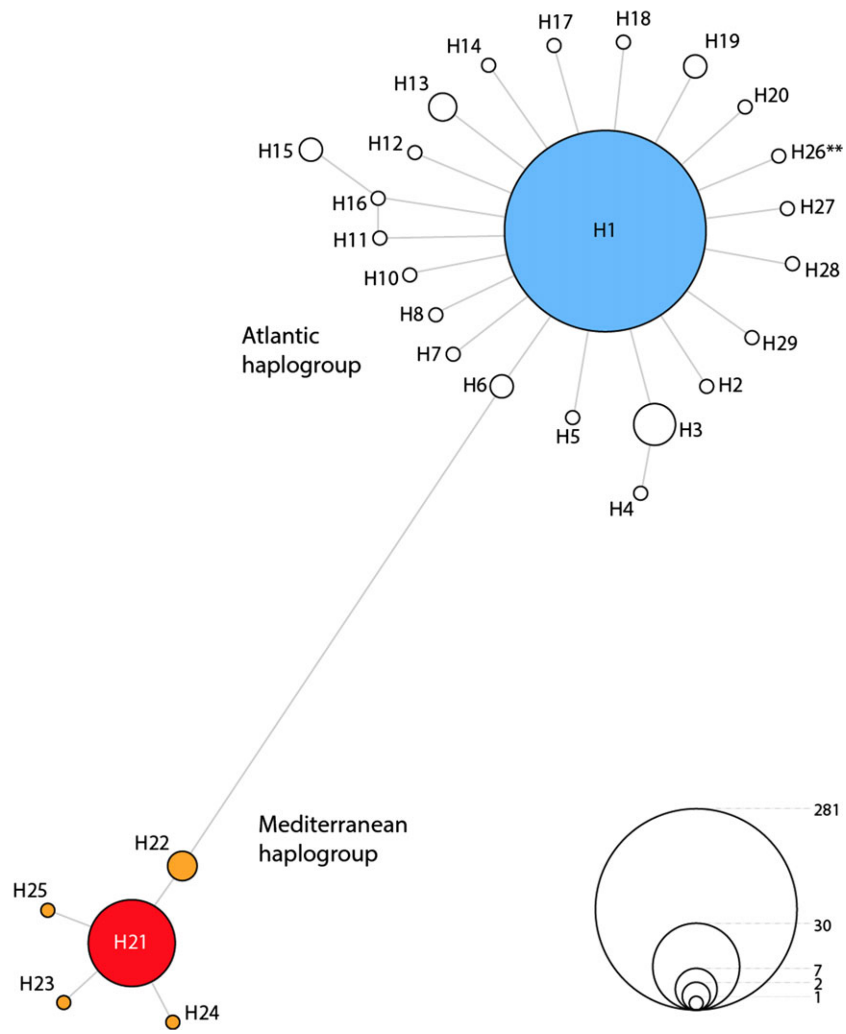

Fig. 1. Median-joining haplotype network. Each circle represents a haplotype, the frequency of which is proportional to circle diameter (legend: bottom right). Distances between haplotypes are proportional to the number of mutational events (see text). The Atlantic haplogroup contains one Mediterranean specimen, represented by haplotype H26 (marked with **).

encountered at Thau; when this site was removed from the group of populations that were not found in contact with *O. inornata*, diversity values dropped significantly ($H_e = 0.179 \pm 0.0368$, $\pi \times 10^{-3} = 0.38 \pm 0.51$). Comparing molecular diversity at the site level revealed the same pattern (Welch two-sample *t*-test, including Thau, for H_e: $t = -0.83$, df = 6.22, P = 0.44; for π: $t = -0.16$, df = 12.81, P = 0.88. In both cases, results were also non-significant when the Thau population was removed).

Except for one case, the *D* and *F*s statistics were never positive (Table 2). Furthermore, the only slightly positive *D* value (deviation from zero non-significant) was observed when sites where the invasive was absent were pooled, including Thau, and this result was therefore likely influenced by the underlying population structure (see Genetic differentiation among populations section below). There is therefore no supporting evidence that *O. erinacea* populations exposed to *O. inornata* suffered a population decline. Some sites exhibited significant negative values of *D* and *F*s, which can be interpreted as signs of purifying selection, selective sweep and/or population expansion. Particularly, the pooled Atlantic sites showed significantly negative values for both tests, regardless of whether the invasive was present or not. These molecular signatures must, however, be interpreted with care, as they may reflect older demographic events.

Genealogical relationships and spatial distribution of haplotypes

Two haplogroups, separated by 18 mutational steps, were observed using the median-joining network (Fig. 1). The first haplogroup (23 haplotypes) was mainly composed of Atlantic specimens, and included one specimen from Thau, characterized by haplotype H26 (separated from other Mediterranean haplotypes by 20–22 mutational steps). Haplotypes from this group diverged by ≤2 mutations. Haplotype H1 was common (represented in 89% of Atlantic individuals) and central to the Atlantic haplogroup, while the other 22 haplotypes were rare (seven individuals for H3, three for H13, two for H6, H9, H15, H19 and a single individual for the others) and peripheral to H1. The second haplogroup was strictly composed of Mediterranean individuals. Of six haplotypes from this group, one was common (H21, represented in 82% of Thau individuals) and four were rare (three individuals for H22 and a single individual for H23–25). Haplotypes from this group diverged by one mutation. The Atlantic samples share no haplotypes with the Mediterranean sample (Fig. 2). Except for H1 (present at all sites except Thau), H3 (shared between Loix and Aytré) and H13 (shared among Loix, Aytré and Trébeurden), all haplotypes are private (observed only within one population).

Genetic differentiation among populations

Genetic differentiation among pairs of populations was measured using Φ_{ST}. The population from Thau was significantly differentiated from all other populations. Pairwise Φ_{ST} values ranged between 0.94 and 0.96, corresponding to substantial genetic differentiation between the Mediterranean and Atlantic populations

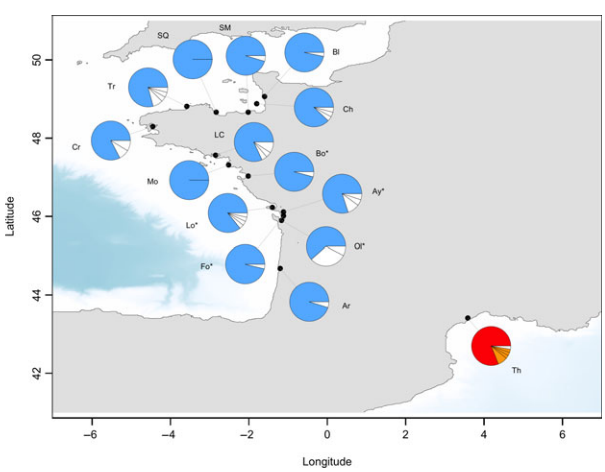

Fig. 2. Distribution of haplotype frequencies along the French coast. Abbreviations for site names are detailed in Table 1. Haplotype colors correspond to the colors used in Fig. 1. The stations where both *Ocenebra erinacea* and *Ocenebra inornata* were observed are marked with an asterisk. Map constructed with R package marmap (Pante & Simon-Bouhet 2013).

(Table 3). Inside the Atlantic group, no significant differentiation was observed after sequential Bonferroni correction (lowest corrected alpha level for Atlantic populations: 0.00055).

Levels of intra-specific divergence in *Ocenebra* compared with *Nucella*

Given the differences in haplotype composition and divergence between Mediterranean and Atlantic sites, we investigated whether the genetic distances correspond to intra- or inter-specific divergence by comparing *Ocenebra* with its close relative *Nucella*. The pairwise K2P distance between *Ocenebra erinacea* haplotypes ranged between 0.18% and 4.54% (maximum observed between haplotypes 20 from Loire and 25 from Thau Lagoon). Within *Nucella*, K2P was calculated for 532 sequences and 117 haplotypes distributed among six species, along a 434-bp stretch of *cox1*. Intra-specific distances ranged from 0% to 3.32%, while inter-specific distance ranged from 4.81% to 12.2%, for specimens distributed over 1000–2000 km (east and west coasts of North America, respectively; BOLD database). For comparison, Zou *et al.* (2011), analysing 108 neogastropod *cox1* sequences (same gene region as analysed here; not including *Ocenebra* or *Nucella*) found maximum intra-specific K2P distances of 2.2% and minimum inter-specific distances of 2.1%.

Discussion

Relationship between *Ocenebra erinacea* and the invasive species *Ocenebra inornata*

While a decrease in genetic diversity in response to invaders has been reported in the past (Kim *et al.* 2003), we did not detect such a pattern among *Ocenebra erinacea* exposed to the invasive species *Ocenebra inornata*. The relatively low polymorphism levels recorded may have hampered our ability to detect genetic effects of the invasive on the native species, and the use of additional molecular markers such as nuclear microsatellites or single nucleotide polymorphisms might further help detect possible demographic events associated with the presence of *O. inornata*. However, this remains to be tested, as even genome-wide scans can fail at detecting recent demographic events and selective pressures. Riquet *et al.* (2013), for instance, used amplified fragment length polymorphisms to compare native and invasive populations of the marine mollusk *Crepidula fornicata*. They reported little genetic differentiation among these populations, and detected no F_{ST} outliers out of 344 tested loci. An alternative hypothesis explaining the apparent absence of genetic effects of the invasive on the native is that the

Table 3. Pairwise Φ_{ST} values calculated using the Kimura two-parameter model of nucleotide substitution. Only pairwise comparisons involving the population of Thau were statistically significant after sequential Bonferroni correction.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. Blainville	0														
2. Chausey	0.0048	0													
3. Saint Malo	0.00242	−0.00244	0												
4. Saint Quay	−0.01001	−0.00356	0.00207	0											
5. Trebeurden	0.02194	0.01045	0.00921	0.01207	0										
6. Crozon	0.05037	0.02772	0.03452	0.04529	0.02682	0									
7. Le Croisic	0.03001	0.01442	0.01577	0.02118	0.01654	0.01135	0								
8. Morbihan	−0.03708	−0.03371	−0.03067	0	−0.02156	0.00476	−0.01543	0							
9. Bourgneuf	0.00458	−0.00021	−0.00102	−0.00181	0.01083	0.0316	0.01598	−0.03274	0						
10. Loix	0.00242	−0.0008	−0.00559	−0.00747	0.00258	0.02217	0.012	−0.0358	−0.01337	0					
11. Aytré	0.03811	0.01838	0.02156	0.0293	0.00956	0.03281	0.02045	−0.01049	−0.00555	−0.01096	0				
12. Oleron	0.28743	0.18725	0.23275	0.28709	0.1342	0.16138	0.13706	0.18898	0.15611	0.12163	0.04004	0			
13. Fouras	0.0004	0.00162	0.00096	−0.00611	0.01656	0.04351	0.02394	−0.03502	0.00196	−0.00096	0.03104	0.26425	0		
14. Arcachon	0.0032	−0.00323	0.00014	0.00443	0.00763	0.03266	0.01404	−0.02941	−0.00153	−0.00658	0.01955	0.22606	0.00145	0	
15. Thau	0.96208	0.95559	0.95617	0.95856	0.94988	0.95236	0.95001	0.94928	0.95614	0.9547	0.9484	0.94167	0.95959	0.95541	0

Φ_{ST}, The differentiation index.

competitive and selective pressures inflicted on *O. erinacea* are too low to have genetic effects (*e.g.* Wittmann *et al.* 2013). Finally, deviations from neutrality observed for the pooled Atlantic sites suggest that selection may have shaped the current genetic diversity and blurred the signatures of demographic processes.

Ocenebra inornata was first documented on the French Atlantic coast in 1995 (De Montaudouin & Sauriau 2000; Pigeot *et al.* 2000), and the specimens of *O. erinacea* used in this study were collected between 1999 and 2004. The introduction of *O. inornata* may have been too recent at the time of sampling for genetic consequences on the native species to be detectable. About 10 years later, the distributional landscape of *O. inornata* on Atlantic coasts has significantly changed, and the invasive is now found as far north as the entrance of the Baltic Sea (Lützen *et al.* 2012). A new survey of the genetic diversity of *O. erinacea* might today uncover the genetic consequences of the invasion by *O. inornata*, and this study therefore provides a snapshot in time that may help better understand the temporal dynamics of loss of genetic diversity. In addition to sampling in the field, we searched for *Ocenebra* specimens in the collections of the Museum national d'Histoire naturelle in Paris (France), in order to look for genetic diversity in *O. erinacea* specimens collected prior to, or soon after, the invasion by *O. inornata* (MNHN voucher numbers IM-2008-7101, IM-2008-7102, IM-2008-7103). Unfortunately, we were not able to amplify the *cox1* marker from these specimens.

Remarkably low genetic diversity of *Ocenebra erinacea* populations

Mitochondrial genetic diversity, as measured using part of *cox1*, was low relative to what has been observed previously in other marine mollusks. Overall, *Ocenebra erinacea* haplotype and nucleotide diversities were (disregarding the sample from Thau, see below) $H_e = 0.18$–0.25 and $\pi \times 10^{-3} = 0.38$–$0.57$ (Table 2). Comparatively, $H_e = 0.684$ and $\pi \times 10^{-3} = 2.25$ for *Ocenebra inornata* in its natural range (data from Martel *et al.* 2004a), $H_e = 0.734$ and $\pi \times 10^{-3} = 14.78$ in the gastropod *Cyclope neritea* (Simon-Bouhet *et al.* 2006), and $H_e = 0.720$ and $\pi \times 10^{-3} = 89.84$ in the bivalve *Macoma balthica* (Becquet *et al.* 2012).

The low genetic diversity observed at *cox1* was consistent with the low diversity observed using allozymes: Martel (2003) reported that the number of alleles (N_{all}) and the observed heterozygosity (H_o) characterizing the Atlantic populations of *O. erinacea* are respectively two to four times, and 20–30 times lower ($N_{all} = 1.1 \pm 0.1$; $H_o = 0.01 \pm 0.01$; mean $\pm$ SD) than in other marine gastropods sampled in their native range, such as *Bedeva hanleyi* (N_{all} = 2.2 ± 0.1, H_o = 0.30 ± 0.02; Hoskin 2000), *Drupella* sp. (N_{all} = 2.3 ± 1.0, H_o = 0.25; Johnson & Cumming 1995) and *Littorina striata* (N_{all} = 4.2 ± 1.0, H_o = 0.18 ± 0.17; De Wolf *et al.* 2000). Congruent patterns across mitochondrial and allozyme markers thus suggest low genetic diversity in these populations rather than an absence of variability at *cox1*. Still, our sampling remains restricted compared with the native range of the species, and additional monitoring may reveal new patterns of genetic diversity.

Genetic diversity and connectivity among populations

The genetic diversity of the Mediterranean population was among the highest (Table 2). The oyster farmers of the Thau Lagoon, one of the main shell fish farming areas of the French Mediterranean coasts, carry out commercial exchanges with distant production sites, and *Ocenebra erinacea* is likely to be transferred during these exchanges. In fact, the morphological survey carried out by Berrou *et al.* (2004) evidenced exchanges from Oléron Island to Thau Lagoon (this is congruent with the Atlantic haplotype H26 being observed at Thau; Figs 1 and 2). In our study, the high genetic diversity observed in Thau Lagoon could be the result of the introduction of Mediterranean specimens imported from other production sites such as Oléron. As no Mediterranean site was sampled other than Thau Lagoon, the artificial mixing induced by shellfish exchanges cannot be further evaluated here.

Alternatively, the difference in genetic diversity observed between the Atlantic and the Mediterranean population of Thau Lagoon might be explained by historical and biogeographic factors, and/or selection. *Ocenebra erinacea* is not well adapted to cold water and Belgium currently constitutes the northern limit of its natural range (Graham 1998). Consequently, the species may have found, as have other marine species (*e.g.* Nikula & Väinölä 2003; Ladhar-Chaabouni *et al.* 2010), a refugium on the Iberian coast or in the Mediterranean basin during past glaciations. *Ocenebra erinacea* may have disappeared from the French Atlantic coasts during the Würm Glacial period (115,000 to 10,000 years BP) but survived on the coasts of the Iberian peninsula, which is known as one of the major Pleistocene refugia (Gómez & Lunt 2007). At the end of this climatic crisis, a reduced number of individuals from southern refuges may have reached northern coasts. Maggs *et al.* (2008), reviewing molecular signatures of glacial refugia on marine species, made predictions of low genetic and haplotype diversity in northern regions previously covered by ice sheets, and comparatively high diversity in refugial southern regions (and see Hewitt 1996). These predictions are generally met for *O. erinacea*, but additional sampling from the Iberian peninsula and the Mediterranean Sea would be necessary to further characterize the historical biogeography of this species. Given *O. erinacea*'s maladaptation to cold water, another possibility is that selection (either purifying selection or selective sweeps) linked to differences in water temperature between Thau and the Atlantic sites produced the observed patterns of genetic diversity. The negative Tajima's *D* and Fu's *F*s observed for the Atlantic population (in the absence and presence of the invasive) would support this scenario.

One potential consequence of biogeographic divergence between Atlantic and Mediterranean populations is the emergence of new species (Hewitt 1996, 2004). Recently, Salicini *et al.* (2013) have shown that in the bat *Myotis naterreri*, a complex of four cryptic species exists in the Western Palearctic region (Central and Southern Europe, Northwestern Maghreb), each species coinciding with a glacial refugium. In *O. erinacea*, inter-clade divergence overlaps with the inter-specific distances observed in the closely related genus *Nucella* (although the geographic distances separating *Nucella* specimens were greater than the distances separating *O. erinacea* specimens; see Bergsten *et al.* 2012). In addition, *O. erinacea* specimens from the Atlantic and Mediterranean can readily be distinguished using morphology, and the morphological distance between Atlantic and Mediterranean *O. erinacea* is comparable to what is observed between *O. erinacea* and *Ocenebra brevirobusta* Houart 2000 (Berrou *et al.* 2004). It is therefore possible that the Atlantic and Mediterranean clades sampled for this study belong to groups in incipient stages of speciation, or even undescribed species.

Acknowledgements

We thank Stéphane Robert (IFREMER, La Tremblade, France), Yvonne Pascal, Michel Pascal (INRA Rennes, France), François Siorat (LPO), Armel Deniau (LPO), Marie-Gabrielle Vitre, Paul Wendling, Francoise Dreau, Christian Bussière, Isabelle Kuhne, Jean-Paul Egreteau, José Simone Guézou, Sandrine Sourisseau, Xavier de Montaudouin (University of Bordeaux, France) and Pierre-Guy Sauriau (IFREMER, L'Houmeau, France) for their help with field sampling, Jacques Pigeot for his insights and help with identifications, Stéphane Robert (IFREMER, La Tremblade, France), Philippe Goulletquer (IFREMER, La Tremblade, France) and Yves Pichot (IFREMER, Sète, France) for information on shellfish transfers in France, Pierre Lozouet at the Muséum national d'Histoire naturelle (Paris, France) for granting access to the *Ocenebra* collection and Alice Saunier for her help in the lab. This work was funded by the Conseil Général de

Charente-Maritime and the Ministère de l'Ecologie et du Développement Durable (Program INVABIO no. D4E/SRP/01115). Salaries for E.P. and A.V. were covered by a grant to the Poitou-Charentes region (Contrat de Projet État-Région 2007–2013), by a grant from the Fond Européen de Développement Régional (EP), and by Actions Internationales et Rayonnement of the University of La Rochelle (AV). We thank the three anonymous reviewers and the editor, whose comments significantly improved the manuscript. This work is dedicated to the memory of Michel Pascal.

References

Amano K., Vermeij G.J. (1998) Taxonomy and evolution of the genus *Ocinebrellus* (Gastropoda: Muricidae) in Japan. *Paleontological Research*, **2**, 199–212.

Bandelt H., Forster P., Rohl A. (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, **16**, 37–48.

Barrett R., Hebert P.D.N. (2005) Identifying spiders through DNA barcodes. *Canadian Journal of Zoology*, **83**, 481–491.

Becquet V., Simon-Bouhet B., Pante E., Hummel H., Garcia P. (2012) Glacial refugium *versus* range limit: conservation genetics of *Macoma balthica*, a key species in the Bay of Biscay (France). *Journal of Experimental Marine Biology and Ecology*, **432–433**, 73–82.

Bergsten J., Bilton D.T., Fujisawa T., Elliott M., Monaghan M.T., Balke M., Hendrich L., Geijer J., Herrmann J., Foster G.N., Ribera I., Nilsson A.N., Barraclough T.G., Vogler A.P. (2012) The effect of geographical scale of sampling on DNA barcoding. *Systematic Biology*, **61**, 851–869.

Berrou V., Merle D., Dommergues J.L., Crônier C., Néraudeau D. (2004) Comparative morphology of Pliocene, Quaternary and Recent shells of *Ocenebra erinaceus* (Linnaeus, 1758) and *O. brevirobusta* Houart, 2000 (Mollusca, Muricidae, Ocenebrinae): reflections on the intra- and interspecific variations. *Geodiversitas*, **26**, 263–295.

Bouchet P., Houart R. (2014). *Ocenebra inornata* (Récluz, 1851). Accessed through: World Register of Marine Species at http://www.marinespecies.org/aphia.php?p=taxdetails&id=403745 on 2014-05-02.

Carlton J.T. (1989) Man's role in changing the face of the ocean: biological invasions and implications for conservation of near shore environments. *Conservation Biology*, **3**, 265–273.

Carlton J.T. (1992) Introduced marine and estuarine mollusks of North America: an end-of-the-20th-century perspective. *Journal of Shellfish Research*, **11**, 489–505.

Choe B.L., Park J.K. (1997) Description of Muricid species (Gastropoda: Neogastropoda) collected from the coastal areas of South Korea. *Journal of Biological Science*, **1**, 281–296.

De Montaudouin X., Sauriau P.-G. (2000) Contribution to a synopsis of marine species richness in the Pertuis Charentais Sea with new insights in soft-bottom macrofauna of the Marennes-Oléron Bay. *Cahiers de Biologie Marine*, **41**, 188–222.

De Wolf H., Verhagen R., Backeljau T. (2000) Large scale population structure and gene flow in the planktonic developing periwinkle *Littorina striata* (Mollusca: Gastropoda), in Macronésia. *Journal of Experimental Marine Biology and Ecology*, **246**, 69–83.

Elton C.S. (1958) *The Ecology of Invasions by Animals and Plants*. Methuen and Co. Ltd., London: 196 pp.

Excoffier L., Lischer H.E.L. (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, **10**, 564–567.

Excoffier L., Smouse P.E., Quattro J.M. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Folmer O., Black M., Hoeh W., Lutz R., Vrijenhoek R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**, 294–299.

Frankham R. (1995) Conservation genetics. *Annual Review of Genetics*, **29**, 305–327.

Fu Y.-X. (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, **147**, 915–925.

Garton D.W., Haag W.R. (1991) Heterozygosity, shell length and metabolism in the European mussel, *Dreissena polymorpha*, from a recently established population in lake Erie. *Comparative Biochemistry and Physiology*, **99**, 45–48.

Gómez A., Lunt D.H. (2007) Refugia within Refugia: patterns of Phylogeographic Concordance in the Iberian Peninsula. In: Weiss S., Ferrand N. (Eds), *Phylogeography of Southern European Refugia*, volume **III**. Springer, Netherlands: 155–188.

Goulletquer P., Bachelet G., Sauriau P.G., Noel P. (2002) Open Atlantic coast of Europe - a century of introduced species into French waters. In: Leppäkoski E., Gollash S., Olenin S. (Eds), *Invasive Species of Europe – Distribution, Impact and Management*. Kluwer Academic Publishers, Dordrecht/Boston/London: 276–290.

Graham A. (1988) In: Brill E.J., Backhuys W. (Eds), *Molluscs: Prosobranch and Pyramidellid Gastropods: Keys and Notes for the Identification of the Species*, 2nd edn. Synopses of the British fauna (new series), Vol. 2. Leiden/New York, UK: 662 pp. ISBN 90-04-08771-0. vii.

Hebert P.D.N., Cywinska A., Ball S.L., deWaard J.R. (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **270**, 313–321.

Hedgecock D., Jing-Zhong L., DeCola S., Haudenschild C.D., Meyer E., Manahan D.T., Bowen B. (2007) Transcriptomic analysis of growth heterosis in larval Pacific oysters (*Crassostrea gigas*). *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 2313–2318.

Hewitt G.W. (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society*, **58**, 247–276.

Hewitt G.M. (2004) Genetic consequences of climatic oscillations in the quaternary. *Philosophical transactions of the Royal Society of London Series B, Biological Sciences*, **359**, 183–195.

Hoskin M.G. (2000) Effects of the east Australian current on the genetic structure of a direct developing muricid snail (*Bedeva hanleyi*, Angas): variability within and among local populations. *Biological Journal of the Linnean Society*, **69**, 245–262.

Houart R., Sirenko B.I. (2003) Review of the Recent species of *Ocenebra* Gray, 1847 and *Ocinebrellus* Jousseaume, 1880 in the Northwestern Pacific. *Ruthenica*, **13**, 53–74.

Johnson M.S., Cumming R.L. (1995) Genetic distinctness of three widespread and morphologically variable species of *Drupella* (Gastropoda: Muricidae). *Coral Reefs*, **14**, 71–78.

Kim M.S., Brunsfeld S.J., McDonald G.I., Klopfenstein N.B. (2003) Effect of white pine blister rust (*Cronartium ribicola*) and rust-resistance breeding on genetic variation in western white pine (*Pinus monticola*). *Theoretical and Applied Genetics*, **106**, 1004–1010.

Kimura M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide-sequences. *Journal of Molecular Evolution*, **16**, 111–120.

Krug P. (2011) Patterns of speciation in marine gastropods: a review of the phylogenetic evidence for localized radiations in the sea. *American Malacological Bulletin*, **29**, 169–186.

Ladhar-Chaabouni R., Hamza-Chaffai1 A., Hardivillier Y., Chénais B., Denis F. (2010) A pilot study of genetic differentiation between two phenotypes of a Mediterranean population of the bivalve *Cerastoderma glaucum* and genetic discrimination with other *Cerastoderma glaucum* and *Cerastoderma edule* populations outside the Mediterranean. *Marine Ecology*, **31**, 355–363.

Lande R. (1995) Mutation and conservation. *Conservation Biology*, **9**, 782–791.

Launey S., Hedgecock D. (2001) High genetic load in the Pacific oyster *Crassostrea gigas*. *Genetics*, **159**, 255–265.

Librado P., Rozas J. (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, **25**, 1451–1452.

Lockwood J.L., Hoopes M.F., Marchetti M.P. (2007) *Invasion Ecology*. Blackwell Publishing, Oxford: 304.

Lützen J., Faasse M., Gittenberger A., Glenner H., Hoffmann E. (2012) The Japanese oyster drill *Ocinebrellus inornatus* (Récluz, 1851) (Mollusca, Gastropoda, Muricidae), introduced to the Limfjord, Denmark. *Aquatic Invasions*, **7**, 181–191.

Mack R.N., Simberloff D., Lonsdale W.M., Evans H., Clout M., Bazzaz F.A. (2000) Biotic invasions: causes, epidemiology, global consequences and control. *Ecological Application*, **10**, 690–710.

Maggs C.A., Castilho R., *et al.* (2008) Evaluating signatures of glacial refugia for North Atlantic benthic marine taxa. *Ecology*, **89**, S108–S122.

Martel C. (2003) Invasions biologiques et perturbations anthropiques des écosystèmes littoraux: source, profil d'expansion et impacts d'un gastéropode marin, *Ocinebrellus inornatus*, introduit accidentellement sur les côtes atlantiques françaises. Doctoral thesis, Université de La Rochelle: 122 pp and annexes.

Martel C., Viard F., Bourguet D., Garcia-Meunier P. (2004a) Invasion by the marine gastropod *Ocinebrellus inornatus* in France. I. Scenario for the source of introduction. *Journal of Experimental Marine Biology and Ecology*, **305**, 155–170.

Martel C., Viard F., Bourguet D., Garcia-Meunier P. (2004b) Invasion by the marine gastropod *Ocinebrellus inornatus* in France. II. Expansion along the Atlantic coast. *Marine Ecology Progress Series*, **273**, 163–172.

Martel C., Guarini J.M., Blanchard G., Sauriau P.G., Trichet C., Robert S., Garcia-Meunier P. (2004c) Invasion by the marine gastropod *Ocinebrellus inornatus* in France. III. Comparison of biological traits with the resident species *Ocenebra erinacea*. *Marine Biology*, **146**, 93–102.

Mitton J.B., Grant M.C. (1984) Associations among protein heterozygosity, growth rate and developmental homeostasis. *Annual review of Ecology and Systematics*, **15**, 479–499.

Mooney H.A., Cleland E.E. (2001) The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 5446–5451.

Nei M. (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York: 512.

Nikula R., Väinölä R. (2003) Phylogeography of *Cerastoderma glaucum* (Bivalvia: Cardiidae) across Europe: a major break in the Eastern Mediterranean. *Marine Biology*, **143**, 339–350.

Pante E., Simon-Bouhet B. (2013) marmap: a package for importing, plotting and analyzing bathymetric and topographic data in R. *PLoS One*, **8**, e73051.

Parmesan C., Yohe G. (2003) A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, **421**, 37–42.

Pascal P.-Y. (2004) Rôle des espèces autochtones dans les processus d'invasions biologiques marines: l'exemple du perceur invasif *Ocinebrellus inornatus* et du perceur autochtone *Ocenebra erinacea* sur les côtes françaises. Masters thesis, Université de La Rochelle, 47 pp.

Pigeot J., Miramand P., Garcia-Meunier P., Guyot T., Séguignes M. (2000) Présence d'un nouveau prédateur de l'huître creuse, *Ocinebrellus inornatus* (Récluz, 1851), dans le bassin conchylicole de Marennes-Oléron. *Comptes-rendus de l'Académie des Sciences de Paris*, **323**, 697–703.

Ratnasingham S., Hebert P.D.N. (2007) BOLD: the Barcode of Life Data System BOLD: the Barcode of Life Data System. *Molecular Ecology Notes*, **7**, 355–364. http://www.barcodinglife.org.

Riquet F., Daguin-Thiébaut C., Ballenghien M., Bierne N., Viard F. (2013) Contrasting patterns of genome-wide polymorphism in the native and invasive range of the marine mollusc *Crepidula fornicata*. *Molecular Ecology*, **7**, 355–364.

Ruiz G.M., Rawlings T.K., Dobbs F.C., Drake L.A., Mullady T., Huq A., Colwell R.R. (2000) Global spread of microorganisms by ships: ballast water discharged from vessels harbours a cocktail of potential pathogens. *Nature*, **408**, 49–50.

Sakai A.K., Allendorf F.W., Holt J.S., Lodge D.M., Molofsky J., With K.A., Baughman S., Cabin R.J., Cohen J.E., Ellstrand N.C., McCauley D.E., O'Neil P., Parker I.M., Thompson J.N., Weller S.G. (2001) The population biology of invasive species. *Annual Review of Ecology and Systematics*, **32**, 305–332.

Salicini I., Ibáñez C., Juste J. (2013) Deep differentiation between and within Mediterranean glacial refugia in a flying mammal, the *Myotis nattereri* bat complex. *Journal of Biogeography*, **40**, 1182–1193.

Seebens H., Gastner M.T., Blasius B. (2013) The risk of marine bioinvasion caused by global shipping. *Ecology Letters*, **6**, 782–790.

Simon-Bouhet B., Garcia-Meunier P., Viard F. (2006) Multiple introductions promote range expansion of the mollusc *Cyclope neritea* (Nassariidae) in France: evidence from mitochondrial sequence data. *Molecular Ecology*, **15**, 1699–1711.

Strauss S.Y., Lau J.A., Carroll S.P. (2006) Evolutionary responses of natives to introduced species: what do introductions tell us about natural communities? *Ecology Letters*, **9**, 357–374.

Tajima F. (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, **123**, 585–595.

Thompson J.D., Gibson T.J., Plewniak F., Jeanmougin F., Higgins D.G. (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acid Research*, **24**, 4876–4882.

Wittmann M.J., Hutzenthaler M., Gabriel W., Metzler D (2013) Ecological and genetic effects of introduced species on their native competitors. *Theoretical Population Biology*, **84**, 25–35.

Woolley S.M., Posada D., Crandall K.A. (2008) A comparison of phylogenetic network methods using computer simulation. *PLoS One*, **3**, e1913.

Wright S. (1951) The genetical structure of populations. *Annals of Human Genetics*, **15**, 323–354.

Zou S., Li Q., Kong L., Yu H., Zheng X. (2011) Comparing the usefulness of distance, monophyly and character-based DNA barcoding methods in species identification: a case study of Neogastropoda. *PLoS One*, **6**, e26619.

Zou S., Li Q., Kong L. (2012) Multigene barcoding and phylogeny of geographically widespread muricids (Gastropoda: Neogastropoda) along the coast of China. *Marine Biotechnology*, **14**, 21–34.

Zouros E. (1993) Associative overdominance evaluating the effects of inbreeding and linkage disequilibrium. *Genetica*, **89**, 35–46.