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Genetic structure of *Carcinus maenas* within its native range: larval dispersal and oceanographic variability

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ABSTRACT: Unravelling the interactions between life-history strategies and oceanography is central to our understanding of gene flow and connectivity in the marine environment. In the present study, we investigated the population genetic structure of the shore crab in its native range in relation to oceanographic characteristics and dispersal potential. Using 10 microsatellite markers over 2 yr, we surveyed 18 locations distributed along ~4200 km within the species native range, from Sweden to Morocco, assessed the population structure by means of F_{ST} and Bayesian clustering analysis and tested the hypothesis of isolation-by-distance (IBD) with a Mantel test. We focused particular attention along a 1200 km stretch of the Iberian Peninsula. We found no evidence of genetic structure (F_{ST} = 0.0001, $p > 0.05$) along the Iberian coast, and patterns were temporally stable over 2 yr. Across the more extensive geographic spatial scale, overall genetic differentiation was low (F_{ST} = 0.001) but statistically significant ($p < 0.001$). Furthermore, clustering analysis grouped the samples into 3 genetic units from (1) Sweden, (2) Wales and the Iberian Peninsula and (3) Morocco. While the correlation between genetic and geographic distances was significant, the pattern was not consistent with an IBD pattern. Results suggests that, in the absence of barriers to gene flow, shore crab populations are genetically similar across thousands of kilometres, but isolated populations still may occur within the species native range. Local oceanography and larval behaviour may have a significant influence on the structuring of the populations under study.

KEY WORDS: Population structure · Gene flow · Larval dispersal · Physical oceanography · Microsatellite DNA · *Carcinus maenas*

INTRODUCTION

Most coastal marine invertebrates and fish develop from planktonic larvae that drift for days to months in the oceanic realm. Such development strategies therefore represent an important mechanism for the dispersal of marine species for adults that are either sedentary or exhibit limited mobility during the adult phases. Highly mobile larvae have the potential to be transported several meters to hundreds of kilometres and in the apparent absence of barriers to gene flow, even distant regions might be connected genetically (Kinlan & Gaines 2003, Palumbi 2003, Thorrold 2006). Examples of ongoing long distance gene flow are widespread in marine systems (Bohonak 1999), though unrecognized barriers to dispersal often result in a disparity between the paradigm of large-scale connectivity and empirical observations (reviewed in Hauser & Carvalho 2008). In fact, numerous studies report the presence of population subdivision in species with

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extensive potential for dispersal, and sometimes, across a surprisingly small scale (Shaw et al. 1999, Hutchinson et al. 2001, Taylor & Hellberg 2003, Bekkevold et al. 2005, Bilodeau et al. 2005, Weetman et al. 2007). Moreover, self-recruitment may be more common than previously recognized, as evidenced by genetic studies (e.g. Jones et al. 2005, Carreras-Carbonell et al. 2007).

Isolation-by-distance (IBD) theory states that the genetic distances between populations increases with greater geographic distance, and that genetic distance declines with increased dispersal radius, producing a clear geographic genetic structure (Hellberg et al. 2002, Palumbi 2003). Genetic IBD is evident in several species ranging from fish (Pogson et al. 2001, Purcell et al. 2006, Johansson et al. 2008), to marine invertebrates (Palumbi et al. 1997, Launey et al. 2002, Couceiro et al. 2007), and has proven to be a powerful approach to interpreting the dynamics of gene flow (Palumbi 2003). It is now recognized that the relationship between dispersal radius and population connectivity can be very complex and many factors such as the species' life history, behavioural adaptations, oceanographic circulation patterns or historical events (Pringle & Wares 2007) influence contemporary patterns of gene flow. The use of novel and more informative genetic markers and chemical tags, together with enhanced sampling design, data analysis and individual-based coupled physical–biological models incorporating oceanography and larval biology, provide a robust amalgam of tools to explore effectively larval dispersal and population dynamics (reviewed in Levin 2006, Cowen & Sponaugle 2009).

Here, we analyse microsatellite variation among populations of the decapod crustacean *Carcinus maenas* within the native species range. *C. maenas* is one of the most abundant and intensively studied invertebrates in the world and, accordingly, is suited as a biological model to address a number of important issues in marine ecology related with gene flow and population connectivity. *C. maenas* has a native geographical distribution that extends from Norway to Mauritania, including Iceland, the Faroe Isles and the British Isles, where it inhabits estuaries and rocky shores during its juvenile and adult stages (Almaça 1962, Crothers 1968). Crabs from the genus *Carcinus* are very successful predators and tolerate a wide range of environmental conditions. *Carcinus* has become a global invader during the last century, establishing populations in the east and west coasts of North America, South Africa, Japan, Australia and Argentina (Carlton & Cohen 2003). In some areas, its range is still expanding, with measurable impacts on native communities (Yamada & Gillespie 2008). Dispersal is via a planktonic larval phase that consists of 4 zoeal stages and a megalopal stage that develops in the water column from late winter to early summer for 4 to 6 wk depending on water temperature (Queiroga 1996). Larval *Carcinus* spend considerable time in the plankton, a life-history strategy that predicts long dispersal distances that will be strongly influenced by coastal and oceanic circulation regimes (Shanks et al. 2003, Peliz et al. 2007). In addition, several studies (Queiroga & Blanton 2005, Queiroga et al. 2007) show how shore crab larvae take advantage of the capacity to perform tidally-synchronized migrations to maximize their export out to the sea, followed by supply back to estuaries and rocky shores where adults live. Larvae are also known to perform extensive diel vertical migrations, exploiting currents at different depths that can be important for larval retention in shelf waters, especially in strongly vertically sheared flows (e. g. cross-shore upwelling circulation in stratified shelves), as described by Marta-Almeida et al. (2006).

Mitochondrial DNA variation across the European range of *Carcinus maenas* based on cytochrome c oxidase I (COI) gene was previously surveyed by Roman & Palumbi (2004) who found significant genetic differences between the off-shelf populations of Faeroe Islands and Iceland and the continental populations, as well as slight genetic structuring between the central North Sea and populations to the south along the Atlantic coast up to southern Spain. More recently, Darling et al. (2008) used both the COI gene and 9 microsatellite loci to investigate the genetic patterns of *Carcinus* introductions around the globe. Some native *C. maenas* populations, from where just a few individuals were sampled, were analysed during the study but the data revealed little detectable genetic differentiation within the native species' range. The genetic structuring that was recovered was dominated by differences between the off-shelf population at Torshavn, Faeroe Islands, and the other native populations. Here, we analysed a broader sampling of populations, individuals and microsatellite loci covering the main distribution of *C. maenas* in its native European range, including 1 location in North Africa not studied previously.

The circulation of coastal waters of the Western Iberian Peninsula is highly dynamic and characterised by seasonal and short term variations in current patterns. A predominantly equatorward flow occurs after the spring transition until the end of the summer, when northerly, upwelling-favourable winds dominate the circulation (Fiúza et al. 1982), while in winter, a predominantly poleward flow is observed (Iberian Poleward Current, IPC) (Peliz et al. 2005). During the non-upwelling season, the shelf is under the influence of another local feature resulting from river runoff of several rivers located on the northwest coast of Portugal and Spain that generates a low salinity

surface layer: the Western Iberia Buoyant Plume (WIBP) (Peliz et al. 2002). Previous studies in this region based on physical modelling indicate that larval transport processes can be strongly dependent on mesoscale features associated with both the WIBP and the IPC (Santos et al. 2004), as well as with different wind regimes, coastal orientation and river plumes (Peliz et al. 2007). Moreover, Peliz et al. (2007) hypothesized that dispersal conditions in northwest Iberia may be significantly different from the regions to the south of the Estremadura promontory and north of Cape Finisterre due to changes in coastal topography and local oceanography. A previous survey carried out in the Portuguese coast found, indeed, weak but significant genetic structuring between *Carcinus maenas* populations from north and south of the Estremadura promontory (Pascoal et al. 2009). We then expect that variation in oceanographic features, which can vary seasonally or interannually, associated with different segments of the coast, should affect larval dispersal and population connectivity of the shore crab. To test this hypothesis, we surveyed the genetic connectivity in neighbouring populations sampled along 1200 km of the Iberian Peninsula coastline and examined the temporal stability of microsatellite allele frequencies from 2 consecutive yr. In order to place regional differentiation into a broader geographic scale, we also surveyed the levels of population differentiation and tested the hypothesis of IBD in populations outside the Iberian Peninsula from samples collected in a third year. In total, our sampling comprised 18 locations distributed from Gullmarsfjord in Sweden to Oued Tahadart in Morocco.

MATERIALS AND METHODS

Sample collection. *Carcinus maenas* occurs in estuaries and rias along the Iberian Peninsula. We collected samples from 14 populations distributed along a 1200 km stretch of the south, west and north coasts (Fig. 1, Table 1) in 2006, and again from the same sites in 2007, allowing a test of temporal variation. To assess relationships across a broad geographic scale, 4 locations within the species' native distributional range and 1 location in the Mediterranean were sampled in 2008: Gullmarsfjord (Sweden), Menai Strait (Wales, UK), Cadiz Bay (South West Spain), Oued Tahadart (Morocco) and Ebro Delta (Catalan coast). At each location, ~50 crabs were caught with baited hoop nets in the months of June to August, in order to minimize possible seasonal differences. From each specimen, muscle tissue was removed from 1 periopod and preserved in 96% ethanol until DNA extraction.

DNA extraction and microsatellite genotyping. Total genomic DNA was extracted in 96-well format from muscle tissue using overnight digestion with proteinase K following a modified salt extraction protocol (Aljanabi & Martinez 1997). DNA was resuspended in a volume of 100 µl of 1× TE buffer (10 nM Tris-Cl, 1 nM EDTA, pH 8.0) and stored at −20°C. We selected 12 microsatellite loci developed for *Carcinus maenas*: 10 loci from Tepolt et al. (2006) (Cma01EPA, Cma02EPA, Cma03EPA, Cma04EPA, Cma05EPA, Cma08EPA, Cma09EPA, Cma10EPA, Cma12EPA, Cma14EPA) and 2 loci from Pascoal et al. (2009) (SP107, SP495). Loci were amplified in 2 multiplex PCR reactions using forward 6-FAM, VIC, NED or PET fluorescently labelled primers. PCR amplifications contained ~20 to 100 ng of template DNA, 1× QIAGEN Multiplex PCR Master Mix (Qiagen) and 0.1 to 0.3 µM of each primer in a total reaction volume of 10 µl. Reactions were performed in Bio-Rad Tetrad 2 Peltier Thermal Cyclers under the following conditions: 95°C for 15 min followed by 30 cycles of 94°C for 30 s, 55°C for 90 s, and 72°C for 60 s followed by a final extension at 60°C for 30 min. Resulting products were then resolved on a ABI 3130xl Genetic Analyzer (Applied Biosystems) and sized using GeneScan LIZ-500 internal size standard and GENEMAPPER version 4.0. software (Applied Biosystems). During initial testing, 100 individuals were amplified independently 2 times across all loci to assess the reliability of PCR and genotyping error rate.

Statistical analysis. The potential presence of null alleles and scoring errors due to stuttering and large allele drop-out was tested using MICRO-CHECKER version 2.2.3 software (van Oosterhout et al. 2004). Allele frequencies and measures of genetic diversity such as expected heterozygosity (H_e), observed heterozygosity (H_o), number of alleles (N_A) and allelic richness (A) were calculated by FSTAT version 2.9.3.2 (Goudet 2001) and by GENETIX version 4.05 (Belkhir et al. 1996–2004). FSTAT was also used to assess deviations from Hardy-Weinberg equilibrium across all loci and populations using the inbreeding coefficient F_{IS} as estimated by f (Weir & Cockerham 1984) and to estimate overall levels of population differentiation using F_{ST} as estimated by f (Weir & Cockerham 1984). The significance of F_{IS} and F_{ST} was tested based on a random permutation procedure, and confidence intervals (CI) calculated by bootstrapping over loci (Goudet 2001). Pairwise F_{ST} (θ) values between all population pairs were calculated with GENETIX, with their significances tested using 10 000 permutations. Annual, within-location samples that did not show significant genetic differentiation in these tests were pooled in subsequent analysis. Linkage disequilibrium between pairs of loci was tested using the exact test implemented in GENEPOP version 4.0 (Rousset 2008), with

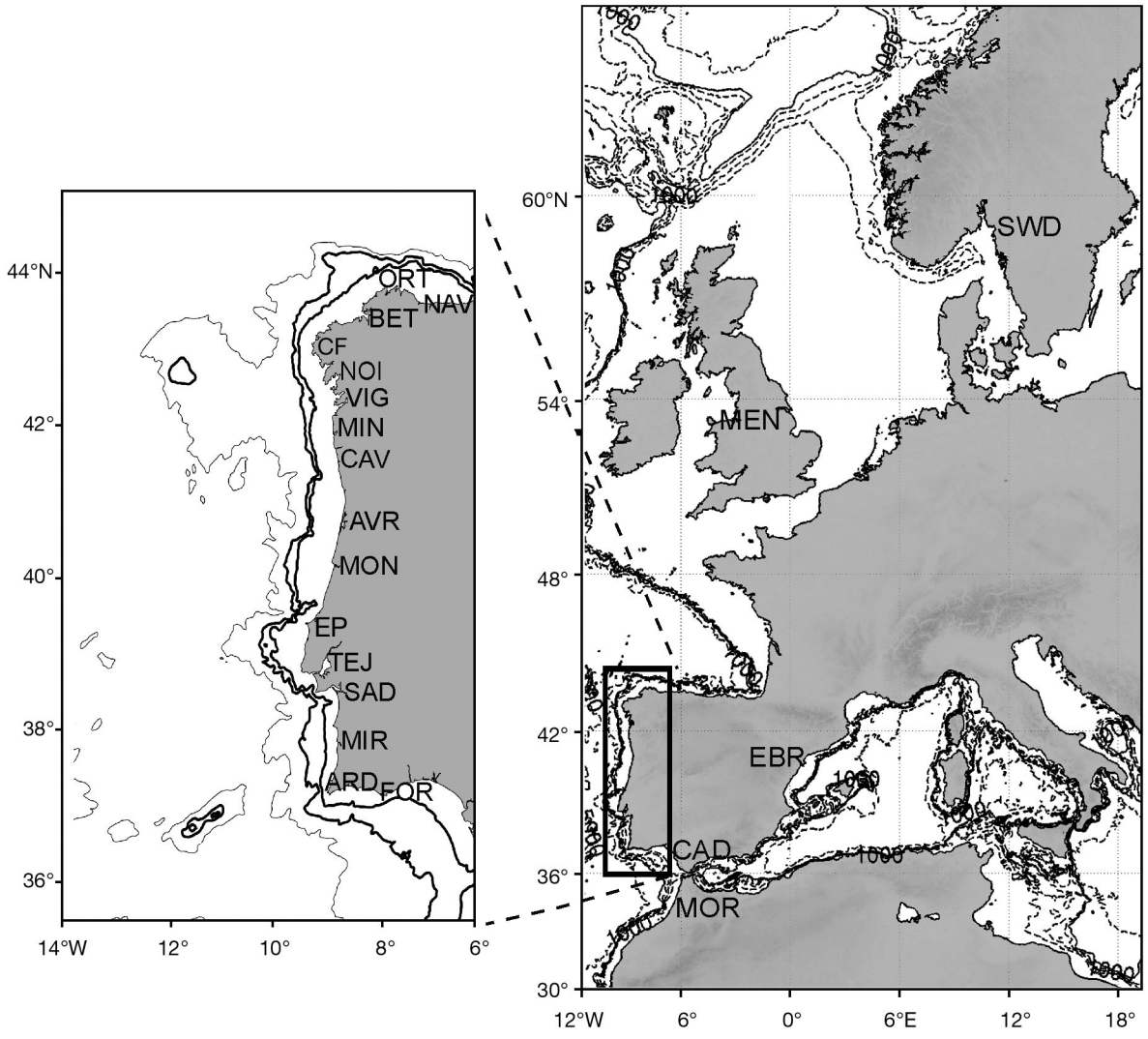

Fig. 1. *Carcinus maenas.* Sampling sites. See Table 1 for location codes. CF: Cape Finisterre; EP: Estremadura Promontory

significance levels determined by the Markov chain method (dememorization = 5000, batches = 500, iterations = 10 000). Where multiple comparisons were involved, we used the sequential Bonferroni procedure (Rice 1989) at the 5% level to adjust the statistical significance. IBD was assessed by plotting pairwise F_{ST}/(1 – F_{ST}) values (Rousset 1997) against the logarithm of the geographic distances (measured as the shortest distance by sea in km) between all sample sites. Mantel test (30 000 permutations) and reduced major axis (RMA) regression were conducted to assess the significance and strength of the relationship between genetic and geographic distances with the software IBDWS (Jensen et al. 2005). The same standard population genetic analysis described above was performed on the inferred populations identified by GENELAND (Guillot et al. 2005) (see below).

Power analysis. Statistical power for detecting genetic differentiation using the microsatellite markers characterised by given levels of allelic diversity and sample sizes was analysed with the program POWSIM (Ryman & Palm 2006). Computer simulations mimic sampling from populations at various levels of expected divergence under a classical Wright-Fisher model without migration or mutation. To test the power to detect an expected divergence of F_{ST} = 0.001 among subpopulations, 1000 simulations, over 20 generations each, were run employing sample sizes corresponding to those from our sampling regions and the allele frequencies from the current data set as a starting point.

Bayesian clustering analysis. We used the Bayesian clustering methodology of GENELAND version 3.1.4 software (Guillot et al. 2005) in the R-PACKAGE

Table 1. *Carcinus maenas*. Sampling sites, position and number of individuals collected each year (2006 to 2008). Samples from the same location showing no genetic divergence were pooled, so that a total of 18 samples from *C. maenas* populations were included in the statistical processing. Code: sample location abbreviation

Location	Code	Geographic position	Sample size		
			2006	2007	2008
Gullmarsfjord	SWD	58°15′N, 11°25′E	–	–	50
Menai Strait	MEN	53°14′N, 4°10′W	–	–	50
Navia	NAV	43°32′N, 6°43′W	39	20	–
Ortigueira	ORT	43°42′N, 7°52′W	49	50	–
Betanzos	BET	43°21′N, 8°12′W	48	50	–
Noia	NOI	42°48′N, 8°54′W	50	50	–
Vigo	VIG	42°20′N, 8°38′W	50	48	–
Minho	MIN	41°52′N, 8°50′W	49	50	–
Cavado	CAV	41°31′N, 8°46′W	50	50	–
Aveiro	AVR	40°37′N, 8°44′W	50	50	–
Mondego	MON	40°08′N, 8°50′W	50	50	–
Tejo	TEJ	38°44′N, 8°55′W	49	50	–
Sado	SAD	38°24′N, 8°45′W	27	50	–
Mira	MIR	37°39′N, 8°43′W	–	49	–
Arade	ARD	37°09′N, 8°29′W	34	50	–
Formosa	FOR	37°00′N, 7°58′W	50	50	–
Cadiz	CAD	36°28′N, 6°11′W	–	–	50
Oued Tahadart	MOR	35°34′N, 6°00′W	–	–	30
Ebro Delta	EBR	40°38′N, 0°43′W	–	–	40

(Ihaka & Gentleman 1996) to detect and determine the level of genetic structure in the data set. GENELAND integrates the spatial coordinates of individuals together with the genetic information and so provides an improved definition of the spatial genetic units when compared with non-spatial clustering methods. All the unknown parameters are processed simultaneously through Markov chain Monte Carlo (MCMC) computations. Due to substantial algorithm improvement implemented in the recent versions of GENELAND software (from version 3.0.0 onwards), we used the correlated frequency model that allowed us to detect subtle structures in the presence of low genetic differentiation that would probably remain undetected using an uncorrelated frequencies model (Guillot 2008). Additionally, improvements in the post-processing scheme allowed estimation of the number of populations (K), as well as the assignment of individuals to the inferred populations in a single step, treating the number of clusters as unknown. We placed an independent Gamma prior on the drift coefficients with parameters (2, 20). GENELAND was then run 50 times for each dataset with 500 000 MCMC iterations and a burn-in of 100 000 iterations in the post-processing. Next, we calculated the mean logarithm of posterior probability distribution of the data for each of the 50 runs and selected only the 10 with the highest posterior distribution to be considered in the analysis. We finally checked visually for the consistency of results across the 10 runs.

RESULTS

Microsatellite amplification

The presence of null alleles, assessed with MICRO-CHECKER (van Oosterhout et al. 2004), was detected in 2 loci (Cma02EPA and Cma12EPA), and scoring errors due to stuttering were found at 1 locus (Cma12EPA). These 2 loci were therefore removed from subsequent analysis of population genetic structure. For the remaining 10 loci, PCR products from repeated amplifications of the same individual consistently produced the same genotype and all reliably amplified in every sample except the locus SP495, which did not amplify in the samples collected in Ebro Delta. During our study, we identified that individuals from this population are in fact *Carcinus aestuarii*, a sibling species of *C. maenas* that occurs in the Mediterranean Sea. For this reason, the Ebro population was discarded from the analysis.

Genetic diversity, Hardy-Weinberg and linkage equilibrium

All microsatellite loci displayed moderate to high levels of polymorphism (Table 2): the number of alleles per locus ranged from 2 to 54 (mean = 19.8) and the

Table 2. *Carcinus maenas*. Summary statistics for 10 microsatellite loci pooled from 18 populations. Significant F_{IS} values of the tests for heterozygosity deficiency and significant F_{ST} values of the tests for genetic differentiation, following Bonferroni correction (Rice 1989), are denoted in **bold**. Asterisks refer to values that were significant before applying correction ($^{*}p < 0.05$; $^{**}p < 0.01$). N_A: number of alleles; H_o: observed heterozygosity; H_e: expected heterozygosity; F_{IS} (f) and F_{ST} (θ) values calculated after Weir & Cockerham (1984)

Locus	N_A	H_o	H_e	F_{IS}	F_{ST}
Cma01EPA	8	0.426	0.415	−0.010	0.001
Cma03EPA	16	0.798	0.806	0.007	0.002*
Cma04EPA	28	0.912	0.903	−0.012	0.000
Cma05EPA	2	0.414	0.425	0.034	0.003
Cma08EPA	33	0.934	0.952	0.009	0.000
Cma09EPA	22	0.778	0.792	0.021	0.001
Cma10EPA	54	0.953	0.959	0.008	0.000
Cma14EPA	11	0.365	0.377	0.022	0.004**
SP107	15	0.558	0.586	**0.049**	**0.006**
SP495	9	0.612	0.644	**0.051**	0.001
Overall	19.8	0.674	0.686	**0.016**	**0.001**

expected heterozygosity from 0.377 to 0.959 (mean = 0.686). Global F_{IS} was 0.016 (95% CI = 0.004–0.029, $p < 0.001$) and there was a significant heterozygote deficiency over all loci due to loci SP107 and SP495. The small but significant heterozygote deficiency suggests that inbreeding or a subtle spatial structure (i.e. Wahlund effect) exists within the data set (null alleles detected by MICRO-CHECKER were eliminated prior to analysis). Significant linkage disequilibrium was found in 42 out of 810 pairwise comparisons among 10 loci for all populations; however, none was significant after sequential Bonferroni correction (Rice 1989).

Power analysis

The simulations undertaken using POWSIM (Ryman & Palm 2006) indicate that the number of loci, the number of alleles per locus, their frequency distributions and the sample sizes used were sufficient to reveal population structure at a true F_{ST} as low as 0.001 with a statistical power of >99%.

Population differentiation

Comparison of allele frequencies among samples collected along the Iberian Peninsula in 2006 and 2007, from the same locations, exhibited no significant genetic differences (F_{ST} ranged from –0.0030 to 0.0052). Since the signal of genetic differentiation detected appears to be stable, at least over 2 yr, we pooled the samples from the same geographical location. Considering solely the Iberian Peninsula samples, no significant differentiation was detected using F_{ST} (F_{ST} = 0.000, 95% CI = –0.000–0.001, $p > 0.05$). Moreover, no structure was observed when these samples were pooled into north (NAV, ORT, BET, NOI, VIG, MIN, CAV, AVR, MON) and south (TEJ, SAD, MIR, ARD, FOR) of the Estremadura Promontory, allowing testing for regional differences of the coast (F_{ST} = –0.000, 95% CI = –0.000–0.000, $p > 0.05$) (see Table 1 for sample location code).

Over a large geographical scale, an overall F_{ST} value of 0.001 (95% CI = 0.001–0.003, $p < 0.001$, Table 2), indicates low, but significant genetic differentiation among samples.

Pairwise comparisons were assessed among all samples collected from Sweden to Morocco (Table 3). Samples from the Iberian Peninsula together with the sample from Wales, UK, appeared homogeneous, with no significant differentiation observed from the Menai Straits to Cadiz following the use of sequential Bonferroni correction for multiple comparisons (pairwise F_{ST} ranged from –0.0025 to 0.0038). Comparisons between

Table 3. *Carcinus maenas*. Estimates of pairwise genetic differentiation (F_{ST} values estimated by θ) among 18 populations. Significant F_{ST} values following sequential Bonferroni correction (Rice 1989) for 153 multiple comparisons are in **bold**. Asterisks: values that were significant before applying correction ($^{*}p < 0.05$; $^{**}p < 0.01$). See Table 1 for sample location codes

	SWD	MEN	NAV	ORT	BET	NOI	VIG	MIN	CAV	AVR	MON	TEJ	SAD	MIR	ARD	FOR	CAD
MEN	0.0059*																
NAV	**0.0132**	0.0004															
ORT	**0.0147**	0.0018	0.0006														
BET	**0.0117**	0.0004	–0.0003	–0.0001													
NOI	**0.0136**	–0.0004	–0.0013	0.0010	–0.0003												
VIG	**0.0159**	0.0006	0.0008	0.0016	–0.0017	0.0005											
MIN	**0.0153**	0.0011	0.0000	0.0000	–0.0014	–0.0007	–0.0015										
CAV	**0.0161**	0.0024	0.0008	0.0010	–0.0002	0.0017	0.0005	0.0001									
AVR	**0.0149**	0.0016	0.0032*	0.0030*	–0.0004	0.0038**	0.0004	0.0009	0.0015								
MON	**0.0122**	0.0007	0.0009	0.0001	0.0001	0.0014	0.0009	–0.0001	0.0003	–0.0002							
TEJ	**0.0158**	0.0005	–0.0010	–0.0012	–0.0012	0.0007	–0.0003	–0.0011	–0.0007	0.0019	–0.0005						
SAD	**0.0106**	–0.0003	–0.0005	0.0008	–0.0015	0.0001	–0.0002	–0.0009	–0.0010	–0.0002	–0.0006	0.0002					
MIR	**0.0146**	0.0028	0.0008	0.0016	–0.0003	0.0019	0.0018	0.0010	–0.0014	0.0025	0.0011	0.0009	–0.0014				
ARD	**0.0159**	0.0015	–0.0006	–0.0002	–0.0014	0.0007	–0.0009	–0.0009	–0.0010	0.0006	–0.0008	–0.0011	–0.0001	–0.0001			
FOR	**0.0193**	0.0021	0.0004	–0.0003	–0.0006	0.0011	–0.0009	–0.0012	0.0009	0.0007	0.0007	–0.0005	–0.0002	0.0013	–0.0006		
CAD	**0.0102**	–0.0010	0.0004	–0.0020	–0.0011	0.0006	0.0005	0.0001	0.0002	0.0012	–0.0012	0.0007	0.0001	–0.0025	–0.0014	0.0002	
MOR	**0.0289**	0.0110**	0.0064*	0.0041	0.0054*	0.0061*	0.0038	0.0032	0.0060*	0.0023	0.0034	0.0061*	0.0062*	0.0070*	0.0018	0.0011	0.0043

the previous samples (Menai Strait to Cadiz) with the Moroccan sample indicated significant genetic differentiation in 8 out of the 16 comparisons before sequential Bonferroni correction, though none were significant after correction (pairwise F_{ST} ranged from 0.0011 to 0.0110). Samples from Sweden, in the Skagerrak region, exhibited significant differences with all other locations, after correcting for multiple comparisons, except with the Menai Straits (Wales) sample: F_{ST} = 0.0059, $p < 0.05$ (SWD and MEN); F_{ST} = 0.0289, $p < 0.001$ (SWD and MOR) and against the Iberian Peninsula samples, F_{ST} = 0.0102 to 0.0193, $p < 0.001$. The Mantel test correlation between genetic and the logarithm of geographic distances was positive and significant ($Z = 1.20$, $r = 0.55$, $p < 0.01$), with geographic distance accounting for 30% of the variation in genetic differentiation (Fig. 2). When populations from the Iberian Peninsula were considered on their own, no significant IBD was observed ($Z = 0.02$, $r = -0.03$, $p > 0.60$) (Fig. 2).

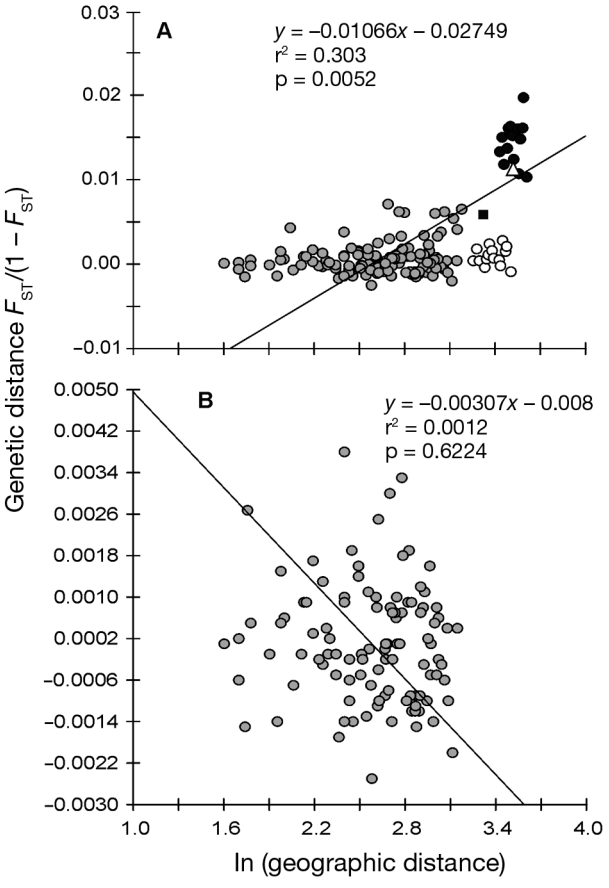

Fig. 2. *Carcinus maenas.* Relationship between genetic differences ($F_{ST}/[1 - F_{ST}]$) and the logarithm (geographical distance) at (A) all sampling sites (SWD to MOR) and (B) at the Iberian Peninsula (NAV to CAD). Comparisons between (A) Sweden and Menai Strait (■); Sweden and the remaining samples (●); Menai Strait and Morocco (△); Menai Strait and the remaining samples (○); the remaining comparisons (◍), and (B) all comparisons between samples from Navia to Cadiz (◍). For abbreviations see Table 1

Bayesian clustering analysis

We investigated the number of clusters along the native range of *Carcinus maenas* based on 18 locations using GENELAND (Guillot et al. 2005), a Bayesian method that uses both genetic and spatial data. Posterior distributions of K displayed a clear mode at $K = 3$ across the 10 replicates (Fig. 3). The Geneland model identified 3 spatially coherent clusters (Fig. 4) in 8 out of 10 replicates: the first includes *C. maenas* from the Swedish population (SWD); the second, *C. maenas* from Menai Straits with the Iberian Peninsula (MEN-IP); and the third, *C. maenas* from the Morocco population (MOR). Each cluster had a probability of 0.8 of belonging to their regional group, thereby providing strong support to the respective cluster.

Population genetic parameters of the inferred populations

The 3 identified clusters displayed comparable levels of genetic diversity that were relatively high, they were assessed using expected heterozygosity and allelic richness, the latter corrected for difference in sample size (Table 4). In the Swedish and Moroccan populations, F_{IS} was −0.003 and 0.053, respectively, and there was no evidence of departure from Hardy-Weinberg equilibrium. For the cluster comprising samples from Menai Straits and the Iberian Peninsula, F_{IS} = 0.016, with a significant deficiency in heterozygosity ($p < 0.001$). In the 3 inferred populations, there was no significant linkage disequilibrium. Global F_{ST} = 0.011 (95% CI = 0.004 – 0.020, $p < 0.001$), and pairwise F_{ST} among clusters were 0.0141 ($p < 0.001$; SWD and MEN-IP), 0.0289 ($p < 0.001$; SWD and MOR) and 0.0045 ($p < 0.05$; MEN-IP and MOR).

DISCUSSION

Spatio-temporal structure across the Iberian Peninsula

In this study, we used a combination of approaches (F_{ST}, IBD and Bayesian clustering analysis) to investigate genetic differentiation in *Carcinus maenas* within the Atlantic native species range and across the Iberian Peninsula in particular. Along the Iberian Penin-

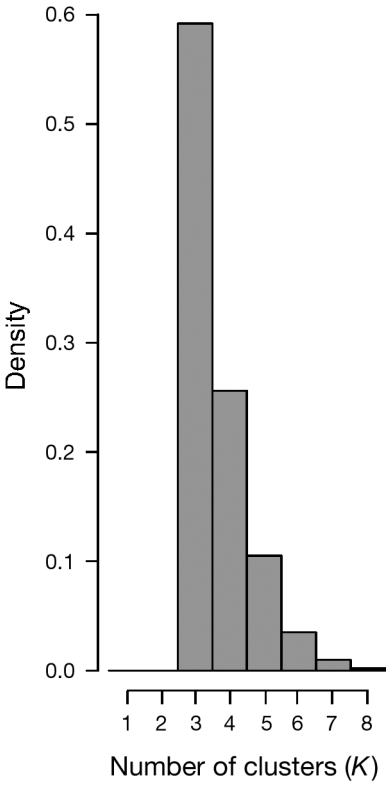

Fig. 3. *Carcinus maenas.* Posterior density distribution of the number of clusters estimated from GENELAND analysis in 10 replicates

sula, differentiation might be predicted across the Cape Finisterre and the Estremadura Promontory based on differences in oceanographic regime (Peliz et al. 2007). Diekmann et al. (2005) reported a split between northern and southern seagrass *Zostera noltii* populations along the west Iberian coast, coinciding with the Estremadura promontory, caused partly by geographical features that act as barriers to dispersal and by ocean surface currents. Pascoal et al. (2009) found weak genetic structure among *C. maenas* populations sampled during 2005 across a 450 km stretch of the Portuguese coast. Our data, based on samples collected with a spatial resolution of a few 10s to over 1000s of km, indicates genetic homogeneity among sites separated by several 100s of km along a 1200 km extent of the Iberian coast, suggesting that genetic exchange (effective migration) is occurring over this scale. Sampling in the same area over 2 consecutive yr confirmed such a view, suggesting that apparent genetic similarity of populations is temporally stable and unlikely to be caused by sampling artifacts (Waples 1998). Although interaction of diel vertical migration behaviour with upwelling circulation may retain larvae inshore (Marta-Almeida et al. 2006), the prevailing current regimes and oceanographic features along the Iberian coast do not seem to act as barriers to larval dispersal. The close proximity of estuaries may also facilitate the exchange of migrants between rivers through larval drift, in accordance with dispersal distances in the order of 120 km during the larval life of the shore crab, on the Western Iberian Shelf, as estimated by Peliz et al. (2007). *C. maenas* does not undergo philopatric migrations that could be a mechanism promoting population differentiation. Also, crabs collected by Pascoal et al. (2009) were sampled at the adult stage, precluding some 'Allendorf-Phelps effect' (Waples 1998). We regard the results obtained by Pascoal et al. (2009) as reflecting the potentially transient nature of population structuring in local Portuguese shore crab populations caused by 'sweepstakes' recruitment (Hedgecock 1994) that no longer persists in the absence of a strong physical barrier or diversifying selection (Pringle & Wares 2007). Additionally, in the Pascoal et al. (2009) study, the presence of null alleles was detected in 3 out of the 9 loci used to assess population structure, which may have driven the differences between the present and the former study.

Population structure within the native range of *Carcinus maenas*

Across larger geographical scales, F_{ST} estimates, IBD and Bayesian clustering analysis were all consistent in finding significant genetic heterogeneity among samples. Values of the pairwise tests of differentiation have shown significant differences between Sweden and the remaining Atlantic samples, with no significant differences for the group of samples extending from the Menai Straits in the UK, to Morocco. The IBD relationship found, although statistically significant, is apparently non-linear and essentially depends on the effect of the most divergent Swedish sample (Fig. 2). Furthermore, results from GENELAND supported the existence of 3 genetic clusters in the data set: Sweden (SWD), Menai Strait together with Iberian Peninsula (MEN-IP) and Morocco (MOR) (Fig. 4). These clusters were characterized by comparable levels of genetic diversity. Expected heterozygosity values were 0.696 (SWD), 0.686 (MEN-IP) and 0.663 (MOR), which are similar to values reported for microsatellites for other decapod crustaceans (e.g. H_e = 0.5 to 0.6 for *Pachygrapsus marmoratus* [Silva et al. 2009]; H_e = 0.7 to 0.8 for *Maja brachydactyla* [Sotelo et al. 2008]). The GENELAND model identified the Moroccan sample as an individual cluster, despite the failure of the F_{ST} approach to distinguish clearly Morocco from MEN-IP samples in individual comparisons. Some methods are expected to perform better under particular scenarios,

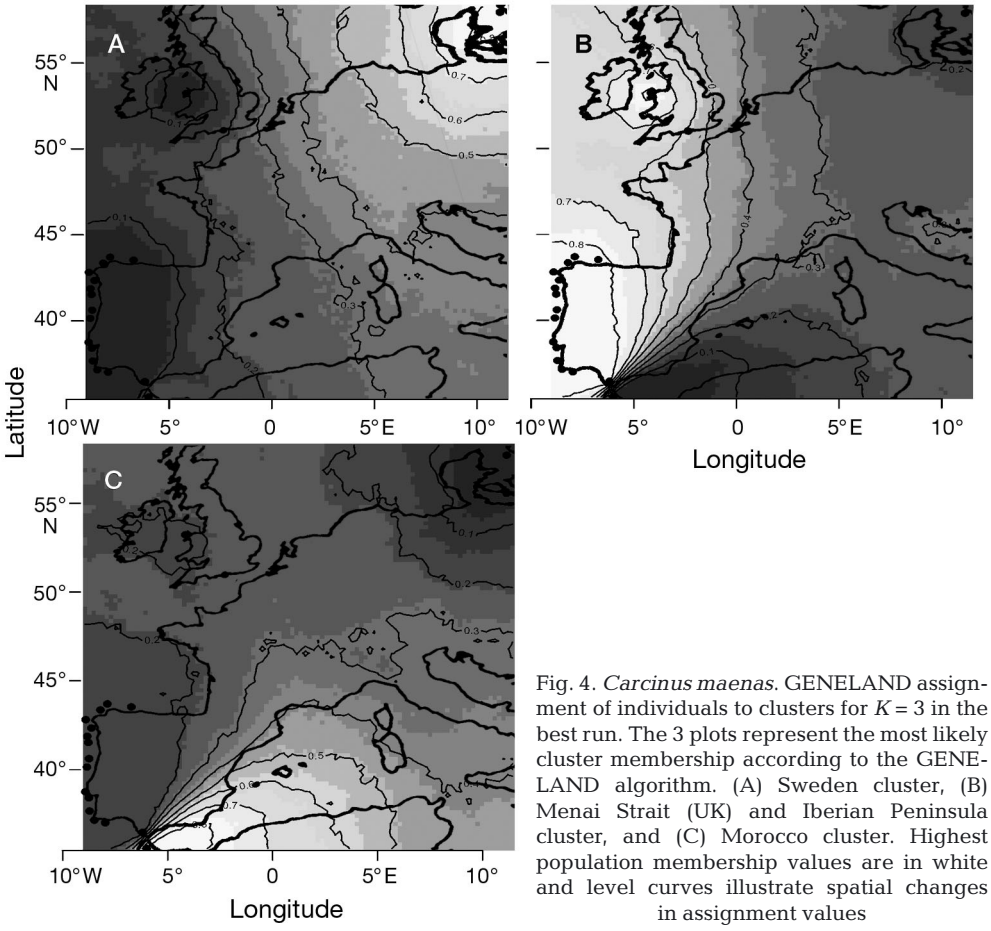

Fig. 4. *Carcinus maenas*. GENELAND assignment of individuals to clusters for $K = 3$ in the best run. The 3 plots represent the most likely cluster membership according to the GENELAND algorithm. (A) Sweden cluster, (B) Menai Strait (UK) and Iberian Peninsula cluster, and (C) Morocco cluster. Highest population membership values are in white and level curves illustrate spatial changes in assignment values

Table 4. *Carcinus maenas*. Summary statistics for 10 microsatellite loci for SWD, MEN-IP and MOR populations inferred from the cluster analyses. Significant F_{IS} values of the tests for heterozygosity deficiency, following Bonferroni correction (Rice 1989), are in **bold**. Asterisks: values that were significant before applying correction ($^{*}p < 0.05$; $^{**}p < 0.01$). n: sample size; A: allelic richness (estimated for n = 25); H_o: observed heterozygosity; H_e: expected heterozygosity; F_{IS} (f) and F_{ST} (θ) values calculated after Weir & Cockerham (1984)

Locus	Sweden (n = 50)				Menai Strait and Peninsula (n = 1362)				Morocco (n = 30)			
	A	H_o	H_e	F_{IS}	A	H_o	H_e	F_{IS}	A	H_o	H_e	F_{IS}
Cma01EPA	2.8	0.520	0.466	−0.117	3.3	0.415	0.415	−0.001	2.8	0.433	0.352	−0.236
Cma03EPA	6.9	0.780	0.742	−0.052	9.1	0.802	0.806	0.005	10.6	0.733	0.852	0.141*
Cma04EPA	14.0	0.920	0.909	−0.012	14.0	0.914	0.903	−0.012	12.5	0.900	0.909	0.010
Cma05EPA	2.0	0.420	0.357	−0.176	2.0	0.411	0.429	0.040	2.0	0.467	0.499	0.067
Cma08EPA	19.4	0.880	0.948	0.072*	19.9	0.948	0.952	0.004	19.8	0.833	0.951	0.125*
Cma09EPA	8.9	0.900	0.826	−0.089	8.4	0.770	0.787	0.022	9.7	0.724	0.813	0.111
Cma10EPA	25.5	0.960	0.963	0.003	23.3	0.951	0.959	0.009	19.0	0.960	0.952	−0.009
Cma14EPA	5.7	0.440	0.488	0.099	5.7	0.366	0.371	0.020	6.4	0.266	0.250	0.060
SP107	5.8	0.620	0.645	0.039	6.8	0.560	0.590	**0.051**	5.6	0.448	0.433	−0.036
SP495	4.4	0.540	0.616	0.124	5.2	0.619	0.649	0.047*	3.9	0.536	0.603	0.114
Overall	9.5	0.698	0.696	−0.003	9.8	0.675	0.686	**0.016**	9.3	0.628	0.663	0.053

such as high or low gene flow, and GENELAND spatial analysis may be a more sensitive approach especially when dealing with samples with very low levels of differentiation (Guillot 2008).

Our results suggest that shore crab larvae are not being exchanged continuously between the Sweden location and the remaining Atlantic samples. Such apparent isolation could arise from the geographical

separation of populations following an IBD pattern, as stated above. However, the pattern of IBD between the Menai Straits sample with the other samples is not observed (except with Morocco), even for geographic distances ranging up to 2700 to 3200 km (Fig. 2, white circles), the same distances that separates Sweden from some Atlantic locations. Such an observation indicates that, in the absence of barriers to gene flow, shore crab populations are genetically similar across thousands of kilometres. Therefore, in addition to geographical distance itself, the subdivision detected between Sweden and other sites is likely to be reinforced by regional current patterns and/or by the presence of an oceanographic barrier. The main inflow into the North Sea is from North Atlantic water that spreads along the shelf break into the Norwegian Trench. Atlantic water also enters the southern North Sea through the English Channel (Danielssen et al. 1997). These water inflows may transport larvae that originate in Atlantic populations. However, these larvae may be retained by the counter-clockwise gyre formed in the north-eastern end of the Skagerrak that can also block the inflow of waters from the southern part of the North Sea into the Skagerrak–Kattegat area (Danielssen et al. 1997). Such a scenario has been previously suggested to retain Norway lobster larvae *Nephrops norvegicus* along the Swedish west coast (Øresland 1998). Furthermore, the Norwegian Coastal Current, which represents the main outflow from the Skagerrak, flows predominantly northward along the west coast of Norway (Danielssen et al. 1997) and may be flushing larvae to northern areas. Additional sampling between Sweden and the UK is needed to clarify whether the differentiation of the Swedish sample was actually due to limited larval exchange among populations in accordance with geographical distances or to a genetic break.

Tide-related vertical migratory behaviour is present in several brachyuran larvae and is known to influence the direction and extent of horizontal transport in estuarine species, mainly preventing larvae from being retained inside the estuary or the shore (Queiroga et al. 1997, DiBacco et al. 2001, Bilton et al. 2002, Queiroga & Blanton 2005). Nevertheless, *Carcinus maenas* larvae from Skagerrak lack a tidal rhythm because they inhabit areas where tides are of very small amplitude and have little effect on water circulation (Queiroga et al. 2002). Since tidal vertical migration was demonstrated to be inherited in crabs from meso-tidal areas as in the British Isles (Zeng & Naylor 1996), a possible effect of Skagerrak isolation may be reflected in the loss of tidal rhythm behaviour in *C. maenas* larvae from this region. The coincidence of a genetic break between Sweden and the rest of the Atlantic populations with the split in genetically determined behavioural patterns is interesting and deserves further investigation. Reproductive isolation can be promoted in populations that developed under different environmental conditions (Palumbi 1994). With our data we can only speculate if the populations from Sweden are, or may become, reproductively isolated from populations from meso-tidal systems. However, if a significant level of reproductive isolation was achieved in the latter scenario, there would clearly be enhanced opportunities for speciation. A parallel example, supported by genetic studies (Geller et al. 1997, Roman & Palumbi 2004, Darling et al. 2008), is seen in the sibling species relationship between *C. maenas* and *C. aestuarii* from the Mediterranean Sea. The divergence between the 2 *Carcinus* forms was estimated to have commenced ~5 to 8 million yr ago (Roman & Palumbi 2004) and is probably maintained by the Almeria–Oran front (Patarnello et al. 2007), which prevents frequent migration of larvae. The divergence between the Skagerrak and the Atlantic *C. maenas* is much shallower and may be the result of recently separated populations that have had insufficient time to diverge compared to the Atlantic and the Mediterranean forms. Indeed, the eastern North Sea coastlines where the Skagerrak is inserted attained their present circulation ~8500 yr ago following periods of change during the late Pleistocene and Holocene that included the deglaciation of the region (Gyllencreutz et al. 2006).

The Moroccan sample is also distinct from the other samples in the GENELAND analysis and is more closely related to the Arade (F_{ST} = 0.0018, $p > 0.05$) and Formosa (F_{ST} = 0.0011, $p > 0.05$) samples in south Portugal, and more genetically distinct from the northernmost samples in Sweden (F_{ST} = 0.0289, $p < 0.001$) and the Menai Strait (F_{ST} = 0.0110, $p < 0.01$). Thus, IBD could be responsible for the genetic divergence of this sample from the European samples.

Although here we found evidence of restricted gene flow, it is worth highlighting the apparent genetic homogeneity observed among *Carcinus maenas* populations across oceanic distances as long as 3000 km, the approximate distance that separates Menai Straits from Cadiz Bay in SW Spain. These findings are consistent with weak genetic drift driven by presumed large effective population sizes, high fecundity and extended pelagic larval duration, which may translate into enhanced dispersal abilities. The presence of a larval export strategy from estuaries into shelf waters using selective tidal stream transport (Queiroga et al. 1997) enhances net transport of larvae between coastal populations that will drift with ocean currents. Also, *C. maenas* can spawn throughout winter and spring/summer seasons, depending on latitude, thereby ensuring that larvae are released under a wide variety of

oceanographic conditions. Other marine crabs with high larval dispersal capacity show similar patterns of low genetic differentiation over extensive spatial scales (McMillen-Jackson & Bert 2004, Pfeiler et al. 2005, Cassone & Boulding 2006, Ungfors et al. 2009). Furthermore, weak or a complete lack of genetic differentiation along the European Atlantic coast has also been documented for other invertebrate species with high dispersal potential, such as the sea urchin *Paracentrotus lividus* (Duran et al. 2004), the European lobster *Homarus gammarus* (Triantafyllidis et al. 2005), the netted dog whelk *Nassarius reticulatus* (Couceiro et al. 2007), the spiny spider crab *Maja brachydactyla* (Sotelo et al. 2008) and the velvet swimming crab *Necora puber* (Sotelo et al. 2009). Although it is apparent therefore that taxa exhibiting similar life-history parameters and high potential vagility may often show apparent panmixia along the Atlantic coast, exceptions continue to emerge (e.g. Shaw et al. 1999, Papetti et al. 2005, Diekmann et al. 2005). Such complexity is likely to arise from the interplay of local and regional oceanographic features with biological traits arising from variance in behaviour, developmental stage, predation and resource availability.

Comparison with previous studies

Previous studies of *Carcinus maenas* using mitochondrial DNA analysis indicated a significant genetic break between populations in Western and Northern Europe (Roman & Palumbi 2004, Darling et al. 2008), a pattern hereby confirmed with microsatellite markers. A recent study (Darling et al. 2008) examined genetic structuring of several native *C. maenas* samples in a global study of the invasion genetics of the genus *Carcinus* using 9 microsatellites, 8 of which were also employed in the present study. Darling et al. (2008) reported the lack of regional geographic structure as shown by pairwise F_{ST} and R_{ST} analysis among samples from Northern Europe (including 1 sample from Sweden) and Western Europe (including samples from Betanzos, Aveiro and Cadiz) and also failed to identify any clustering in the data when using the Bayesian algorithm implemented in STRUCTURE (Pritchard et al. 2000). Such findings contrast with those reported here, where *C. maenas* from the Swedish region were clearly distinct from all other samples. The performance of the analytical tests is known to be sensitive to sample size and number and variability at loci screened, especially in samples with low differentiation (Ruzzante 1998, Cornuet et al. 1999): an observation typical of many marine taxa with high gene flow. In the study of Darling et al. (2008), as few as 8 individuals were used in the analysis, potentially compromising their resulting observations. In our study, the employment of larger sample sizes are expected to enhance precision for differentiation estimators, as indicated by the power analysis, which yielded a power of >99% of detecting even low levels of population structure.

CONCLUSIONS

In summary, estimates of F_{ST} were generally low among *Carcinus maenas* populations, in common with many marine species, and as expected for highly polymorphic microsatellite markers regardless of population structure (Slatkin 1995). Furthermore, traditional *F* statistics augmented by Bayesian methods can prove useful in assessing genetic differentiation in such circumstances. Microsatellite data from *C. maenas* across its native range indicated the existence of genetically distinct populations across large geographic scales, providing evidence of a more complex population structure than suggested previously and confirming the utility of microsatellite markers in detecting subtle genetic differentiation in a highly mobile species.

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