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A global invader at home: population structure of the green crab, *Carcinus maenas*, in Europe

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Abstract

The European green crab, *Carcinus maenas*, has a native distribution that extends from Norway to Mauritania. It has attracted attention because of its recent invasions of Australia, Tasmania, South Africa, Japan and both coasts of North America. To examine the population structure of this global invader in its native range, we analysed a 502-base-pair fragment of the mitochondrial cytochrome *c* oxidase I (COI) gene from 217 crabs collected in the North Atlantic and 13 specimens from the Mediterranean. A clear genetic break (11% sequence divergence) occurs between the Mediterranean and Atlantic, supporting the species-level status of these two forms. Populations in the Faeroe Islands and Iceland were genetically distinct from continental populations (F_{ST} = 0.264–0.678), with Iceland represented by a single lineage also found in the Faeroes. This break is consistent with a deep-water barrier to dispersal in green crabs. Although there are relatively high levels of gene flow along the Atlantic coast of Europe, slight population structure was found between the central North Sea and populations to the south. Analysis of variance, multidimensional scaling, and the distribution of private haplotypes support this break, located between Bremerhaven, Germany, and Hoek van Holland. Similar biogeographical and genetic associations for other species, such as benthic algae and freshwater eels, suggest that the marine fauna of Europe may be generally subdivided into the areas of Mediterranean, western Europe and northern Europe.

Keywords: *Carcinus aestuarii*, *Carcinus maenas*, genetic structure, marine invasions, mitochondrial DNA

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Introduction

Common, large and easily found, the green crab, *Carcinus maenas*, is a familiar intertidal animal throughout its native range in Europe and North Africa. This highly adaptable crab — capable of surviving air exposure for at least 10 days (Crothers 1968) and tolerant of short-term exposure to temperatures as low as 0 °C and as high as 33 °C (Broekhuysen 1936; Eriksson *et al*. 1975) — is well equipped to endure ocean voyages and plane rides (Yamada 2001). Such traits, along with high fecundity and a long planktonic larval stage, combine to make *C. maenas* an ideal global invader. It has successfully colonized Australia, Tasmania, South Africa, Japan and both coasts of North America (Brenchley 1982; Lafferty & Kuris 1996; Yamada 2001). Once established, this species has become the dominant intertidal crab in some areas, affecting the abundance, size structure and defence response of native species (Tyrell & Harris 1999; Yamada 2001).

To understand the history of these invasions, it is important to elucidate the biogeography of the green crab in its native range. *Carcinus maenas* is found in coastal areas from Norway to Mauritania, with populations on the Faeroe Islands and Iceland. Though its species or subspecies status has been disputed, a distinct form, given the name *C. aestuarii*, is found in the Mediterranean Sea (Rice & Ingle 1975; Cohen *et al*. 1995; Bulnheim & Bahns 1996; Yamada 2001). We collected individuals of both forms but focused on the North Atlantic coastline, the source for most global invasions (Geller *et al*. 1997).

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The same life-history characteristics that allow for successful invasions — wide environmental tolerance, high fecundity and long larval stages — can lead to seemingly unstructured populations of species (Waples 1998). Indeed, distinct populations are often difficult to detect in the marine environment, and it has been suggested that many species may be organized into large panmictic populations (Palumbi 1992, 1994; McQuinn 1997). Most marine invertebrates with high dispersal potential lack sharp geographical differentiation, with genetic variation occurring almost exclusively within populations (Palumbi 1996; Burton 1997). Yet a growing number of genetic surveys have found evidence of restricted dispersal in marine organisms with planktonic larvae (e.g. Barber *et al*. 2000; Kojima *et al*. 2000; Taylor & Hellberg 2003). With high-resolution genetic markers, patterns of restricted gene flow have been found in European species such as the eel *Anguilla anguilla*, once widely accepted as forming a single, panmictic population (Wirth & Bernatchez 2001; Maes & Volckaert 2002).

Recent studies using the cytochrome *c* oxidase I (COI) gene in marine invertebrates have also revealed population subdivision in European species such as the mud snails *Hydrobia acuta* and *H. glyca* (Wilke & Pfenninger 2002) and the bivalve *Macoma balthica* (Luttikhuizen *et al*. 2003), implying that genetic exchange is restricted for these populations. Both of these studies reveal a break between western Europe and northern Europe along the English Channel, which roughly aligns with the Boreal–Lusitanian regions proposed by Briggs (1970, 1974). Our study was designed to explore the population structure of onshore populations of *C. maenas* and to determine if a break occurred between these onshore populations and offshore islands such as the Faeroes and Iceland.

Previous studies of the green crab suggest that larval release at nocturnal high tide and inherited vertical-migration rhythms may enhance long-distance offshore dispersal (Zeng & Naylor 1996; Queiroga *et al*. 1997; Zeng *et al*. 1999). Bulnheim & Bahns (1996) reported a slight geographical cline in *C. maenas* from north to south in the *Pgm* allozyme locus; however, no variation was detected in several other allozymes. Bagley & Geller (1999) found no population structure in a microsatellite DNA study of Atlantic European crabs. Yet both of these studies were restricted to few collection locales. Bagley & Geller (1999) sampled two locations, the Netherlands and Spain, and Bulnheim & Bahns (1996) sampled six locales: Bergen, Norway; Heikendorf and Helgoland, Germany; Roscoff and Arcachon, France; and Cadiz, Spain. We gathered samples from 15 populations in Europe — including major ports along the continental shelf, the offshore Faeroe Islands and Iceland, and Naples, Italy — to look for evidence of clinal variation, large-scale population structure and restricted gene flow. This extensive sampling regime should allow us to discern if population structure occurs in an intertidal species renowned for offshore larval dispersal. The knowledge of these phylogeographical patterns may help track human-mediated dispersal pathways and potentially prevent or mitigate future invasions.

Materials and methods

We sequenced a 502-base-pair (bp) fragment of the mitochondrial COI gene from 230 crabs collected in 15 locations in Europe, including the Mediterranean, North Atlantic coast, Iceland and Faeroe Islands (Fig. 1, Table 1). Samples were collected from beneath cobbles, in mussel beds and along docks. All samples were preserved in 95% ethanol, except for 24 crabs collected in Mongstadt, Norway, which were stored in 80-proof vodka (40% alcohol, Absolut). DNA was extracted with a 10% Chelex (Bio-Rad) solution from gills or muscle tissue in periopods (Walsh *et al*. 1991). Polymerase chain reaction amplifications (30 cycles of 1 min at 94 °C, 1 min at 50 °C and 1 min at 72 °C) were conducted with 2.5 μL 10× buffer, 1.5 mM $MgCl_2$, 0.5 mM dNTPs, 0.4 unit *Taq* DNA polymerase, and 1 μM of each primer (5′-GCT TGA GCT GGC ATA GTA GG-3′, 5′-GAA TGA GGT GTT TAG ATT TCG-3′). These primers, internal to those produced by Folmer *et al*. (1994), were designed for *Carcinus maenas*. The polymerase chain reaction fragments were sequenced on an ABI 3100 using BigDye (Perkin Elmer) terminator chemistry. All samples were sequenced in the forward and reverse direction; with no gaps, they were aligned by eye using the program SEQUENCHER.

A minimum-spanning tree was constructed using the software package ARLEQUIN (Schneider *et al*. 1997). PAUP* 4.0 (Swofford 1998) was used to conduct a heuristic search,

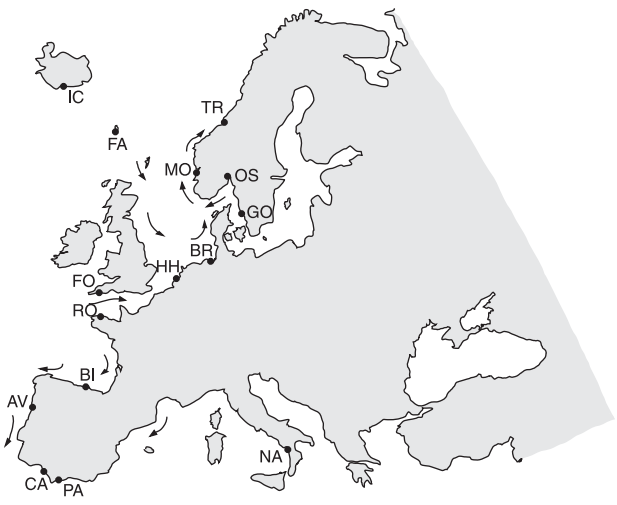

Fig. 1 Sampling locations of *Carcinus* in Europe. Arrows indicate general circulation patterns; see Table 1 for abbreviations.

Table 1 Collection locations, number of individuals per sampling site (*N*) and summary statistics of genetic variability for *Carcinus maenas* and *C. aestuarii* in Europe

Sampling location	Code	*N*	Sampling date	Haplotype diversity (*H*)	Nucleotide diversity (π)
Naples, Italy	NA	13	March 2001	0.79 ± 0.11	0.0102 ± 0.0060
Palmones, Spain	PA	10	June 2000	0.93 ± 0.07	0.0049 ± 0.0033
Cádiz, Spain	CA	12	August 2001	0.85 ± 0.07	0.0039 ± 0.0027
Aveiro, Portugal	AV	24	September 2001	0.81 ± 0.06	0.0036 ± 0.0024
Bilbao, Spain	BI	15	September 2001	0.65 ± 0.13	0.0029 ± 0.0021
Roscoff, France	RO	16	Summer 1999	0.86 ± 0.08	0.0040 ± 0.0026
Fowey, UK	FO	14	August 2001	0.89 ± 0.06	0.0045 ± 0.0030
Hoek van Holland, the Netherlands	HH	19	August 2001	0.81 ± 0.06	0.0038 ± 0.0025
Bremerhaven, Germany	BR	17	August 2001	0.87 ± 0.07	0.0055 ± 0.0035
Göteborg, Sweden	GO	15	August 2001	0.93 ± 0.04	0.0065 ± 0.0040
Oslo, Norway	OS	9	September 2001	0.81 ± 0.12	0.0064 ± 0.0041
Mongstadt, Norway	MO	24	September 2001	0.90 ± 0.05	0.0048 ± 0.0030
Trondheim, Norway	TR	7	Summer 1999	0.95 ± 0.10	0.0057 ± 0.0039
Tórshavn, Faeroe Islands	FA	20	September 2001	0.56 ± 0.06	0.0033 ± 0.0023
Seltjarnarnes, Iceland	IC	15	August 2002	0.00 ± 0.00	0.0000 ± 0.0000
Total		230			

H is a measure of haplotype diversity; π is nucleotide diversity per site.

with 1000 random sequence additions and tree bisection–reconnection branch swapping. Kimura's two-parameter model, which corrects for multiple hits, was used to calculate divergence between *C. maenas* and *C. aestuarii* (Kimura 1980).

An analysis of molecular variance (AMOVA) (Excoffier *et al*. 1992) in ARLEQUIN was used to test the hierarchical distribution of molecular variance at different geographical scales. Uncorrected pairwise distances were used to estimate the relative contribution of molecular variance at two levels: (i) among biogeographical regions (Φ_{CT}, the regions are northern Europe, including Bremerhaven, Germany, and Scandinavian populations; western Europe, including Hoek van Holland, Fowey, UK, and French and Iberian populations; and the off-shelf Faeroes and Iceland populations); and (ii) among populations (Φ_{ST}, correlation of haplotypes within populations relative to those from the whole species). Onshore and offshore regions were determined by previous work on marine fish (Foss *et al*. 1998; Shaw *et al*. 1999; Hoarau *et al*. 2002).

Because there are no major biogeographical boundaries or deep splits in the data along the continental shelf, three analyses were used to test for genetic structure across this region. (i) A multidimensional scaling approach was used to determine if this multivariate technique supported the split between Bremerhaven and Hoek van Holland. A Bray–Curtis analysis of similarity was performed with the software package PRIMER 5.0 (http://www.primer-e.com) to test whether this north–south cluster of populations was statistically distinct from other populations. (ii) A method employed by Sotka & Palumbi (in preparation; Sotka, personal communication) was used to test the distribution of haplotypes among *C. maenas* populations. The number of observed private haplotypes, those restricted to northern or southern populations, was compared to the distribution expected by chance using a χ^2 test. Because singletons are by definition private haplotypes, all genotypes that were found only once were removed from this analysis. (iii) A Mantel test, which assesses the significance of a regression between genetic and geographical distance by calculating the sum of the element-by-element products and comparing these results with a null distribution of values, and a reduced major axis regression of these values were performed in IBD (Bohonak 2002) to analyse isolation by distance. For log transformation of genetic distances, negative and zero values were changed to a small constant (0.0001) prior to transformation.

Results

Fifty-three COI haplotypes were recovered among the 230 individuals collected. (Common haplotype sequences were deposited in GenBank, accession numbers AY616437–AY616445.) Substitutions were generally in the third position and all were silent. With an 11.0% sequence divergence between the Naples and North Atlantic populations, the largest break occurred between the two putative sibling species, *Carcinus maenas* and *C. aestuarii*. Given this split, Mediterranean samples were removed from the analysis of population structure in *C. maenas*.

In Atlantic populations, divergence is shallow among haplotypes — most vary by a single base pair — and structure is slight along the coast. When groups are split between western Europe, northern Europe and Faeroes/Iceland, most of the genetic diversity was found within populations (80.5%). A smaller but significant amount of diversity ($P = 0.008$) was found among these groups (17.4%), and less among populations (2.1%). The AMOVA showed high levels of genetic structuring among these groups: $\Phi_{SC} = 0.03$, $\Phi_{ST} = 0.20$, $\Phi_{CT} = 0.17$. With a single haplotype recovered in Iceland ($n = 15$), no haplotype or nucleotide diversity was found on this offshore island. Much of the genetic structure was between the offshore Faeroes/Iceland group and the continental-shelf populations; when these offshore populations were removed from the analysis, the among group variation fell to a smaller, but still significant 1.2% ($P = 0.027$).

The existence of the slight north–south break between Bremerhaven, Germany, and Hoek van Holland is supported by both the multidimensional scaling and the haplotype frequency analyses. The multidimensional scaling plot revealed that the northern and western populations form separate clusters (Fig. 2), and an analysis of similarity showed that this clustering was significant (ANOSIM; $P < 0.04$). The frequencies in Table 2 reveal a greater number of private haplotypes, those found exclusively in north or south populations, than would be expected by chance ($\chi^2 = 134$, d.f. = 3, $P < 0.005$). For example, all eight individuals with haplotype 23 were found in northern populations.

The parsimony network reveals biogeographical clustering of genotypes. Of the five clades with 100% majority-rule consensus support, three were associated with geographical regions. A clade of three haplotypes (in purple, Figs 3 and 4) was found exclusively in the Faeroes and Iceland. Seventeen of 18 individuals forming a boreal clade (in blue, Figs 3 and 4) were located in the North Sea. One individual from this clade was recovered in Fowey, UK, along the western edge of the English Channel. A southern clade, comprised of seven individuals, was restricted to Fowey, UK, and points south.

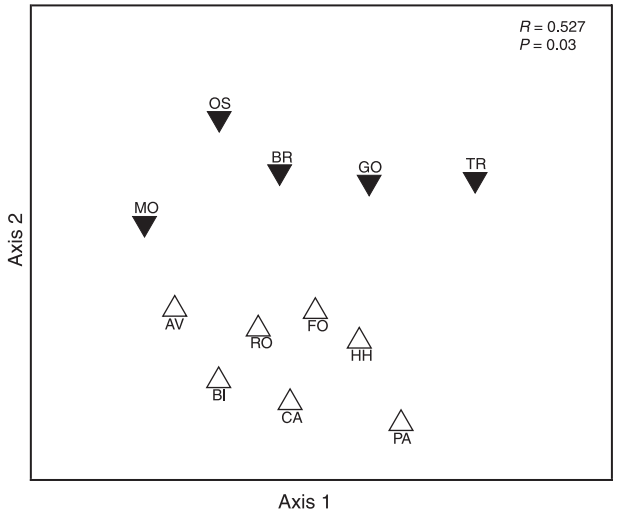

Fig. 2 Multidimensional scaling plot of green crab haplotypes from Atlantic Europe. An analysis of similarity supports significant clustering of northern (black) and southern (white) populations; see Table 1 for abbreviations.

Isolation by distance revealed a shallow slope for *C. maenas* along the Atlantic coast (genetic distance vs. geographical distance: $P = 0.101$, slope $= 2.6 \times 10^{-6}$; log genetic distance

Table 2 Distribution of haplotypes among *Carcinus maenas* populations along the continental shelf

	Western Europe							Northern Europe					
Haplotype	PA	CA	AV	BI	RO	FO	HH	BR	GO	OS	MO	TR	Total
1	1	4	9	9	6	4	6	6	3	4	7	1	60
5	0	0	0	0	0	0	0	0	0	0	1	1	2*
6	3	2	2	2	2	2	6	2	3	0	1	2	27
8	0	0	0	0	1	0	1	0	0	0	0	0	2*
9	1	0	0	0	1	0	2	0	1	1	0	0	6
10	1	1	6	1	2	3	2	0	0	0	4	0	20
14	1	0	0	1	0	1	0	0	0	0	1	0	4
23	0	0	0	0	0	0	0	2	2	2	1	1	8*
24	0	0	1	0	0	0	0	2	0	1	1	0	5
25	0	0	0	0	0	0	0	0	1	0	1	0	2*
27	0	0	0	0	0	1	0	1	0	0	1	1	4
28	0	0	0	0	0	0	0	0	0	0	2	0	2*
38	0	0	0	0	0	0	0	0	1	0	1	0	2*
43	1	3	0	0	0	1	0	0	0	0	0	0	5*

Haplotypes with an asterisk are restricted to populations in western Europe or northern Europe. Singleton haplotypes are not included in the table.

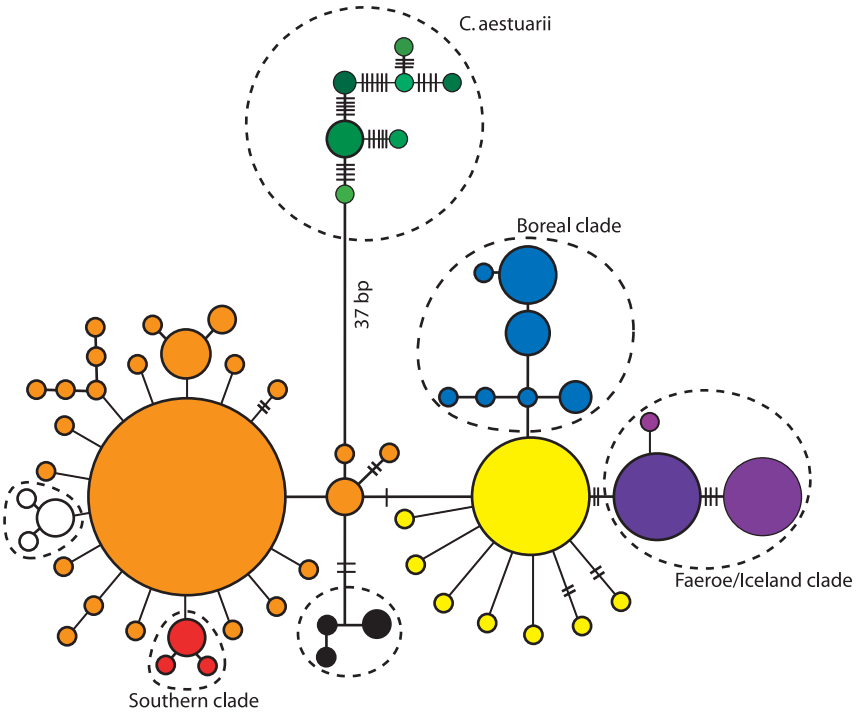

Fig. 3 Maximum-parsimony mitochondrial DNA network of *Carcinus maenas* and *C. aestuarii* haplotypes. All clades circled by dashed lines have 100% majority-rule consensus support.

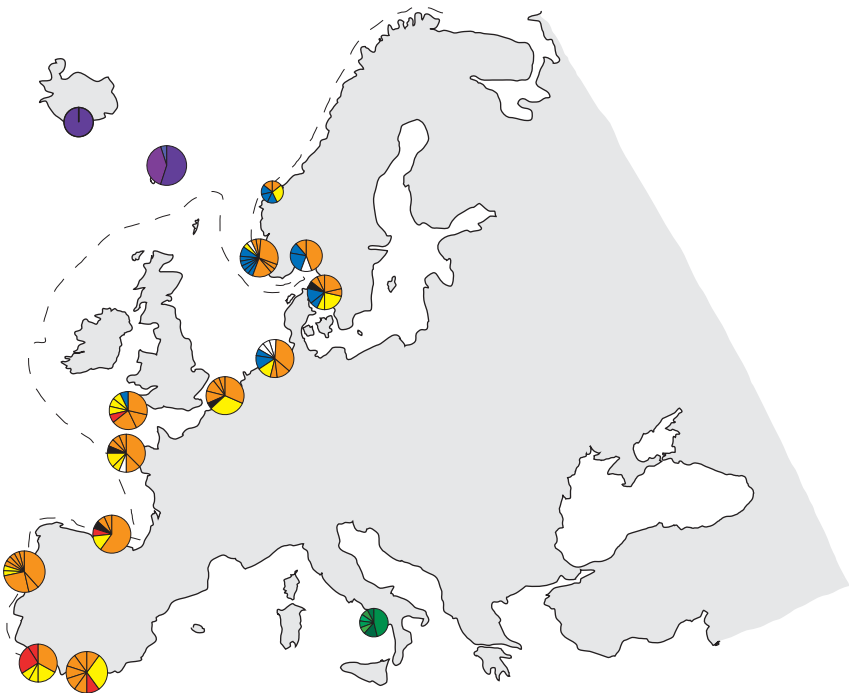

Fig. 4 Haplotype frequencies for *Carcinus maenas* and *C. aestuarii* in Europe. The dashed line indicates the 200-m contour of the continental shelf.

vs. log geographical distance: $P = 0.056$, slope 3.3), but neither analysis was significant at the 95% level.

Discussion

There was considerable genetic diversity in the COI gene of *Carcinus* along the continental shelf, with low diversity among the offshore populations of the Faeroe Islands and Iceland. Although the deepest split occurs between the Mediterranean and Atlantic forms, coastal populations of *Carcinus maenas* showed regional genetic structure. Multidimensional scaling, AMOVA, and an analysis of private haplotype frequencies all support a slight but significant break between western and northern Europe (located

between Hoek van Holland and Bremerhaven, Germany). Although this break is slightly north of the Boreal–Lusitania faunal break suggested by Briggs (1970, 1974), which occurs along the western edge of the English Channel, the subdivision could reflect regional current patterns: larval dispersal in the central and northern North Sea may be retained by the internal counterclockwise, or cyclonic, circulation patterns in the sea (Brown *et al*. 1999). Adey & Steneck (2001) recently proposed biogeographical regions, based on a time-integrated temperature model, for benthic algae in rocky sublittoral zones. The genetic division in crabs along the European coast aligns well with the proposed regions: Subarctic (northern), Celtic and Lusitanian (western), and Mediterranean. Crabs from Palmones, Spain, along the western edge of the Mediterranean Basin, however, cluster morphologically and genetically with *C. maenas*.

How does this finding compare with previous genetic studies of *Carcinus*? Initial studies of *C. maenas* found no population structure along the Atlantic coast of Europe (Geller *et al*. 1997; Bagley & Geller 1999) except for clinal variation in one allozyme marker that was probably imposed by selection (Bulnheim & Bahns 1996). The microsatellite study, however, was based on just two North Atlantic locations, both of which were south of our split in the central North Sea (Bagley & Geller 1999), and the 16S rRNA study found only one haplotype in the North Atlantic and one in the Mediterranean (Geller *et al*. 1997). Although nearly all of the allozyme loci analysed by Bulnheim & Bahns (1996) were monomorphic, with populations showing little genetic variation, they found a slight cline at the *Pgm* locus as latitude decreased between populations from Norway to Spain and the Mediterranean. The clear genetic signal in our study is probably the result of an extensive sampling regime and the utility of the COI gene for exploring population structure.

This finding is consistent with an emerging pattern indicating that some marine organisms, even those known to migrate long distances to reproduce or with the potential for widespread larval dispersal, can retain significant population structure. Recent genetic studies of European eels, for example, have shown evidence of restricted gene flow (Wirth & Bernatchez 2001; Maes & Volckaert 2002) despite earlier studies that supported panmixia in these long-distance migrants (Lintas *et al*. 1998). Using multidimensional scaling analysis for seven eel populations, Maes & Volckaert (2002) identified three distinct groups — northern Europe (Norway), western Europe (the Netherlands, Ireland and France) and the Mediterranean — which align with our findings for *Carcinus*. This split between the central North Sea and southern populations may be the result of historically separated populations, selective forces, isolation by distance or a combination of the above.

The deepest division in the COI data occurs between the Mediterranean and North Atlantic basins, where the status of the two forms of *Carcinus* continues to be debated. In their study of larval development, Rice & Ingle (1975) suggested a subspecific status based on a lack of distinction in the larval stages. Similarly, allozyme data prompted Bulnheim & Bahns (1996) to reject a species-level break between the Mediterranean and Atlantic forms. Yet the adult stages show morphological distinctions that indicate a species split between the forms of these two basins (Yamada 2001), and a recent genetic study using 16S rRNA sequences confirms this break (Geller *et al*. 1997). Our work supports these recent findings. Variation in the COI gene between populations from Naples and those of Atlantic Europe and the Iberian Peninsula shows a mean distance of 11.0%, indicating long-term division of the two forms. With divergence rates for COI estimated to be from approximately 1.4% per million years (*Alpheus* snapping shrimp, Knowlton & Weigt 1998) to 2.3% per million years (*Sesarma* grapsid crabs, Schubart *et al*. 1998), we infer that these *Carcinus* forms split about 5–8 million years ago.

Previous studies using the 16s rRNA gene discriminated between *Carcinus meanas* and *C. aestuarii* invasions (Geller *et al*. 1997). The COI gene can similarly be used to detect the Mediterranean or Atlantic origin of green crab invasions. However, the 14 sample sites included in our analysis of *C. maenas* may also allow us to discern source populations within the North Atlantic. The slight but significant break between north and western Europe and the sharp break between onshore and offshore populations will help to determine the region of origin for this global invader, which has become established in Japan, Tasmania, western North America and South Africa in the past few decades.

The deepest genetic division for *C. maenas* occurs between the offshore Faeroe Islands and Iceland and all continental-shelf populations. Three unique haplotypes were found in these two offshore areas, indicating that the island populations are closed — with long-term isolation from the European Atlantic coast. The deep-water barrier to dispersal between the continental shelf and these offshore islands may be responsible for the break, a pattern consistent with marine fishes in the region such as plaice (*Pleuronectes platessa*, Hoarau *et al*. 2002), halibut (*Hippoglussus hippoglussus*, Foss *et al*. 1998), and herring (*Clupea harengus*, Shaw *et al*. 1999). Although Briggs (1974) found no evidence of species endemism on the Faeroe Islands, the genetic breaks in at least four species suggest that an investigation of this island group's faunal relationship to continental Europe is warranted. The reduced diversity observed in the Icelandic population, where only a single haplotype was recovered among 15 crabs, suggests that the island was colonized relatively recently. This haplotype, which matches the most common genotype in the Faeroes, is probably derived from the island group. A future study of

the relationship of these offshore islands could test for evidence of a stepping-stone range expansion from the northern UK to the Shetland Islands, Faeroe Islands and Iceland. The distinct nature of the phylogeographical pattern on these offshore islands suggests that conservation attention should be paid to the marine fauna of these regions.

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This study is part of Joe Roman's PhD thesis exploring anthropogenic change in the North Atlantic using genetic tools. He is a conservation biologist interested in marine environmental history and changing perceptions of the oceans. Steve Palumbi is a marine ecologist and evolutionist at Stanford's Department of Biological Sciences; he is based at the Hopkins Marine Station.
