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Genetic patterns across multiple introductions of the globally invasive crab genus *Carcinus*

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Abstract

The European green crab *Carcinus maenas* is one of the world's most successful aquatic invaders, having established populations on every continent with temperate shores. Here we describe patterns of genetic diversity across both the native and introduced ranges of *C. maenas* and its sister species, *C. aestuarii*, including all known non-native populations. The global data set includes sequences from the mitochondrial cytochrome *c* oxidase subunit I gene, as well as multilocus genotype data from nine polymorphic nuclear microsatellite loci. Combined phylogeographic and population genetic analyses clarify the global colonization history of *C. maenas*, providing evidence of multiple invasions to Atlantic North America and South Africa, secondary invasions to the northeastern Pacific, Tasmania, and Argentina, and a strong likelihood of *C. maenas* × *C. aestuarii* hybrids in South Africa and Japan. Successful *C. maenas* invasions vary broadly in the degree to which they retain genetic diversity, although populations with the least variation typically derive from secondary invasions or from introductions that occurred more than 100 years ago.

Keywords: admixture, *Carcinus*, genetic diversity, green crab, invasive species, multiple introductions

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Introduction

The rate of human-mediated introductions of non-native species to coastal and estuarine habitats has risen dramatically in the past century, concurrent with increases in the magnitude and efficiency of global maritime commerce (Carlton & Geller 1993; Cohen & Carlton 1998; Ruiz *et al*. 2000). The resulting disturbance to native ecosystems is now recognized as a fundamental aspect of global environmental change (Ricciardi 2007). Coastal environments face a host of anthropogenic stresses, including pollution, development, overexploitation of resources, climate change and sea level rise, and the physical disruption of habitat through dredging, leveeing, and other modifications (Kappel 2005). Given the accelerating growth of human populations impacting these systems, the additional stress of invasive species represents a significant challenge to the management of coastal resources (Williams & Grosholz 2008).

Of the vast number of marine invasive species, few have achieved the worldwide notoriety of the European green crab, *Carcinus maenas*. The species has a long and complex history of anthropogenic range expansion that began in the early 19th century with the establishment of the first known invasive population on the Atlantic Coast of the USA (Carlton & Cohen 2003). Since then, *C. maenas* has successfully established populations in Australia (in the 1890s), South Africa (1983), Japan (1984), the Pacific coast of North America (1989), the northern Canadian Maritimes (1991), Tasmania (1993), and Argentina (1999) (Carlton & Cohen 2003; Hidalgo *et al*. 2005; Roman 2006). A number of transport vectors have been implicated in these events, including natural dispersal, solid ballast, hull and equipment fouling, ballast water, and contaminated packing material shipped with commercial shellfish (Carlton & Cohen 2003). Patterns of expansion subsequent to establishment have varied widely; whereas some populations have spread rapidly from their initial point of introduction (Grosholz

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& Ruiz 1995; Yamada *et al*. 2005), others have remained relatively stationary (Hampton & Griffiths 2007). Once established, green crabs are capable of causing substantial and costly impacts to recipient ecosystems (Grosholz *et al*. 2000; Walton *et al*. 2002).

The native range of *C. maenas* extends along the Atlantic coast of Europe as far north as Norway, including the British Isles and Iceland, and along the northern Atlantic coast of Africa to Mauritania (Yamada 2000; Carlton & Cohen 2003). This range abuts that of *C. aestuarii*, the only known congener of *C. maenas*, which is native throughout the Mediterranean Sea (Yamada 2000; Carlton & Cohen 2003). The two taxa are distinguishable by morphological traits (Yamada & Hauck 2001), and their status as independent species has been supported by genetic analysis (Roman & Palumbi 2004). While *C. maenas* has established throughout the world, *C. aestuarii* has also been recorded beyond its native range, in South Africa and Japan (Geller *et al*. 1997; Carlton & Cohen 2003). There is no known case of *C. aestuarii* establishing independently of its congener, and the suggestion has been made that *maenas* × *aestuarii* hybrids may exist in poorly studied portions of the native range, or may even have formed in introduced populations following establishment of both species (Bagley & Geller 2000; Geller *et al*. 1997). Although hybridization between introduced taxa has been recognized to enhance invasiveness in some plant systems (Ellstrand & Schierenbeck 2006), the role of this process in animal systems remains poorly studied.

Attempts to understand the mechanisms driving successful marine biological invasions have considered various factors, including the ecological and evolutionary responses of established populations (Sax & Brown 2000; Sax *et al*. 2007) and the crucial roles of vectors of introduction (Drake & Lodge 2004; Ricciardi 2006), propagule pressure (Lockwood *et al*. 2005; Colautti *et al*. 2007) and multiple introductions (Roman & Darling 2007). Recently, it has been recognized that phylogeographic and population genetic analyses can provide crucial insight into patterns of introduction, colonization, and spread of invasive taxa, helping to lay a foundation for deeper understanding of the mechanisms underlying invasion dynamics (Geller 1996; Holland 2000; Sakai *et al*. 2001; Lee 2002). For instance, genetic analysis has played a key role in determining the likely introduction pathways for invasive pests, providing valuable information to those tasked with limiting risks of future introductions (Plantard *et al*. 2008; Bonizzoni *et al*. 2004; Aketarawong *et al*. 2007). In addition, genetic study has advanced understanding of complex patterns of post-establishment spread (Estoup *et al*. 2004; Viard *et al*. 2006), generating important insights relevant to both natural range expansions and expansions recently stimulated by accelerating climate change (Phillips *et al*. 2008).

The degree to which genetic diversity is preserved in introduced populations has also garnered much attention. Genetic studies have in some cases allowed researchers to assess strong genetic bottlenecks and estimate numbers of founders (Ficetola *et al*. 2008), informing risk assessment and management strategy; in others, they have revealed important relationships between genetic diversity and key morphological and life-history traits (Kolbe *et al*. 2007b; Facon *et al*. 2008), providing insights into the potential for local adaptation and invasion success. The possibility that reductions in genetic diversity may limit the potential invasiveness of introduced populations has been raised repeatedly. Although comparisons among invasive taxa have been enlisted to address this hypothesis (Wares *et al*. 2005; Roman & Darling 2007; Dlugosch & Parker 2008), examination of multiple invasions by species such as *C. maenas* may also be informative. Investigation of such cosmopolitan species can reveal common patterns across independent introductions and provide general insight into the correlates of invasion success (Voisin *et al*. 2005).

Despite substantial effort to understand the ecology, history and impacts of *C. maenas* invasions (Yamada 2000; Carlton & Cohen 2003), there has been no comprehensive examination of the genetic patterns associated with this species' global range expansion. Here we build substantially on earlier research (Bagley & Geller 2000; Geller *et al*. 1997; Roman & Palumbi 2004; Roman 2006) to describe genetic patterns throughout the native and invasive ranges of *C. maenas* and its congener *C. aestuarii*, based on both mitochondrial cytochrome *c* oxidase subunit I (COI) sequences and nine highly polymorphic nuclear microsatellite loci. There were three aims for this work: (i) to extend previously described analysis of mitochondrial DNA population structure in the native range (Roman & Palumbi 2004) using both mitochondrial and nuclear DNA data, (ii) to infer colonization histories by exploring the ancestry of introduced populations, and (iii) to compare the genetics of introduced populations and their sources and investigate the role of genetic diversity in invasion success. We relate the results of these analyses to historical knowledge of *Carcinus* invasions and discuss how colonization histories can complicate the search for general patterns across invasion events.

Methods

Molecular methods

Live crabs were collected from 39 sites throughout the native and introduced ranges of *Carcinus maenas* and *C. aestuarii* (Table 1). Specimens were frozen at –20 °C or preserved in 70–95% ethanol for DNA extraction. For samples collected prior to 2000, DNA was extracted from frozen gill tissue using the protocol of Geller *et al*. (1997). Prior to polymerase chain reaction (PCR) amplification, all DNA samples were further purified using DNeasy Tissue

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Table 1 Summary of *Carcinus* collections used in the current study

Sample	Collection location	Region	n_{mtDNA}	n_{msat}	Species
ICE	Seltjarnarnes, Iceland	Off-shelf Europe	18	0	CM
TOR	Torshavn, Faroe Islands		20	19	CM
TRO	Trondheim, Norway	Northern Europe	3	0	CM
MON	Mongstadt, Norway		22	14	CM
OSL	Oslo, Norway		9	8	CM
GOT	Goteborg, Sweden		15	15	CM
HEL	Helgoland, Germany		5	0	CM
BRE	Bremerhaven, Germany		17	17	CM
HOE	Hoek van Holland, the Netherlands		19	19	CM
NET	Den Helder, the Netherlands		45	44	CM
FOW	Fowey, England	Western Europe	14	14	CM
WIG	Isle of Wight, England		4	3	CM
BIL	Bilbao, Spain		15	15	CM
BET	Betanzos, Spain		14	13	CM
AVE	Aveiro, Portugal		24	22	CM
CAD	Cadiz, Spain		47	42	CM
PAL	Palmones, Spain		9	8	CM
BAN	Banyuls-sur-mer, France	Mediterranean Europe	17	16	CA
NAP	Naples, Italy		28	25	CA
			341	294	Total
			18.1	18.4	Mean*
Introduced					
MUR	Murphy's Cove, Nova Scotia, Canada	Nova Scotia	20	20	CM
BAR	Barnstable Harbor, MA, USA	Eastern USA	20	19	CM
MYS	Mystic Harbor, CT, USA		27	28	CM
SFB	San Francisco, CA, USA	Western USA	24	0	CM
RED	Redwood Shores Lagoon, CA, USA		28	35	CM
BOD	Bodega Bay, CA, USA		16	0	CM
TOM	Tomales Bay, CA, USA		0	50	CM
WIL	Willapa Bay, OR, USA		21	123	CM
YAQ	Yaquina Bay, OR, USA		6	0	CM
LEI	Little Espinosa Inlet, BC, Canada		3	0	CM
CAP	Cape Town, South Africa	South Africa	50	51	CM/CA
COR	Coronet, Australia	Australia	21	11	CM
CLI	Clifton Beach, Australia		18	0	CM
FAL	Falmouth, Tasmania	Tasmania	31	31	CM
DOK	Dokai Bay, Japan	Japan	22	18	CM/CA
TOK	Tokyo Bay, Japan		60	50	CM/CA
TUF	Tokyo University of Fisheries, Japan		15	13	CM/CA
JAP	Tokyo Bay, Japan		66	63	CM/CA
SHI	Shinhama Bay, Japan		15	15	CM/CA
ARG	Caleta Carolina, Argentina	South America	15	11	CM
			478	538	Total
			23.9	33.6	Mean*

n, number of individuals tested (mtDNA/microsatellite); CM, *C. maenas*; CA, *C. aestuarii*. Sample sizes are given for both mitochondrial COI data and microsatellite data; *, calculation of mean sample sizes does not include samples with $n = 0$.

Kits (QIAGEN). For samples collected after 2000, DNA was extracted from frozen gill or ethanol-preserved muscle tissue using the DNeasy Tissue Kit according to the manufacturer's protocol.

A fragment of the mitochondrial COI gene was amplified from 822 individuals. For *C. maenas*, PCR amplification of COI was conducted using universal primers LCO1490 (GGTCAACAAATCATAAAGATATTGG) and HCO2198 (TAAACTTCAGGGTGACCAAAAAATCA) (Folmer *et al*. 1994); for *C. aestuarii*, these primers failed to amplify consistently, and degenerate versions COIF-PR115 (TCWACNAAYCAYAARGAYATTGG) and COIR-PR114 (ACYTCNGGRTGNCCRAARARYCA) were used. PCR cycling parameters consisted of an initial denaturation step

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at 94 °C for 5 min followed by 35 cycles of 30 s at 94 °C, 60 s at 50 °C, and 60 s at 72 °C, with a final 15-min extension at 72 °C. COI amplification products were sequenced in both forward and reverse directions using amplification primers.

Analysis of nuclear genetic diversity was performed with nine microsatellite loci. Eight of these loci, and conditions for their amplification, have been previously described (Tepolt *et al*. 2006): Cma01EPA, Cma02EPA, Cma03EPA, Cma04EPA, Cma05EPA, Cma08EPA, Cma09EPA, and Cma14EPA. One additional locus, Cma16EPA (primer sequences: F-TTCAGTCCTACCCAAACATCG and R-CTGTCGTAATTAAAAAGTGCTTGG; annealing temperature, 52 °C), designed and screened as in Tepolt *et al*. (2006), was also used. To confirm genotyping accuracy, 5% of samples, chosen at random, were rerun. Samples which failed to amplify at three or more loci after two attempts were dropped from the data set, leaving 832 specimens in the final data set. For seven collection sites (ICE, TRO, HEL, BOD, YAQ, and LEI), discarding poorly amplifying individuals (fewer than six of nine loci successful) resulted in a complete lack of microsatellite data.

Data analysis

Mitochondrial COI sequences were aligned and edited using Clustal_X (Thompson *et al*. 1997), and 481 bp of overlapping sequence were utilized for all subsequent analyses. We included 95 haplotypes in our analyses; of these, 53 derived from an earlier study on *Carcinus* in its native range (Roman & Palumbi 2004), and 42 were novel. All haplotypes have been deposited in GenBank (Accession nos FJ159008 to FJ159102). All microsatellite data were new to this study. Maximum parsimony analysis was conducted in PAUP* version 4.0 (Swofford 2003), employing a heuristic search with tree-bisection–reconnection (TBR) branch swapping, and a minimum-spanning haplotype tree was constructed manually based on relationships assessed using Arlequin version 3.0 (Excoffier *et al*. 2005). Arlequin was also used to conduct hierarchical analysis of molecular variance (AMOVA) based on mtDNA pairwise uncorrected genetic distance for all *C. maenas* populations in the native range of the species. Molecular variance was partitioned both among populations and among the following biogeographic regions as defined by Roman & Palumbi (2004): Northern Europe, including Germany and Scandinavian populations; Western Europe, including the Netherlands, England, and Atlantic Iberian populations; and the populations outside the continental shelf ('off-shelf') in the Faeroe Islands and Iceland. Similar AMOVA was conducted with microsatellite data based on the same hierarchical geographic structure.

Pairwise Cavalli-Sforza & Edwards chord distances (D_{CE}), proportion of shared alleles, and F_{ST} were calculated based on microsatellite data for all global populations using Microsatellite Analyser (Dieringer & Schlötterer 2002), and pairwise R_{ST} values were calculated using R_{ST}Calc version 2.2 (Goodman 1997). All genetic distance measures were calculated with 1000 randomized permutations to assess statistical support. As one means of assessing likely sources for introduced populations, relatedness trees were constructed based on chord distances using the neighbour-joining algorithm, and a majority rule bootstrap consensus tree was built using the programs Neighbor and Consense in PHYLIP version 3.65 (Felsenstein 1989). Neighbour-joining trees were drawn with the software Unrooted (Perrière & Gouy 1996).

As an alternative means of determining likely ancestry of introduced populations, we assessed genetic structure using a Bayesian clustering approach implemented in the software Structure (Falush *et al*. 2003). To determine the most likely number of clusters (K) supported by the microsatellite data, we conditioned our data on values of K ranging from 1 to 8. In preliminary analyses, values of K greater than 8 consistently resulted in likelihoods equal to or lower than those observed at lower values of K, and thus, these values were not considered in our full analyses in the interest of reducing computation time. For each value of K, five independent Markov chain Monte Carlo (MCMC) runs were conducted, with 100 000 generations discarded as burn-in followed by an additional 1 million generations. We ran models with the total data set (including all native and non-native populations from both *Carcinus* species), all *C. maenas* populations (excluding both native *C. aestuarii* and populations in Japan and Cape Town where C. *aestuarii* haplotypes exist), and only native *C. maenas* populations. We adopted two different approaches to determine the value of K most likely to indicate underlying genetic structure. The first is that of Garnier *et al*. (2004), who recommend choosing the value of K that captures the major structure in the data by assessing the successive increases in posterior probability as K increases; the value of K that precedes a plateau in the plot of posterior probability vs. K is thus chosen as the model most consistent with the data. We also utilized the statistic ΔK of Evanno *et al*. (2005), which calculates the mean of the absolute value of the second order rate of change in the posterior probability (assessed over multiple MCMC runs, in our case five), divided by the standard deviation of the posterior probability.

For all global populations, the number of haplotypes, the effective number of haplotypes, and gene diversity were determined in Arlequin, mean number of alleles, mean allelic richness, and expected heterozygosity at microsatellite loci were determined using FSTAT version 2.9.3.2 (Goudet 2001), and maximum uncorrected pairwise distances between COI haplotypes (P_{max}) was calculated using MEGA version 3.1 (Kumar *et al*. 2004). For allelic richness calculations using rarefaction in FSTAT, the population

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from Isle of Wight (WIG) was removed because of low sample size. For each diversity measure, mean values over all native *C. maenas* populations, all native *C. aestuarii* populations, and all introduced populations were calculated. Introduced populations in Australia (COR and CLI), eastern USA (BAR and MYS), western North America (SFB, RED, BOD, TOM, WIL, YAQ, and LEI), and Japan (DOK, TOK, TUF, JAP, and SHI) were also grouped into regions for determination of mean genetic diversity estimates across those regions. Significance of overall differences in diversity measures between native and introduced *C. maenas* populations were assessed by nested ANOVA, with regional populations nested within either native or introduced groups; a single region was assigned to all native *C. maenas* populations. In addition, significance of differences between individual regional populations was determined using unpaired Student's *t*-tests. With the exception of P_{max} comparisons, Japanese populations were not included in these tests, given the clear presence of both *C. maenas* and *C. aestuarii* in those populations. Statistical tests were performed in JMP version 7 (SAS).

Results

Geographic patterns of haplotype diversity

Hierarchical AMOVA based on mitochondrial data for native *Carcinus maenas* populations reveals that most of the genetic variance is found within sampling sites; however, a large and significant fraction (47.92%, $P < 0.0001$) was also found to partition among biogeographic groups (Table 2). When the off-shelf region is removed from the analysis, the variation observed among groups drops precipitously to 2.96%, although this value is still significant (P = 0.009).

Globally, the most common native *C. maenas* haplotype (observed in all populations except the off-shelf populations of the Faeroes and Iceland, the Isle of Wight, and Betanzos) was also the most common haplotype observed in introduced populations (Figs 1 and 2, dark red); in fact, this was the only *C. maenas* haplotype found in western North America, Argentina, and Japan, and represented all but one of 47 individuals found in the eastern USA. Details on the distribution of haplotypes among all samples are available in Appendix S1, Supporting Information.

The distribution of *Carcinus aestuarii* COI haplotypes in the native range suggests little geographic structure for this species, as the population at Naples consists of divergent haplotypes from both *C. aestuarii* clades (dark green and light green in Figs 1 and 2), resulting in an elevated value of P_{max} (Table 3). *C. aestuarii* haplotypes were found in Japan, where they represented 69% of individuals regionally, and also in South Africa, where a single *C. aestuarii* haplotype was observed among 50 sampled individuals.

Table 2 Hierarchical AMOVA based on mtDNA (top) and microsatellite (bottom) data for *C. maenas* populations in the native range. Analyses were conducted both with and without samples from the off-shelf populations in the Faeroe Islands and Iceland. Significant fixation indices are indicated with asterisks

	With Faeroes and Iceland			Without Faeroes and Iceland		
	Variance components	Percentage of variation	Fixation indices	Variance components	Percentage of variation	Fixation indices
Among groups	0.94493	47.92	0.47918*	0.03274	2.96	0.02958*
Among populations within groups	0.03181	1.61	0.03098*	0.01089	0.98	0.01014
Within populations	0.99523	50.47	0.49531*	1.06312	96.06	0.03942*

	With Faeroes and Iceland			Without Faeroes and Iceland		
	Variance components	Percentage of variation	Fixation indices	Variance components	Percentage of variation	Fixation indices
Among groups	0.02908	1.63	0.01628*	0.01733	0.96	0.00961*
Among populations within groups	0.00842	0.47	0.00480*	0.00758	0.42	0.00424*
Within populations	1.74793	97.90	0.02100*	1.77966	98.62	0.01380*

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Table 3 Diversity measures for native and introduced *Carcinus* populations at microsatellite and mtDNA (COI) markers

	Microsatellite				mtDNA			
Sample	n	N_A	A	H_E	n	N_e	h	P_{max}
Native *C. maenas*	**253**	**8.55**^{AB}	**4.35**^{A}	**0.7098**^{A}	**289**	**4.25**^{A}	**0.7619**^{A}	**0.0101**^{C}
ICE	0	—	—	—	3	1.00	0.0000	0.0000
TOR	19	6.22	3.46	0.5963	20	2.15	0.5632	0.0082
TRO	0	—	—	—	3	3.00	1.0000	0.0100
MON	14	6.56	4.16	0.6855	24	6.26	0.8768	0.0120
OSL	8	6.00	4.34	0.7372	9	3.52	0.8056	0.0139
GOT	15	8.89	4.48	0.7430	15	7.76	0.9333	0.0139
HEL	0	—	—	—	5	1.47	0.4000	0.0020
BRE	17	8.33	4.33	0.7102	17	5.45	0.8676	0.0129
HOE	19	9.67	4.46	0.7084	19	4.35	0.8129	0.0100
NET	44	11.78	4.31	0.7055	45	4.28	0.7838	0.0144
FOW	14	8.33	4.44	0.7204	14	5.76	0.8901	0.0100
WIG	3	—	—	—	4	4.00	1.0000	0.0144
BIL	15	7.67	4.25	0.7110	15	2.53	0.6476	0.0080
BET	13	9.00	4.64	0.7318	14	3.38	0.7582	0.0082
AVE	22	9.56	4.38	0.7201	24	4.50	0.8116	0.0090
CAD	42	11.89	4.43	0.7097	48	6.66	0.8679	0.0145
PAL	8	7.22	4.83	0.7486	10	6.25	0.9333	0.0100
Native *C. aestuarii*	**41**	**8.83**	**4.26**	**0.6481**	**45**	**4.73**	**0.7035**	**0.02265**^{B}
BAN	16	7.78	4.10	0.6380	17	1.91	0.5074	0.0082
NAP	25	9.89	4.43	0.6582	28	7.54	0.8995	0.0371
Introduced	**538**	**4.88**	**3.08**	**0.5688**	**478**	**1.68**	**0.2602**	**0.0326**
Nova Scotia								
MUR	20	6.22^{ABC}	3.89^{ABC}	0.6704^{AB}	20	3.17^{ABC}	0.7211^{AB}	0.0080^{CD}
Eastern North America	***47***	***5.78***^{C}	***3.48***^{CD}	***0.6550***^{AB}	***47***	***1.04***^{BC}	***0.0371***^{C}	***0.0020***^{D}
BAR	19	5.67	3.53	0.6705	20	1.00	0.0000	0.0000
MYS	28	5.89	3.42	0.6395	27	1.08	0.0741	0.0040
Western North America	***208***	***5.30***^{C}	***3.16***^{DE}	***0.6232***^{B}	***98***	***1***^{C}	***0.0000***^{C}	***0.0000***^{D}
SFB	0	—	—	—	24	1.00	0.0000	0.0000
RED	35	5.22	3.16	0.6269	28	1.00	0.0000	0.0000
BOD	0	—	—	—	16	1.00	0.0000	0.0000
TOM	50	5.11	3.12	0.6153	0	—	—	—
WIL	123	5.56	3.19	0.6274	21	1.00	0.0000	0.0000
YAQ	0	—	—	—	6	1.00	0.0000	0.0000
LEI	0	—	—	—	3	1.00	0.0000	0.0000
South Africa								
CAP	51	10.89^{A}	4.30^{AB}	0.6741^{AB}	50	4.86^{AB}	0.8106^{AB}	0.0994^{A}
Australia					***39***	***3***^{ABC}	***0.7024***^{AB}	***0.0083***^{CD}
COR	11	4.89^{BC}	3.68^{BCD}	0.6619^{AB}	21	2.88	0.6857	0.0083
CLI	0	—	—	—	18	3.12	0.7190	0.0083
Tasmania								
FAL	31	4.33^{C}	2.55^{EF}	0.4909^{C}	31	1.30^{ABC}	0.2409^{BC}	0.0062^{CD}
Argentina								
ARG	11	2.56^{C}	2.35^{F}	0.4802^{C}	15	1.00^{ABC}	0.0000^{C}	0.0000^{CD}
Japan	***159***	***3.38***	***2.60***	***0.4751***	***178***	***1.51***	***0.3339***	***0.0969***^{A}
DOK	18	2.67	2.37	0.4355	22	1.31	0.2468	0.0969
TOK	50	3.78	2.68	0.4902	60	1.47	0.3254	0.0969
TUF	13	3.11	2.65	0.4917	15	1.99	0.5333	0.0969
JAP	63	4.00	2.65	0.4727	66	1.50	0.3394	0.0969
SHI	15	3.33	2.63	0.4855	15	1.30	0.2476	0.0969
F		**14.3899**	**63.8701**	**43.8089**		**10.1294**	**22.7657**	**183.5634**
P value		**0.0018**	**< 0.0001**	**< 0.0001**		**0.0041**	**< 0.0001**	**< 0.0001**

n, number of individuals; N_A, number of alleles; A, allelic richness; H_E, expected heterozygosity; N_e, effective number of haplotypes; h, gene diversity; P_{max}, the maximum observed uncorrected pairwise distance. Total sample sizes and mean values for diversity measures are shown in bold for native *C. maenas*, native *C. aestuarii*, and introduced populations. For those regions in the non-native range with multiple populations tested, total sample sizes and mean values for regional diversity measurements are shown in bold italics. F ratios and P values for overall comparisons between native and introduced populations (as tested by ANOVA) are shown. For each diversity index, superscript letters connect regional samples not significantly different at the $\alpha = 0.05$ level, as determined by Student's t-tests. For allelic richness calculations, the Isle of Wight population (WIG) was removed from the analysis due to low sample size. Populations from Japan and *C. aestuarii* were included only in comparisons of P_{max}.

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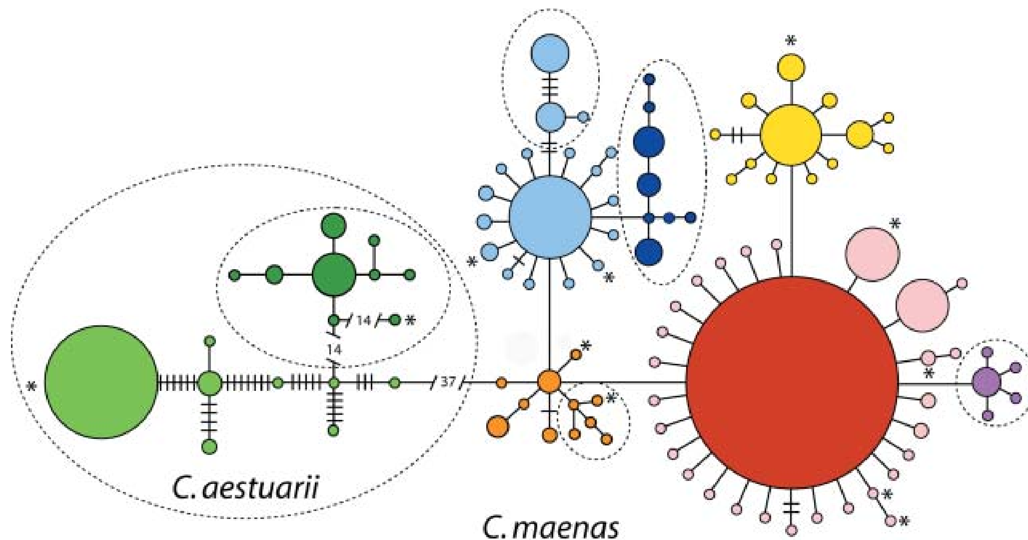

Fig. 1 COI haplotype network for global *Carcinus* populations. Each coloured circle represents one haplotype observed in the global data set; circles are scaled to number of individuals observed with that haplotype. Each connection represents a single nucleotide difference, and thus, connection lengths are not to scale. Additional nucleotide differences between haplotypes are indicated by hashes, or by numbers in the case of widely divergent haplotypes. Haplotypes observed only in introduced populations are marked with asterisks. Haplotypes in green are *C. aestuarii*, all others are *C. maenas*. Dashed lines indicate clades well-supported in maximum parsimony analysis (> 90% majority rule consensus support).

No non-native population consisted solely of *C. aestuarii*, and in total, only two *C. aestuarii* haplotypes were found outside of the species' native range. The identity of these two haplotypes was assessed by phylogenetic analysis: while both clustered with 100% bootstrap support within a clade consisting solely of native *C. aestuarii* COI sequences, neither were observed in the native range and both were divergent from native haplotypes, the Japanese haplotype by 7 substitutions, the South African by 14 (Fig. 1).

Introduced populations in Nova Scotia, South Africa, and Australia possessed a high proportion of haplotypes most commonly observed in northern European populations within the native range. In Nova Scotia, a single haplotype comprising 30% of the population belonged to a well-supported haplotype group occurring solely in populations north of the Netherlands (shown in dark blue, Figs 1 and 2); this same haplotype was observed in the native range only in Mongstadt, a centre for Norwegian oil trade. In Cape Town, a single individual possessed a haplotype belonging to the same northern European clade, appearing in all native populations from Germany through Scandinavia but nowhere else within the native range.

Geographic patterns of microsatellite diversity

Despite the geographic structuring of COI haplotypes within the native range of *C. maenas*, we found considerably weaker evidence of native structure when analysing microsatellite data. Pairwise F_{ST} values were generally low, and after Bonferroni correction, there were few pairwise comparisons that indicated significant genetic differentiation. These were dominated by comparisons between the off-shelf population at Torshavn, Faeroe Islands, and other native populations; all such comparisons were significant, with F_{ST} values ranging from 0.0431 (with Betanzos, Spain) to 0.0955 (with Goteborg, Sweden). Among 66 other comparisons, only 7 were significant after correction; these showed little geographic pattern, with 4 occurring between biogeographic regions and 3 within regions. Differentiation was even less evident when utilizing an estimator that assumes a high-rate stepwise mutation model for the microsatellites (R_{ST}), as no pairwise comparisons were significant after correction for multiple tests. The lack of regional geographic structure relative to that observed with mtDNA data was also evidenced by AMOVA (Table 2), which revealed only 1.63% of genetic variance at microsatellite loci partitioning among biogeographic regions; this value dropped to 0.96% when the Torshavn population was removed from the analysis. Both values did remain significant, however ($P < 0.0001$ and $P = 0.00978$, respectively). In addition, the Bayesian algorithm implemented in Structure failed to predict any hypothetical clusters when run on a data set consisting solely of individuals from the native range of *C. maenas* (not shown).

Neighbour-joining analysis based on Cavalli-Sforza & Edwards chord distance (D_{CE}) reveals a number of well-supported groupings of introduced populations (Fig. 3). For the most part, these represent regional clusters, with

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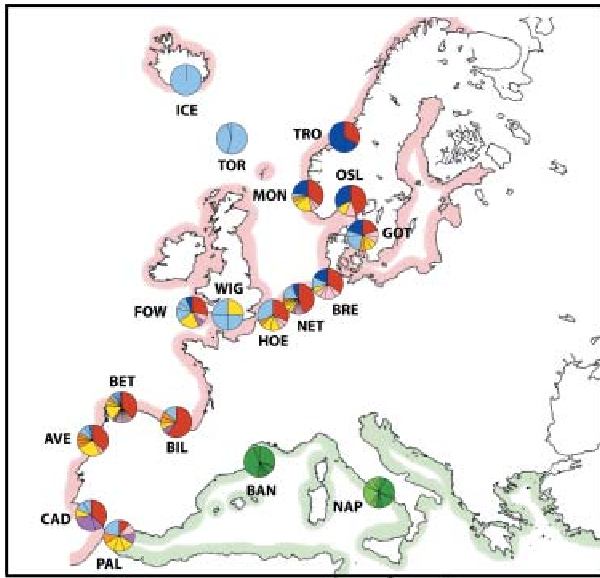

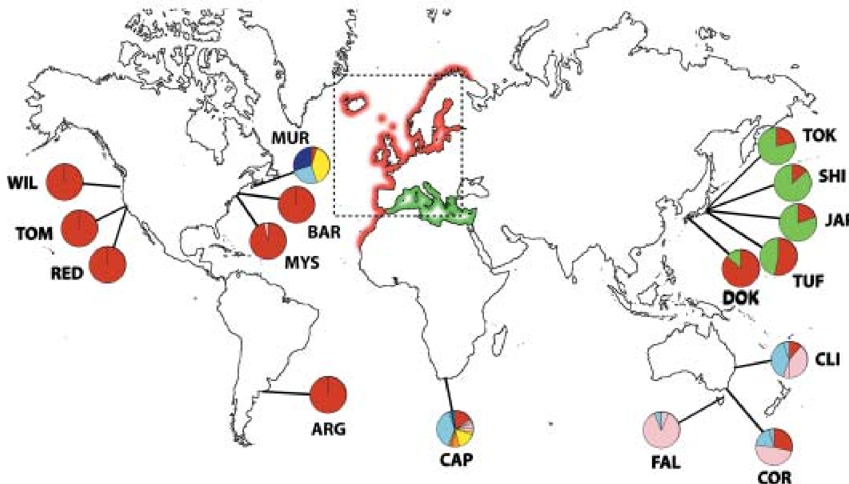

Fig. 2 Distribution of COI haplotypes in the native range (inset) and in introduced populations. The known native range of *Carcinus maenas* is shaded in red, that of *Carcinus aestuarii* in green. Pie charts indicate proportion of haplotype groups observed at each site (colours correspond to those shown in Fig. 1; dark red indicates the single most common haplotype).

populations from North America, Japan, and Australia/ Tasmania forming groups all with bootstrap support greater than 90%. The two sibling species are also well differentiated. The Cape Town population falls within a poorly resolved cluster including all native *C. maenas* populations, whereas the introduced population from Argentina groups most closely with the Australian and Tasmanian populations (70% bootstrap support). The population from Nova Scotia shows no strong affiliation with any of the well-supported clusters, and falls outside of the native *C. maenas* cluster. Neighbour-joining analysis using the proportion of shared alleles as a distance metric produced an almost identical tree topology, with similar bootstrap supports (not shown).

Bayesian clustering analysis reveals similar structure in the microsatellite data. When we considered *C. maenas* alone, both methods of determining the most likely value of K indicated the presence of three distinct clusters. The three hypothetical clusters correspond to: (i) one group including native populations and Nova Scotia; (ii) one including all North American populations outside of Nova Scotia; and (iii) one including populations from Australia, Tasmania, and Argentina (Fig. 4A). When all data were considered, the ΔK statistics predicted four clusters. However, we considered this estimate overly conservative for two reasons. First, the posterior probability continued to increase between $K = 4$ and $K = 5$, despite a sharp decrease in its second order rate of change. Second, the two sibling species were barely distinguishable in the model when $K = 4$, and *C. aestuarii* only clearly formed an independent cluster when $K = 5$. The clear distinction of *C. maenas* and *C. aestuarii* when $K = 5$ provides a biologically relevant argument for accepting this model over the more conservative model. At $K = 5$, populations in Japan, North America (outside of

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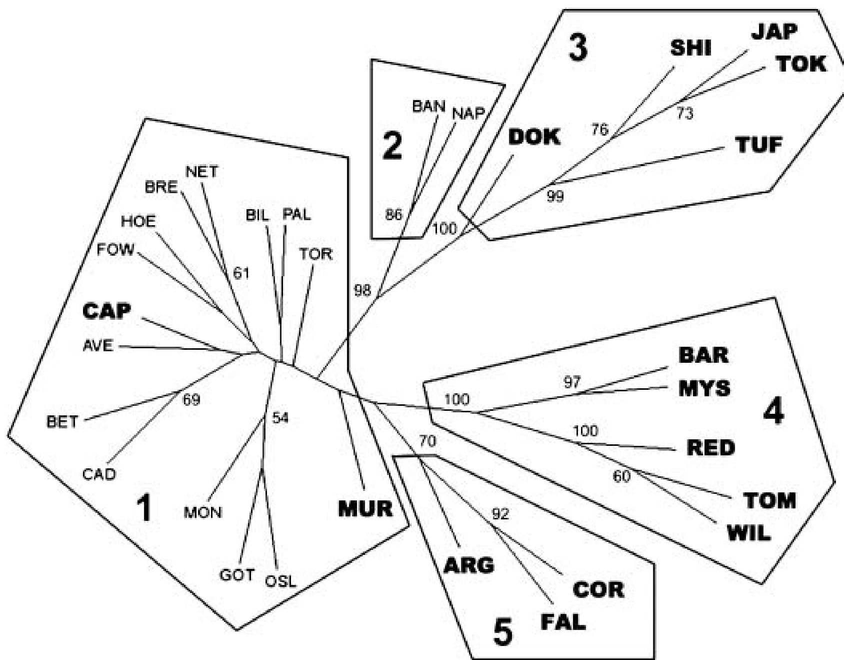

Fig. 3 Neighbour joining tree based on Cavalli-Sforza & Edwards chord distance (D_{CE}). Nodal support is given in per cent of bootstrap replicates (1000 total); only values greater than 50% are shown. Polygons indicate inferred population clusters as determined by Bayesian analysis for all global populations (see Fig. 4B); numbers correspond to cluster definitions shown in Table 4. Tip labels correspond to collection sites in Table 1; introduced populations are labelled in bold.

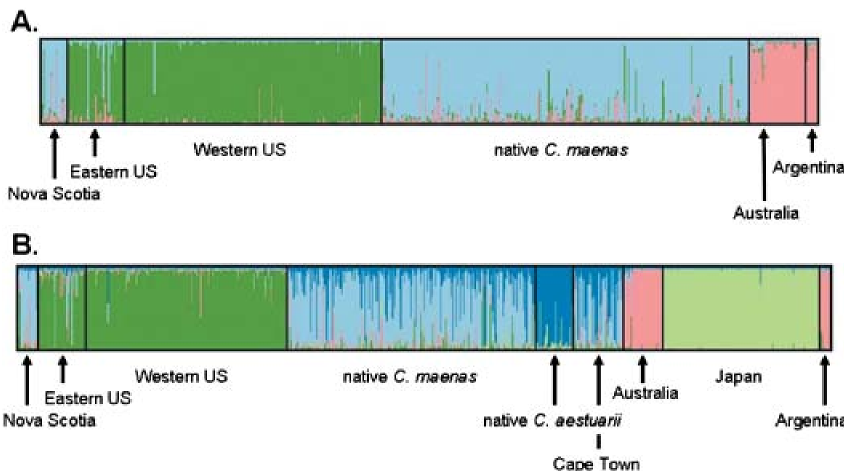

Fig. 4 Results of Bayesian clustering analysis. Each individual is represented by a vertical bar in *K* coloured segments, where *K* is the number of clusters and the length of the segment is proportional to the individual's membership in the corresponding cluster. The run (out of five replicates) with the highest posterior probability is shown. Black vertical bars bisecting the plots delineate regional populations. (A) Structure runs conducted only with *Carcinus maenas* data, with $K = 3$. (B) Structure runs on all populations, with $K = 5$.

Nova Scotia), and Australia (with Tasmania) form three clearly distinct clusters (Fig. 4B). A fourth cluster comprises all native *C. maenas* populations, while a fifth includes native *C. aestuarii*. The population from Argentina clusters with the Australian populations (average co-ancestry within this cluster $\hat{q} = 0.912$; see Table 4), while the population from Nova Scotia shows greatest affinity for the native *C. maenas* cluster ($\hat{q} = 0.782$). Although samples from Cape Town appear to cluster primarily with native *C. maenas* ($\hat{q} = 0.477$), there is indication of significant contributions from native *C. aestuarii* ($\hat{q} = 0.402$).

Genetic diversity in introduced populations

Overall, genetic diversity was significantly lower in introduced populations of *C. maenas* than in native ones. Differences in all five diversity metrics in both nuclear and mitochondrial loci were highly significant (Table 3). However, genetic diversity did vary widely in introduced populations. Only a single mitochondrial haplotype was present in both western North America ($n = 208$) and Argentina ($n = 15$). In the eastern USA, only a single individual was observed with a haplotype other than the most common one ($n = 47$). These populations showed significantly less gene diversity (h) than introduced populations in Nova Scotia, South Africa, and Australia, which were not significantly different from native populations. The population from Tasmania showed intermediate diversity at the COI locus ($n = 31$, $N_e = 1.30$, $h = 0.2409$). Similar patterns were observed for nuclear microsatellite data. Differences between native and introduced populations were most clearly apparent in

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Table 4 Summary of Structure results. Average co-ancestry distributions over five hypothetical clusters (as shown in Fig. 4B). Clusters also correspond to those indicated in Fig. 3: 1. USA; 2. Australia; 3. Japan; 4. native *C. maenas*; 5. native *C. aestuarii*

		Cluster				
Region	Site	1	2	3	4	5
Nova Scotia	MUR	0.065	0.087	0.011	0.782	0.055
Eastern USA	BAR	0.845	0.046	0.013	0.076	0.021
	MYS	0.797	0.063	0.008	0.106	0.026
Western USA	RED	0.943	0.013	0.011	0.013	0.019
	TOM	0.955	0.015	0.012	0.011	0.008
	WIL	0.946	0.024	0.009	0.011	0.009
South Africa	CAP	0.045	0.058	0.018	0.477	0.402
Australia	COR	0.056	0.750	0.008	0.145	0.040
	FAL	0.010	0.973	0.005	0.007	0.005
Japan	DOK	0.006	0.005	0.974	0.007	0.008
	TOK	0.006	0.005	0.979	0.005	0.006
	TUF	0.004	0.005	0.981	0.005	0.005
	JAP	0.006	0.005	0.973	0.007	0.009
	SHI	0.007	0.005	0.978	0.005	0.006
South America	ARG	0.043	0.912	0.006	0.028	0.012

terms of microsatellite allelic richness (A), for which only Nova Scotia ($A = 3.89$) and South Africa ($A = 4.30$) were not significantly less diverse than populations in the native range (mean $A = 4.35$). Western North America, Tasmania, and Argentina were significantly different from native populations for all measures of microsatellite diversity. The eastern USA was significantly different for both allelic richness and number of alleles but not for expected heterozygosity. The population from Australia consistently showed intermediate genetic diversity for microsatellite loci.

Recent studies have shown that detection of multiple introductions is possible through examination of statistics recognizing the co-occurrence of mitochondrial lineages that are geographically separated in the native range (Kolbe *et al*. 2007a; Taylor & Keller 2007). In the case of *Carcinus*, the maximum uncorrected pairwise distance between COI haplotypes (P_{max}) within populations was significantly higher ($P < 0.0001$) in introduced populations compared to native populations of either *C. maenas* or *C. aestuarii* (Table 3), suggesting the contribution of multiple introductions to some samples. However, the significance of this difference appears to be due solely to the presence of both *C. maenas* and *C. aestuarii* haplotypes in two regions. Both Japan ($P_{max} = 0.0969$) and Cape Town, South Africa, ($P_{max} = 0.0994$) had values far higher than those observed in the native range of either *C. maenas* (mean $P_{max} = 0.0101$) or *C. aestuarii* (mean $P_{max} = 0.0227$); all other introduced populations had P_{max} values comparable to or lower than those of native populations. In South Africa, where multiple *C. maenas* haplotypes exist, P_{max} was not significantly different from native populations when the single *C. aestuarii* haplotype was removed from the analysis (not shown).

Discussion

Genetic structure within the native range

Based on mitochondrial haplotype data, Roman & Palumbi (2004) reported a significant genetic break between *Carcinus maenas* populations in Western and Northern Europe, roughly consistent with previously proposed biogeographic boundaries (Adey & Steneck 2001). In addition, populations from the region off the continental shelf in the Faeroe Islands and Iceland were found to be genetically differentiated from continental populations. In the current study, both mitochondrial and microsatellite markers showed significant partitioning of genetic variance between these biogeographic regions (Table 2). The degree of genetic structure observed was much more pronounced in mitochondrial than in nuclear markers, however. AMOVA revealed significant genetic differences at microsatellite loci corresponding to both proposed biogeographic boundaries, but the break between the off-shelf and other populations was considerably less marked than that observed in similar analyses of COI data (Table 2). Similar inconsistencies between mitochondrial and nuclear markers have been reported for other marine invertebrates (Peijnenburg *et al*. 2006; Mathews 2007). The discrepancy may be best explained in the current context by the greater sensitivity of mitochondrial loci to the effects of genetic drift (Shaw *et al*. 2004). However, it is also possible that increased homoplasy in the microsatellite data set has reduced apparent differentiation and masked phylogenetic signals (Estoup *et al*. 2002). This possibility is consistent with the failure of a distance metric based on sum of squared size differences (R_{ST}) to detect significant genetic differentiation within the native range.

The observation of extensive haplotype sharing between populations, in some cases involving haplotypes from well-supported clades, suggests the possibility of infrequent long-distance dispersal events, most likely human-mediated. The most obvious example is the presence in Naples of individuals from two substantially diverged *C. aestuarii* clades. Given the frequency of intracoastal traffic and the obvious proclivity of *Carcinus* for associating with human transport vectors, the potential for anthropogenic mixing of lineages in the native range is significant, and human-mediated gene flow within this region has been reported in other systems (Herborg *et al*. 2007). This could, in part, explain the limited geographic structure observed in Europe, and would contribute to the difficulty associated with assessing intraspecific admixture in introduced populations (see below). However, what geographic structure

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there is suggests that if anthropogenic dispersal has contributed to the contemporary distribution of genetic variation in *Carcinus*, it has been limited enough to preserve much of the signal of evolutionary diversification within the genus.

Sources of Carcinus *introductions*

Preliminary microsatellite data indicated the likelihood that populations on the Pacific coast of North America derived from the earlier 19th century invasion to the Atlantic coast of that continent (Bagley & Geller 2000). The current microsatellite data set clearly reveals the affinity of northeastern Pacific populations with US Atlantic populations. Both distance-based neighbour-joining (Fig. 3) and individual-based Bayesian clustering analyses (Fig. 4; Table 4) provide strong support for grouping Pacific US populations with the earlier Atlantic US invasion. Our microsatellite data also support the conclusion that Tasmanian populations derive from the Australian mainland (Bagley & Geller 2000). The mitochondrial sequence data presented here bolster the conclusion that at least some portion of the recent invasion to Nova Scotia derives from northern European sources (Roman 2006). The most common haplotype observed (six out of 20 individuals) in the Nova Scotia population belongs to a strongly supported haplotype group (dark blue, Fig. 1) the members of which appear only in native populations north of the Netherlands.

Conclusions regarding the invasion history of other introduced populations are less clear. Determination of sources for introduced populations in South Africa and Japan is complicated by the presence of haplotypes characteristic of both sister species and the possibility of interspecific hybridization events. One unexpected result of our analysis is the suggestion that the recently established *C. maenas* population in Argentina may derive from sources in Australia. Both neighbour-joining (whether based on chord distance or proportion of shared alleles) and Bayesian clustering analyses consistently group the Argentine population with populations from Australia and Tasmania (Figs 3 and 4; Table 4). Because the single COI haplotype present in Argentina is the predominant global *C. maenas* haplotype, mitochondrial data can only be said to be consistent with an Australian origin, as the haplotype is common on the Australian mainland (but not present in the derived Tasmanian population). Voisin *et al*. (2005) may have provided a precedent for exchange of marine invasives between Australia and Argentina. They observed that the mitochondrial haplotype composition of an introduced Argentine population of the globally invasive alga *Undaria pinnatifida* was highly similar to the population in Melbourne, Australia, although in that case the authors conclude that the two introductions derived from the same source region. Whereas a common source for Australian and Argentine *C. maenas* populations is possible, the notable differentiation of the Australia–Argentina cluster from all native populations suggests that a secondary invasion of Argentina from Australia is more consistent with the genetic data. It should be noted that the Argentine sample possesses a number of alleles, some at high frequency, which it does not share with Australia, an observation at odds with an Australian origin for this population. However, this patterns has been found in other cases where there is greater independent evidence to suggest secondary introductions; both Tasmania and the US west coast possess relatively common alleles not observed in their putative source regions (Australia and US east coast). In general, given the limited sampling of *C. maenas* in Argentina and pending more thorough investigation of vector strength along this pathway, the possible Australian origin of this population should be considered a hypothesis necessitating further investigation.

Dramatic genetic differences at nuclear loci exist between several of the introduced populations and their sources in the native range. In the case of populations in Japan, the independent clustering of these individuals may reflect a history of hybridization, as discussed below. In the case of Australian and Atlantic US populations, however, the clear genetic distinctions likely result from a combination of founder effects and pronounced drift resulting from long periods of genetic isolation from native sources. These regions appear to have been independent of gene flow from the native range for approximately 120 and 190 years, respectively. The signatures of this isolation have been subsequently inherited by recent secondary invasions deriving from these populations — in the eastern Pacific, Tasmania, and possibly Argentina. Certain introduced populations linked to their native sources by more recent gene flow (e.g. Nova Scotia and Cape Town, established in the past ~20 years) do not exhibit such dramatic differentiation. This pattern has obvious implications for the possibility of accurately sourcing long-established introductions, particularly in the absence of strong genetic structure in the native range.

Multiple introductions and admixture

There has been growing recognition of the role of hybridization in biological invasions (Ellstrand & Schierenbeck 2006; Hanfling 2007), and the potential for intraspecific admixture from multiple native source regions to help introduced populations overcome founder effects and generate novel genetic substrates for selection in the introduced range (Vellend *et al*. 2007). In some cases, admixture from multiple sources has been shown to lead to significant variation in morphology and life-history traits between introduced populations, with potentially important implications for invasion success (Kolbe *et al*. 2007b; Facon *et al*. 2008). Methods capable of detecting admixture in

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introduced populations could thus provide valuable information in assessing risks of invasiveness associated with those populations.

In our analysis, positive indications of multiple sources for *Carcinus* populations are limited to those cases where haplotypes of both sister species are observed. Elevated P_{max} values indicate that introduced populations in both Japan and South Africa harbour significantly greater mtDNA haplotype diversity than native populations. When we applied the more conservative indicator of mean haplotype sequence divergence (Kolbe *et al*. 2007a), we found that only the Japanese population was significantly different from native populations; the presence of the single, highly divergent *C. aestuarii* haplotype in the South Africa population did not significantly raise the mean divergence value.

More telling is the fact that our indicator is incapable of recognizing admixture unless it involves haplotypes from both sister species. The highest possible P_{max} observed in the total *C. maenas* data set (the greatest distance between any two *C. maenas* haplotypes) is 0.0158, not significantly different from the mean value observed within the native range populations (Table 3). Although the unusually high diversity observed among *C. maenas* haplotypes in populations such as Nova Scotia is consistent with the possibility of multiple introductions, the lack of strong genetic structure and deep phylogenetic divergence within the native range of *C. maenas* precludes observation of P_{max} values significantly greater than those observed in source populations. Nova Scotia is particularly interesting in this regard. The occurrence of multiple introductions to eastern North America has been clearly demonstrated, and the Murphy's Cove sample was drawn from a site at the northern end of the putative admixture zone between the early US and the later Canadian introductions (Roman 2006). Populations north of Murphy's Cove lack the common haplotype observed in many other introduced populations and dominant in the eastern USA (dark red, Fig. 2). The Murphy's Cove sample, which does possess this haplotype, may represent a population with genetic contributions from both introductions. Thus, while straightforward observation of haplotype distribution suggests the likelihood of admixture in the Murphy's Cove sample, the screen based on P_{max} is incapable of recognizing it. It should be noted, however, that Bayesian clustering analysis suggests only limited genetic contribution from the cluster including the earlier US introduction (Table 4; Fig. 4B). Although detection of multiple sources is a potentially useful tool in the study of introduced populations (Kolbe *et al*. 2007b; Taylor & Keller 2007), the value of screens such as those employed here and elsewhere are likely to be determined largely by patterns of genetic variation in the native range.

The presence of both *C. maenas* and *C. aestuarii* haplotypes in South Africa has previously prompted the hypothesis of interspecific hybridization in this invasive population (Geller *et al*. 1997). Structure analysis of microsatellite genotypes does suggest that the South Africa population is substantially admixed, with most individuals displaying high proportional contributions from both clusters 4 and 5, which correspond roughly with native *C. maenas* and native *C. aestuarii* (Fig. 4B; summarized in Table 4). While the presence of both sister species' genomes is suggestive of multiple introductions, this cannot be determined conclusively with the available data. Mitochondrial data reveal the presence in South Africa of one COI haplotype found only in native populations north of the Netherlands, suggesting that at least some of the founding crabs may derive from northern European sources. We did observe eight *C. maenas* COI haplotypes in South Africa not present within the native range, indicating that the true source of this population may not have been sampled. It remains possible that unknown single sources exist where the sister species are sympatric and could be entrained and transported by a single vector.

The genetic patterns observed in South Africa are quite different from those observed in Japan, where haplotypes from both sister species exist at high frequencies (69% *C. aestuarii*). Despite the presence of mitochondrial haplotypes from two species, there appears to be very little genetic differentiation at nuclear loci in these populations. In Bayesian analysis of microsatellite data, all Japanese individuals cluster together regardless of their mitochondrial haplotype (see Fig. 4B). This single cluster was highly stable, initially appearing when $K = 2$ and remaining at all higher values of K; no partitioning of the cluster was observed either in overall analyses or in those run independently on solely Japanese samples (not shown). In addition, no significant departure from Hardy–Weinberg equilibrium was observed in any of the Japanese samples (not shown). These patterns are consistent with the decay of cytonuclear disequilibrium within Japan, and strongly suggest a hybrid origin for this population.

We cannot say conclusively whether hybrid origin occurred before or after introduction, although the marked differentiation observed between Japanese crabs and all native *Carcinus* populations indicates considerable divergence from known parental lineages. Admixture analysis reveals that none of the Japanese individuals show significant coefficients of co-ancestry with clusters 4 or 5 (Table 4; Fig. 4B). This observation suggests that the source of the Japanese invasion may be an unsampled European population residing within a contact zone between the two sister species. Alternatively, it is possible that independent introductions of the two sister species were characterized by strong founder effects, resulting in dramatic shifts in allele frequency away from those typically observed in the native range of either species (similar to the effects observed in the USA and Australia). Subsequent homogenization of the

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nuclear gene pool through hybridization would thus result in a single population significantly differentiated from all native sources. Even assuming conservative estimates of generation time for *Carcinus* (3 to 4 years; Yamada 2000), the roughly 20 years between introduction and sampling (five generations, minimum) could provide sufficient time for complete decay of cytonuclear disequilibrium in the population, resulting in the observed discordance between unlinked nuclear and mitochondrial loci.

Genetic diversity in introduced populations

The standard expectation regarding invasive species has long been that introduced populations should experience loss of diversity relative to native sources because of founder effects and post-introduction demographic bottlenecks (Dlugosch & Parker 2008). Recent empirical evidence, however, indicates that many introduced populations exhibit no such reduction in genetic diversity. Some may even become more diverse than native populations, when multiple independent introductions lead to conversion of among-population native diversity to within-population diversity through admixture in the introduced range (Wares *et al*. 2005; Roman & Darling 2007).

Introduced populations of *Carcinus* appear, on average, to have experienced significant genetic diversity loss relative to native populations (Table 3). However, this general trend conceals substantial variation in the genetic diversity retained by particular introduced populations. A number of them, such as western North America, eastern USA, Tasmania, and Argentina exhibit significantly depressed levels of genetic diversity by virtually all measures. Results of microsatellite analysis are similar, indicating significant reductions in nearly all diversity measures in these populations. In contrast, the established populations in South Africa and Nova Scotia possess levels of genetic diversity comparable to those observed in the native range, with no significant reduction in any of the diversity measures, and populations in Australia appear to be of intermediate diversity. The likely hybrid origin of the Japanese population makes assessment of relative diversity impossible, as there is no reasonable native reference against which to compare the observed diversity measures.

Ideally, the availability of diversity measurements for introduced populations allows inference of general patterns in the relationship between invasion dynamics and the changes in diversity that occur with colonization (Wares *et al*. 2005; Dlugosch & Parker 2008). Such generalizations are difficult to make in the case of *Carcinus* (see Table 5). The potential invasion scenarios are complex for a species that has been transported around the globe for approximately two centuries and is associated with no fewer than eight distinct vectors of introduction (Carlton & Cohen 2003). The

Table 5 Summary of global *Carcinus* introductions

Introduced population	Date of introduction	Most likely vector(s) of introduction	Most likely source region	Loss of diversity relative to source?	Loss of diversity relative to native range?	Evidence for multiple introductions?
Eastern USA	1817	Solid ballast, hull fouling	Atlantic Europe	Yes	Yes	No
Australia	1890	Solid ballast, hull fouling	Atlantic Europe	No	No	No
Cape Town	1983	Ballast water, equipment fouling	Northern Europe and Mediterranean	No	No	Yes?
Japan	1984	Ballast water	Mediterranean and Atlantic Europe	Yes	Yes	Yes?
Western USA	1989	Shellfish transport, ballast water	Eastern North America	No	Yes	No
Nova Scotia	1991*	Ballast water	Northern Europe/Scandinavia	No	No	No
Tasmania	1993	Natural dispersal, ballast water	Australia	Yes	Yes	No
Argentina	1999†	Ballast water	Australia/Tasmania	Yes	Yes	No

Dates of introduction and most likely vectors of introduction for Nova Scotia and Argentina were taken from *Roman (2006) and †Hidalgo (2005), respectively; all other date and vector information derives from Carlton & Cohen (2003). Other results are from the current study. Loss of diversity is considered to have occurred if two or more of the estimated diversity indices (Table 3) are significantly lower in the introduced population. See text for discussion.

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marked loss of genetic diversity that accompanied the earliest introduction of crabs to the eastern USA before 1817 is consistent with the most likely vector for this event. The numbers of live juvenile and adult crabs able to survive lengthy trans-Atlantic crossings in solid ballast or hull-fouling assemblages were probably small relative to the number of larval crabs that could survive considerably shorter trips in modern ballast water holds. The significantly higher genetic diversity observed in the late 20th century introduction to Nova Scotia has been attributed in part to the capacity of contemporary shipping vectors to convey large founding populations (Roman 2006).

Populations that may have been introduced through ballast water discharge do not necessarily exhibit high diversity. The Argentine population was established quite recently (*c*. 1999), with ballast water cited as the most likely vector of introduction (Hidalgo *et al*. 2005). Yet, by all measures, this was one of the least diverse populations in our data set. This reduction in diversity is consistent with the hypothesis that Argentina is one of three secondary introductions; in all such cases, we observed reduced diversity relative to immediate sources (Table 5; in the case of the US west coast population, this reduction is not statistically significant). It must be noted that inferences of likely vectors (as opposed to direct observations) should be approached with some caution. It is possible that the vector responsible for bringing *C. maenas* to Argentina is not ballast water, but one less likely to deliver large propagule pools. Also, while ballasting procedures are likely to entrain large numbers of larval individuals, the genetic diversity of that propagule pool may vary greatly depending on adult reproductive patterns (Hedgecock 1994).

Conclusions

The potential of genetic analyses to provide insight into the patterns of introduction, establishment, and spread of invasive species has been widely touted (Lee 2002; Wares *et al*. 2005), and it has been suggested that study of globally invasive taxa may be particularly revealing, offering opportunities to observe general patterns over multiple independent introductions (Voisin *et al*. 2005). Still, the idiosyncratic nature of individual invasion events continues to be recognized as a potential obstacle to useful generalization (Colautti *et al*. 2007). *Carcinus* appears to provide a case in point. This genus has been independently introduced numerous times via numerous vectors over a period spanning almost two centuries. Genetic evidence now suggests important roles for secondary introduction, multiple introduction, and hybridization between sister species. These patterns, along with ignorance of the actual transport vectors in most cases, render prediction of the impacts of introduction on diversity — or inference of introduction history based on observed genetic diversity — an extremely challenging task. Although several introduced populations appear to have lost diversity relative to their immediate sources, this is not generally true — nor does there seem to be a consistent pattern relating to date or vector of introduction and diversity (Table 5).

The case of *Carcinus* contributes to the emerging consensus that there may not be strong correlation between the neutral genetic diversity retained by an introduced population and its potential for spread and impact (Roman & Darling 2007; Dlugosch & Parker 2008). The western US population, one of the least diverse populations in the global data set, has arguably been the most successful, having expanded over nearly 2000 km of coastline in less than 25 years (Yamada *et al*. 2005); at the same time, the highly diverse Cape Town population has remained virtually static since establishment (Hampton & Griffiths 2007). In these cases, the dynamics of expansion clearly depend more on environmental conditions than on neutral genetic diversity. These patterns suggest, among other things, that the significant reduction of diversity observed in the Argentine population provides no necessary barrier to expansion should environmental conditions in that region prove conducive.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Distribution of *Carcinus* COI haplotypes by site

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