

Transcriptome sequencing reveals both neutral and adaptive genome dynamics in a marine invader

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Abstract

Species invasions cause significant ecological and economic damage, and genetic information is important to understanding and managing invasive species. In the ocean, many invasive species have high dispersal and gene flow, lowering the discriminatory power of traditional genetic approaches. High-throughput sequencing holds tremendous promise for increasing resolution and illuminating the relative contributions of selection and drift in marine invasion, but has not yet been used to compare the diversity and dynamics of a high-dispersal invader in its native and invaded ranges. We test a transcriptome-based approach in the European green crab (*Carcinus maenas*), a widespread invasive species with high gene flow and a well-known invasion history, in two native and five invasive populations. A panel of 10 809 transcriptome-derived nuclear SNPs identified significant population structure among highly bottlenecked invasive populations that were previously undifferentiated with traditional markers. Comparing the full data set and a subset of 9246 putatively neutral SNPs strongly suggested that non-neutral processes are the primary driver of population structure within the species' native range, while neutral processes appear to dominate in the invaded range. Non-neutral native range structure coincides with significant differences in intraspecific thermal tolerance, suggesting temperature as a potential selective agent. These results underline the importance of adaptation in shaping intraspecific differences even in high geneflow marine invasive species. They also demonstrate that high-throughput approaches have broad utility in determining neutral structure in recent invasions of such species. Together, neutral and non-neutral data derived from high-throughput approaches may increase the understanding of invasion dynamics in high-dispersal species.

Keywords: adaptation, invasive species, natural selection and contemporary evolution, neutral processes, population genetics – empirical, transcriptomics

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Introduction

Modern biological invasions present a significant threat to ecosystem function and are recognized as a major component of global ecological change (Ricciardi 2007). Invasive species deliver a heavy economic burden in ecosystems – estimated at upwards of \$120 billion annually in the United States alone (Pimentel *et al.*

2005) – and can transform ecosystems. Because of these impacts, managing and preventing species invasions is a conservation priority, and genetic approaches play a growing role in this process (Lodge *et al.* 2006; Rollins *et al.* 2006). Simple demographic data on invasions – such as their source and levels of genetic diversity – have traditionally been the goals of genetic research (Wilson *et al.* 2009). More recently, attention has turned to the role of adaptation in species invasions (Prentis 2008). Local adaptation within a species' native range has been suggested to increase invasion risk, with certain source populations 'pre-adapted' for success in a specific environment, while other native populations

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may be less well suited to their new habitats (Fausch 2008). There is also evidence that invasive species may evolve rapidly in their new ranges (Huey & Pascual 2009; Hodgins *et al.* 2012) and that an ability to quickly adapt to local conditions may facilitate invasion (Vandepitte *et al.* 2014).

Marine invasive species have posed a particular challenge for traditional population genetics approaches. Many of these species have very high dispersal potential, which both facilitates invasion through transport in currents or in ships' ballast water (Carlton & Geller 1993; Byers & Pringle 2006) and complicates the process of tracing invasions because of low population genetic differentiation due to high gene flow (Waples 1998; Palumbi & Pinsky 2013). A recent review suggests that the least structured marine invasive species seem to make the best invaders: low genetic differentiation in the native range is correlated with greater continuous geographic spread in the invasive range (Gaither *et al.* 2013). This, coupled with the short time frame and frequent bottlenecks of contemporary invasions, makes distinguishing invasive populations difficult and can hinder the determination of population structure and dynamics. However, the same traits that reduce resolution with traditional markers also make marine invasions of particular interest to adaptation research. With high dispersal and few small-scale barriers, it has been hypothesized that selection may be the primary contributor to genetic structure in some marine systems (Bierne *et al.* 2003; Moura *et al.* 2014).

Over the past decade, high-throughput sequencing approaches have revolutionized the study of population genetics in nonmodel organisms, in part by providing a much finer resolution view of the genome than traditional sequencing (Luikart *et al.* 2003; Eklom & Galindo 2011). This higher genomic coverage has frequently been leveraged in scans for selection to identify potentially adaptive differences between populations (e.g. Namroud *et al.* 2008; De Wit & Palumbi 2012; White *et al.* 2013). However, potentially selected loci reflect a different demographic and evolutionary process than differentiation of many loci by drift and migration (Wright 1978). A focus on F_{ST} outliers – loci that are more diverged between populations than expected from neutral processes – does not by itself reveal patterns of gene flow or dispersal because such loci are also influenced by selection (Sotka & Palumbi 2006). As a result, a focus on demographic separation of populations requires information on differentiation of neutral loci. Distinguishing loci likely to be under selection from a large panel of markers allows a test of whether the putatively neutral remaining loci show similar population structure (Hess *et al.* 2013; Moore *et al.* 2014), and reveals the relative contributions of

neutral and adaptive processes to differentiation between populations (Lemay & Russello 2015).

Here, we use high-throughput sequencing to examine genetic structure and adaptation in the globally invasive European green crab, *Carcinus maenas*, examining populations spanning the native and invasive ranges. *C. maenas* is native to the northeast Atlantic from Morocco to Iceland and has successfully invaded every continent except Antarctica (Carlton & Cohen 2003; Darling *et al.* 2008; Blakeslee *et al.* 2010). The first documented introduction was to the East Coast of North America circa 1817, spreading south to the Chesapeake Bay and north to Halifax, Nova Scotia, by 1951 (Carlton & Cohen 2003). Starting in the 1980s, the species' range expanded to encompass the Canadian Maritime provinces and Newfoundland, concurrent with a second introduction from northern Europe (Roman 2006; Blakeslee *et al.* 2010). Green crabs are also invasive on the West Coast of North America, where they were first detected in San Francisco Bay in 1989, subsequently spreading north to Vancouver Island in <10 years (Cohen *et al.* 1995; Behrens Yamada & Hunt 2000). While *C. maenas* is a global invader, this study focuses on the native and North American invasive ranges, which represent many of the complex dynamics typical of invasion biology: multiple introductions, serial invasion and bottlenecks and a range of population ages.

Global population genetic analysis has been previously carried out on *C. maenas* using mitochondrial COI sequencing and microsatellites, leading to an overview of the structure and invasion pathways of the species (Roman & Palumbi 2004; Roman 2006; Darling *et al.* 2008; Tepolt *et al.* 2009; Blakeslee *et al.* 2010). However, traditional sequencing approaches have raised or left open important questions for the species. First, in much of the North American invasive range, bottlenecks has caused genetic diversity to drop to the point where prior studies were unable to distinguish between populations (Darling *et al.* 2008; Tepolt *et al.* 2009). Second, the role of selection in these invasions has been suggested by several lines of evidence, but has not been rigorously tested. For example, in the native range, mitochondrial COI shows distinct north–south differentiation, while putatively neutral microsatellites demonstrated very little structure within mainland Europe (Roman & Palumbi 2004; Darling *et al.* 2008). In addition, a physiological study of green crabs shows a pattern of local adaptation in thermal tolerance among populations, particularly in the native range (Tepolt & Somero 2014). Taken together, these data suggest that different introductions from differentially adapted native populations may have contributed to the explosive spread of the species on the East Coast, a fascinating proposition that cannot be addressed by the existing genetic data.

Here, we sequenced the cardiac transcriptomes of 12 individuals from each of seven populations in the native and invasive ranges and identified a panel of 10 809 high-quality SNPs for population genetic analysis. To explore the neutral components and possible impact of selection on shaping this structure, we extracted a subset of 9246 putatively neutral SNPs to contrast with the full SNP set. We used these data in two ways. First, we used the full SNP panel to explore patterns in population structure and genetic diversity, with a focus on increasing resolution in the genetically depauperate invasive range. Second, we compared the full and neutral SNP panels to investigate the relative contribution of neutral and non-neutral processes in shaping differentiation within and between the native and invasive ranges of the species. We show that these analyses illuminate strong patterns of population differentiation despite the recent invasion of these populations, and demonstrate that the balance between neutral and non-neutral processes appears to differ between the native and invasive ranges.

Materials and methods

Sample collection

European green crabs were sampled from seven sites across the native and invasive range: Skitnepollen, Askøy, Norway, and Seixal, Portugal, in Europe; North Harbour, NL, Canada, Walpole, ME, USA, and Tuckerton, NJ, USA, on the East Coast of North America; and Toquart Bay, BC, Canada, and Seadrift Lagoon, CA, USA, on the West Coast of North America (Fig. 1;

Table 1). At each site, 12 adult crabs were collected by trapping from shore.

At each site, six animals were acclimated to 5 °C for 4 weeks and six were acclimated to 25 °C for 3 weeks in the laboratory before tissues were collected for sequencing. These parameters were chosen for a complementary study of cardiac thermal physiology in *Carcinus maenas* and are described in more detail in Tepolt & Somero (2014). After acclimation, animals were sacrificed by severing the ventral nerve cord and whole hearts were placed in an RNA stabilizing reagent. Hearts were stored at 4 °C for 1–5 days to allow the reagent to penetrate the tissues, moved to –20 °C for 1–3 months and then archived at –80 °C. Like acclimation parameters, the use of cardiac tissue was chosen to complement simultaneous work on cardiac thermal physiology (Tepolt & Somero 2014).

Extraction and sequencing

RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA, USA) following the manufacturer's suggested protocol (Simms *et al.* 1993). For each extraction, a piece of cardiac tissue approximately 1 × 1 mm was excised using a razor blade, and the tissue was broken up either manually or by flash-freezing in liquid nitrogen and powdering. At the end of the extraction, RNA pellets were eluted in 25 µL RNase-free ultrapure water and frozen at –80 °C for 1–7 days prior to cDNA preparation.

Total RNA was quantified using the RNA BR assay on a Qubit 2.0 fluorometer (Invitrogen), and approximately 4 µg of RNA was used to construct a cDNA

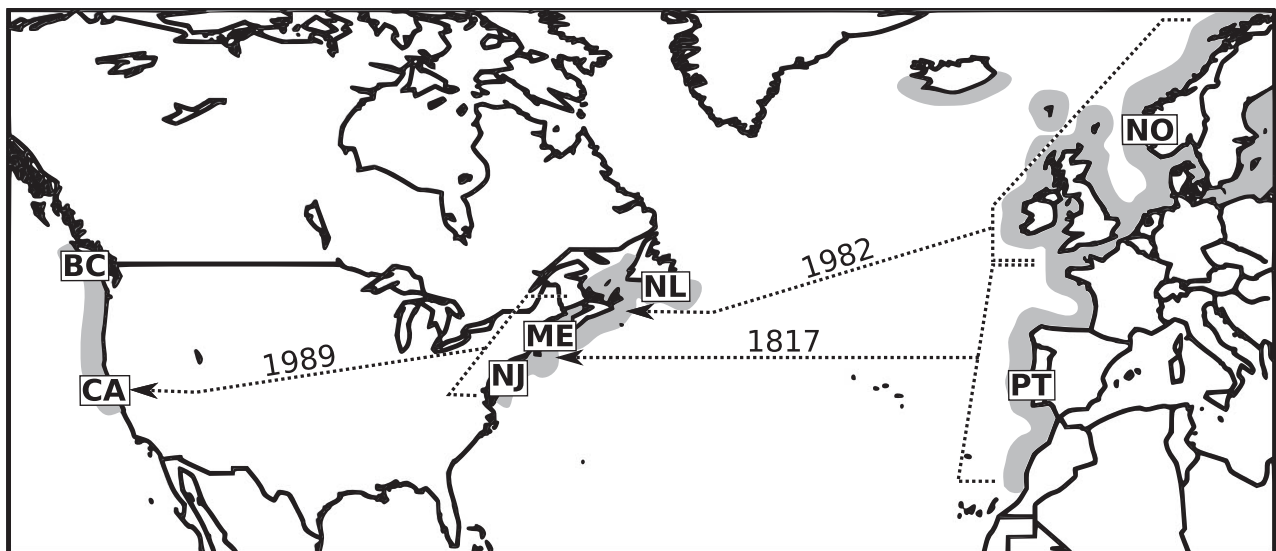


Fig. 1 Map showing collection locations (boxed codes per Table 1) and invasion history. Current range shown in grey. Approximate source populations are shown in dashed brackets, with arrows leading to initial invasion sites. Dates indicate the first record of each invasion.

Table 1 Details of collection locations

Site	Code	N	Lat	Long	Date
Toquart Bay, BC, Canada	BC	12	49.0203	-125.3586	Aug 2010
Seadrift Lagoon, CA, USA	CA	12	37.9079	-122.6726	Apr 2011
North Harbour, NL, Canada	NL	12	47.8459	-54.0969	Sep 2011
Walpole, ME, USA	ME	12	43.9351	-69.5809	Aug 2011
Tuckerton, NJ, USA	NJ	12	39.5095	-74.3248	Jul 2011
Skitnepollen, Askøy, Norway	NO	12	60.5061	5.0226	Aug 2012
Seixal, Portugal	PT	12	38.6455	-9.1036	Jul 2012

N, number of individuals; Lat, latitude; Long, longitude; Date, time of sampling.

library for each sample using the TruSeq RNA Sample Prep Kit from Illumina (San Diego, CA, USA). No in-line control reagents were used, and samples were tagged with one of 12 different adapter index sequences from Illumina for multiplexing.

Libraries were then sent to the University of Utah's Microarray and Genomics Core Facility, where they were quantified using a Bioanalyzer (Agilent, Santa Clara, CA, USA) and pooled into seven lanes of 12 samples each. All lanes contained a mix of samples from 5 to 7 sites. After pooling, samples were sequenced on an Illumina HiSeq 2000 sequencer. One lane was sequenced using 101-bp paired-end reads to aid with transcriptome assembly, while the remaining six lanes were sequenced using 50-bp single-end reads.

Sequence processing and transcriptome assembly

Processing of raw reads was performed using the SFG pipeline and scripts, available at <http://sfg.stanford.edu> (De Wit *et al.* 2012). Reads were initially processed using the FASTX toolkit v0.0.13; after processing, reads were imported into CLC GENOMICS WORKBENCH v6.0.4 (CLC bio, Cambridge, MA, USA) and used to assemble a *de novo* transcriptome. The parameters for this assembly included a mismatch cost of 1, insertion and deletion costs of 2, similarity fraction of 0.9, length fraction of 0.5 and minimum contig length of 200 bp.

Contigs were annotated with UniProt's Swiss-Prot and TrEMBL databases and GenBank's nr protein database using the BLASTX algorithm and NCBI's default parameters. Matches were considered significant at e -values of $\leq 1 \times 10^{-3}$. BLASTing was performed using the University of Oslo's Biportal (<https://www.biportal.uio.no/>).

MEGAN v4.70.4 was used to identify potential noncrab sequences (Huson *et al.* 2011); contigs were removed if they were identified as deriving from Bacteria, Archaea, Fungi or Plantae. Because of the dearth of genomic resources for this species, contigs that could not be identified taxonomically were retained in the assembly. Sequences identified as mitochondrial were also removed from the nuclear assembly used for SNP detection.

Mapping and SNP detection

A modified and updated version of the SFG protocol and scripts were used to map reads and detect SNPs. Initially, all reads were mapped back onto the *de novo* assembly using the BURROWS-WHEELER ALIGNER (BWA) v0.7.4 (<http://bio-bwa.sourceforge.net/>) and default parameters, except for $n = 0.004$ and $k = 5$ (Li & Durbin 2009). The GENOME ANALYSIS TOOLKIT v2.4 was used to detect high-quality nuclear SNPs as described in the SFG protocol (De Wit *et al.* 2012), updated to reflect the GATK v2.4 syntax and recommendations (McKenna *et al.* 2010; DePristo *et al.* 2011).

High-quality SNPs detected by this pipeline (Phred ≥ 30) were further filtered with a custom python script. First, we removed SNPs with >2 alleles and SNPs for which the minor allele frequency was $<1.5\%$. At the individual genotype level, we removed low-quality (Phred < 20) and low-coverage ($<10\times$) genotypes, and heterozygous genotypes where the ratio of one allele to the other was $>4:1$. We retained only SNPs that passed all of these filters and had high-quality genotypes in ≥ 10 individuals per population. Finally, we filtered SNPs to remove likely paralogs by removing SNPs where half of populations were significantly out of Hardy-Weinberg equilibrium (HWE; $P < 0.01$).

Identification of neutral SNPs

In order to reduce our SNP panel to a neutral subset, we used an outlier detection approach with extremely loose criteria for outliers in order to identify and exclude any loci identified as potentially non-neutral. While this approach undoubtedly discarded some truly neutral loci (i.e. false positives), it minimized the retention of weakly selected loci which would bias the neutral SNP panel (i.e. false negatives). Potentially non-neutral SNPs were identified using the *fdist* method for outlier detection (Beaumont & Nichols 1996), as implemented in LOSITAN (Antao *et al.* 2008). We ran LOSITAN 10 separate times, performing 500 000 simulations for each run with the 'Neutral' mean F_{ST}' and 'Force mean F_{ST}' options. Any SNP having an outlier probability ≥ 0.9 ($P \leq 0.1$) in any of these 10 runs was flagged as

potentially under selection and removed from the neutral SNP panel, similar to the approach of Hess *et al.* (2013). These cut-offs were chosen to be particularly conservative in order to build a panel of truly neutral SNPs.

Genetic structure

Principal component analysis (PCA) was performed on both the full and the neutral SNP panels, using the *EIGENSOFT* package (Patterson *et al.* 2006). The full SNP panel was also analysed with *STRUCTURE* v2.3.4 (Pritchard *et al.* 2000; Hubisz *et al.* 2009), using a burn-in period of 50 000 replicates followed by 150 000 MCMC replicates. Optimal K was inferred using the approach of Evanno *et al.* (2005) and implemented in *STRUCTURE HARVESTER* v0.6.93 (Earl & vonHoldt 2011), with 10 replicates each at $K = 1-6$. *CLUMPP* v1.1.2 was used to create composite data from the 10 replicates for each K (Jakobsson & Rosenberg 2007), and results were visualized using *DISTRUCT* v1.1 (Rosenberg 2003). To pick up on more subtle structure, this process was repeated on two subsets of the data: Europe (NO, PT) and populations derived from the initial invasion to the East Coast of North America (CA, BC, NJ, ME).

Pairwise F_{ST} was calculated using *ARLEQUIN* v3.1.5.3 (Excoffier & Lischer 2010), using 10 000 permutations and a significance level of 0.05. This analysis was performed twice, once on the full data set and once on the neutral SNP panel. The difference in F_{ST} between these approaches was calculated for each population pair by dividing pairwise F_{ST} from the neutral data set by pairwise F_{ST} from the full data set, and multiplying by 100 to get a percentage.

Genetic diversity

For the full data set, observed and expected heterozygosity (as $1-Q_{intra}$ and $1-Q_{inter}$) and F_{IS} were calculated for each population using *GENEPOP* v4.2.1 (Rousset 2008). The significance of F_{IS} was assessed using a score test for heterozygote excess implemented in *GENEPOP*. The proportion of all SNPs that were polymorphic in each population (P_n) was calculated in *ARLEQUIN*. Allelic richness and private allelic richness (A_r and pA_r) across all SNPs were calculated in *ADZE* v1.0 (Szpiech *et al.* 2008). The significance of differences in A_r and pA_r between locations was calculated from the mean and standard error for these values (from *ADZE*) and sample size ($N = 12$ in all cases) using unpaired t -tests implemented in *GRAPHPAD QUICKCALCS* online (<http://graphpad.com/quickcalcs/ttest2/>).

All plotting was carried out using the R package *GGPLOT2* (Wickham 2009; R Core Team 2012).

Results

Sequencing, transcriptome assembly, and SNP genotyping

mRNA sequencing resulted in an average of 15.2 million (m) single-end sequenced reads (11.8 m–21.0 m) and 14.0 m paired reads (12.0 m–17.3 m) per individual, for a total of 1.43 billion reads. After trimming and clipping of low-quality bases, and removal of duplicate reads, 1.35 billion reads were used for transcriptome assembly and genotyping. The initial assembly comprised 117 189 contigs ranging from 160 to 21 325 bp with an N50 of 1016 bp. A total of 948 contigs were identified as noncrab contamination and were removed. The final working assembly consisted of 116 241 contigs, of which 32 913 (28%) were annotated. Thirty-nine of these contigs were identified as mitochondrial and were excluded from nuclear SNP detection analyses.

Initial SNP detection and screening resulted in 531 675 high-quality nuclear SNPs with Phred ≥ 30 . We further filtered these SNPs to retain only those that were biallelic with a minor allele frequency of $>1.5\%$, and which had high coverage ($\geq 10\times$) and high-quality genotypes (Phred ≥ 20 ; allele ratio $<4:1$ for heterozygotes) for ten or more individuals per population; this screening resulted in 10 811 SNPs. Two of these were identified as potential paralogs due to being out of HWE in three or more populations and were also removed. The resulting full SNP panel comprised 10 809 nuclear SNPs in 1673 contigs, of which 1299 (78%) were annotated.

Neutral subset

The subset of neutral SNPs were identified by conservatively removing all SNPs that showed higher levels of F_{ST} than expected by chance using the *FDIST* method (Beaumont & Nichols 1996). We ran *LOSITAN* (Antao *et al.* 2008) 10 times and removed all SNPs that showed an outlier probability ≥ 0.9 ($P \leq 0.1$) in any of these 10 runs. Although these methods undoubtedly remove many neutral SNPs, these cut-offs were chosen to be particularly conservative to identify and remove as many non-neutral SNPs as possible. A total of 1563 of our SNPs fell inside the 10% threshold for non-neutrality and were removed to make a putatively neutral panel of 9246 SNPs.

Overall population structure

For the full SNP panel, all pairwise population comparisons showed significant genetic differentiation (Table 2), in contrast to previous studies. Within-region

Table 2 Pairwise F_{ST} (below diagonal) and significance (above diagonal) for all 10 809 SNPs

	NO	PT	NL	ME	NJ	BC	CA
NO	—	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001
PT	0.049	—	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001
NL	0.062	0.083	—	<0.00001	<0.00001	<0.00001	<0.00001
ME	0.100	0.107	0.039	—	0.02	<0.00001	<0.00001
NJ	0.100	0.093	0.043	0.003	—	<0.00001	<0.00001
BC	0.115	0.112	0.054	0.015	0.015	—	<0.00001
CA	0.134	0.133	0.072	0.031	0.032	0.021	—

Sites as in Table 1, Fig. 1.

differentiation was strongest in the native European range, with a pairwise F_{ST} between locations of 0.049. Differentiation in the East Coast was highest between Newfoundland and both other sites (F_{ST} with ME: 0.039; with NJ: 0.043) and lowest between Maine and New Jersey ($F_{ST} = 0.003$; $P = 0.02$). On the West Coast, locations in British Columbia and California were significantly differentiated ($F_{ST} = 0.021$, $P < 0.0001$). Of the two West Coast sites, California was more differentiated

from all other populations than was British Columbia, despite their recent derivation from the same source (Table 2).

The PCA shows similar features and also reflects differences between regions. Our sampled sites between Europe (NO, PT) are distinct from one another and are the most separate from the rest of the samples (Fig. 2). The North American populations form a cluster, except for Newfoundland, which appears intermediate

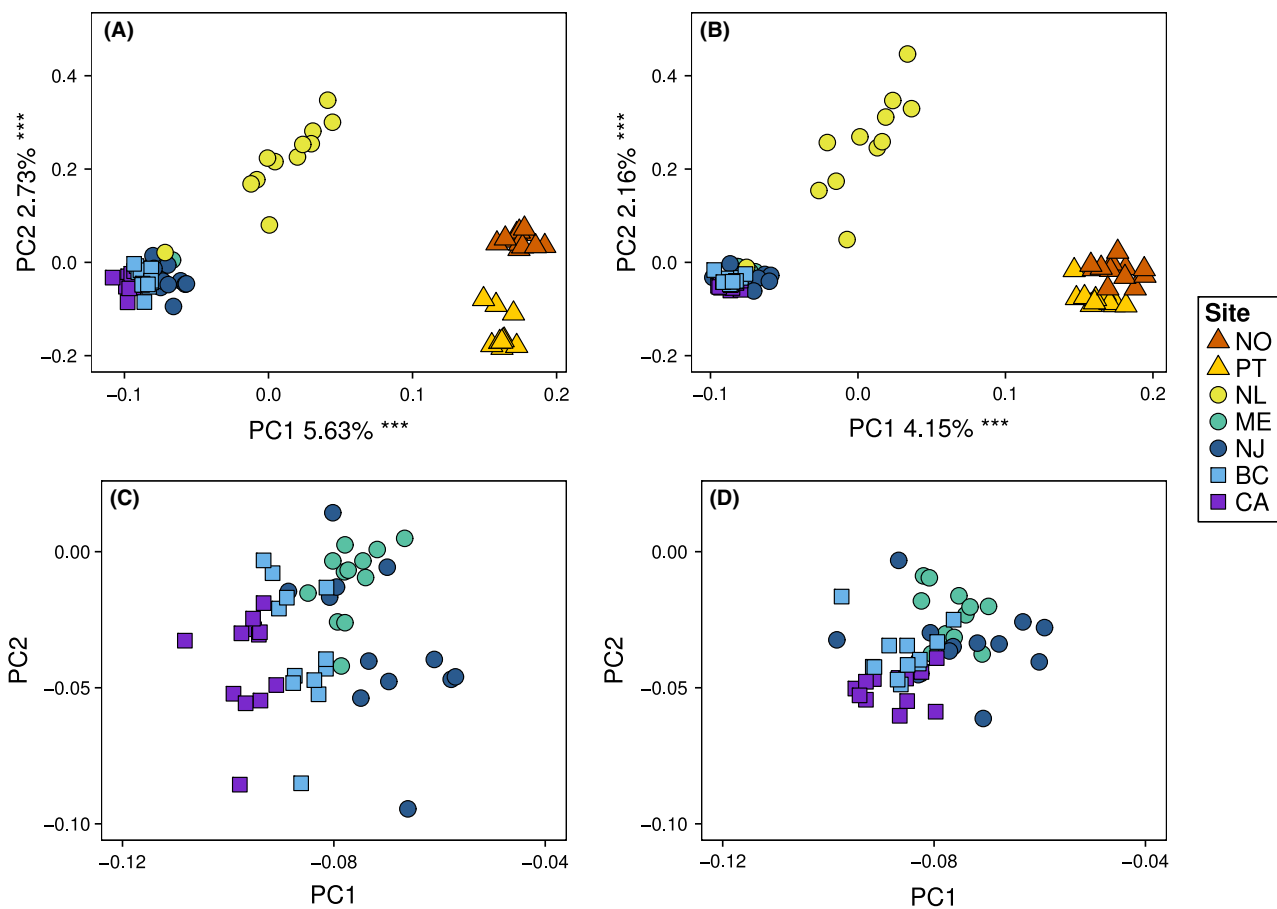


Fig. 2 Principal component analysis showing genetic structure for (A) all sites, all SNPs, and (B) all sites, neutral SNPs only. C and D show structure in Maine, New Jersey, California and British Columbia, expanded for clarity; C shows all SNPs, while D shows neutral SNPs only. Asterisks indicate highly significant PC axes ($P < 0.001$).

between the rest of North America and Europe (Fig. 2). Analysis with *STRUCTURE* also showed a split between Europe and the East Coast, with Newfoundland appearing to be the product of extensive introgression between these clusters (Fig. 3). When *STRUCTURE* was rerun on the European and East Coast clusters alone, each group was split into two subclusters. In Europe, locations were neatly separated, while on the East Coast, there was a less obvious East–West Coast split, with California differentiated from both East Coast sites while individuals from British Columbia were given equal assignment probability to both groups (Fig. 3).

Neutral population structure

The putatively neutral subset of SNPs (9246 of 10 809) showed a lower magnitude of population differentiation than the full SNP panel, as expected given that high- F_{ST} SNPs were removed from this data set. Yet, pairwise

F_{ST} remained significant for all population pairs except Maine–New Jersey ($P = 0.5$; Table 3). Within-region differentiation was strongest between Newfoundland and the other two sites in the invasive East Coast range, with pairwise F_{ST} between locations of 0.024 (ME; $P < 0.0001$) and 0.026 (NJ; $P < 0.0001$). Differentiation in the West Coast was somewhat lower, yet still significant, with an F_{ST} of 0.014 ($P < 0.0001$). Populations in Europe showed a particularly low neutral F_{ST} of 0.009 ($P < 0.0001$), in striking contrast to an F_{ST} of 0.049 in the full SNP set. Of the two West Coast sites, California remained more differentiated from all other populations than did British Columbia (Table 3).

The magnitude of difference in pairwise F_{ST} calculated with the neutral and full data sets showed that, for most population pairs, neutral SNPs demonstrated approximately 50–70% as much differentiation as did all SNPs (Fig. 4). The exceptions were the Maine–New Jersey and Norway–Portugal comparisons. In the case of Maine and New Jersey, F_{ST} was very low with the full SNP set and dropped effectively to 0 with neutral SNPs (Tables 2 and 3). Within Europe, the F_{ST} calculated with neutral markers was only 18% of the F_{ST} calculated using the full data set, a much larger proportional drop than any other pairwise comparison except Maine–New Jersey (Tables 2 and 3; Fig. 4).

The neutral PCA reflects these differences: there is an overall lower level of pairwise differentiation, particularly along axis 2, with less separation evident between sites and individuals (Fig. 2B). This compression is especially obvious between Portugal and Norway in Europe. While each of these sites still forms a distinct cluster, these two European groups are nearly overlapping, without the clear separation evident in the full data set (Fig. 2A, B). This decreasing differentiation between sites is also evident, albeit less extreme, for the cluster made up of Maine, New Jersey, British Columbia and California (Fig. 2D). While the overall position of the loose Newfoundland cluster is unchanged, individuals from Newfoundland appear more dispersed along axis 2 when considering only neutral SNPs (Fig. 2A, B).

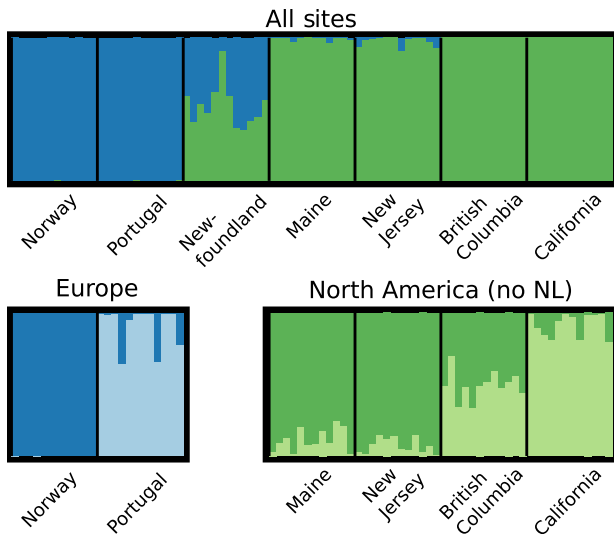


Fig. 3 *STRUCTURE* plot of all sites, all SNPs with $K = 2$ (optimal), and plots of Europe only ($K = 2$, optimal) and sites derived from the initial invasion to North America ($K = 2$, optimal $K = 3$ but did not provide additional information).

Table 3 Pairwise F_{ST} (below diagonal) and significance (above diagonal) for 9246 putatively neutral SNPs

	NO	PT	NL	ME	NJ	BC	CA
NO	—	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001
PT	0.009	—	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001
NL	0.038	0.041	—	<0.00001	<0.00001	<0.00001	<0.00001
ME	0.058	0.056	0.024	—	0.5	0.0001	<0.00001
NJ	0.056	0.053	0.026	–0.001	—	<0.00001	<0.00001
BC	0.065	0.065	0.033	0.010	0.010	—	<0.00001
CA	0.076	0.073	0.045	0.023	0.023	0.014	—

Sites as in Table 1, Fig. 1.

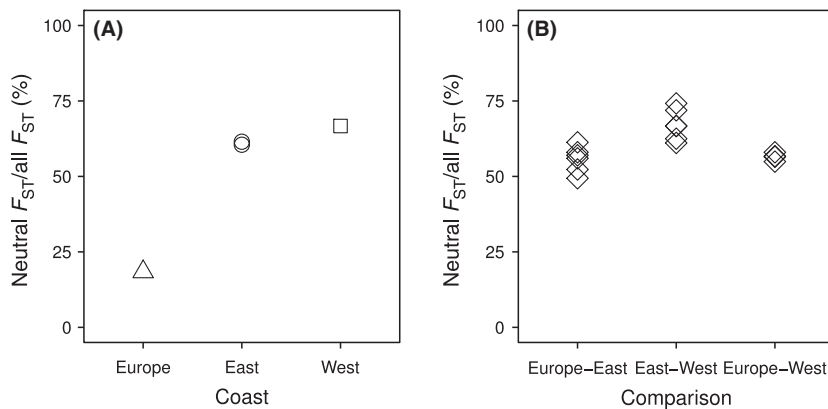


Fig. 4 Percentage change in pairwise F_{ST} using the neutral vs. full SNP set, between (A) sites within a region and (B) sites between regions. In panel A, the ME–NJ comparison has been omitted for clarity.

Genetic diversity

By all measures, genetic diversity was highest in the European native range and lowest in the West Coast (Table 4; Fig. 5). Allelic richness (A_r) ranged from 1.43 in CA to 1.55 in NO, with sites derived from the initial North American introduction all below 1.49 (1.43–1.48) and those in Europe both at 1.55 (Because this study employed only biallelic SNPs, the maximum possible A_r is 2.). As befits its hybrid origin, NL was more diverse than any of the other North American sites ($A_r = 1.53$), but not as diverse as populations in the native range (Table 4). Private allelic richness followed a similar but more extreme trend (Fig. 5), ranging from <0.011 in North American sites (0.007–0.010) through 0.049 in NL to >0.072 in Europe (0.073–0.076). Likewise, the proportion of global SNPs that were variable in a given population ranged from just over half (50.2%) in CA to more than two-thirds (68.5%) in PT (Table 4).

Discussion

Our study represents the first application of high-throughput sequencing to an invasive marine species

Table 4 Genetic diversity by location

Site	Pn	A_r	pAr	H_o	H_E	F_{IS}
NO	0.683	1.551	0.076	0.173	0.174	0.004
PT	0.685	1.550	0.073	0.174	0.174	–0.000
NL	0.642	1.533	0.049	0.175	0.175	–0.002
ME	0.560	1.474	0.009	0.162	0.162	0.002
NJ	0.568	1.483	0.010	0.170	0.166	–0.025***
BC	0.532	1.456	0.007	0.159	0.157	–0.009
CA	0.502	1.432	0.007	0.152	0.151	–0.006

Pn, proportion of polymorphic loci; A_r , allelic richness; pAr, private allelic richness; H_o , observed heterozygosity; H_E , expected heterozygosity; F_{IS} , Wright's inbreeding coefficient [asterisks indicate a significant heterozygote excess ($P < 0.001$)].

in both its native and its invasive ranges. The increased resolution of genomewide SNP markers uncovered previously undetected population structure in the highly bottlenecked invasive range, confirming significant divergence in as little as 20 years. By isolating a subset of putatively neutral SNPs, we were able to contrast overall population structure with neutral structure. These comparisons strongly suggest that non-neutral forces are the primary driver of genetic structure in the native range, with native range F_{ST} dropping to $<20\%$ of its overall value after the removal of potentially non-neutral SNPs. In contrast, F_{ST} between invasive populations was much more robust to the removal of non-neutral SNPs. These data suggest that neutral forces such as drift predominantly shape the species in its invasive range, while selection may drive intraspecific differences in the older and more stable native range. The genetic data presented here are consistent with significant differences in thermal physiology between the same populations, especially in the native range, suggesting that temperature may be a major driver of selection in the native range. Further study is required to determine whether these overall patterns reflect selection on specific genomic regions, and to more thoroughly explore the potential role of temperature as a selective force. Our study illustrates the utility of high-throughput sequencing for understanding the complex population dynamics characteristic of invasion and highlights the importance of selection in shaping structure in high-dispersal marine invasive species.

Population structure

This SNP panel represents a considerably larger portion of the genome than previous approaches, covering at least 1673 regions. These data showed significant genetic structure between all locations, even between invasive populations that appeared undifferentiated when using mtDNA and microsatellites (Darling *et al.*

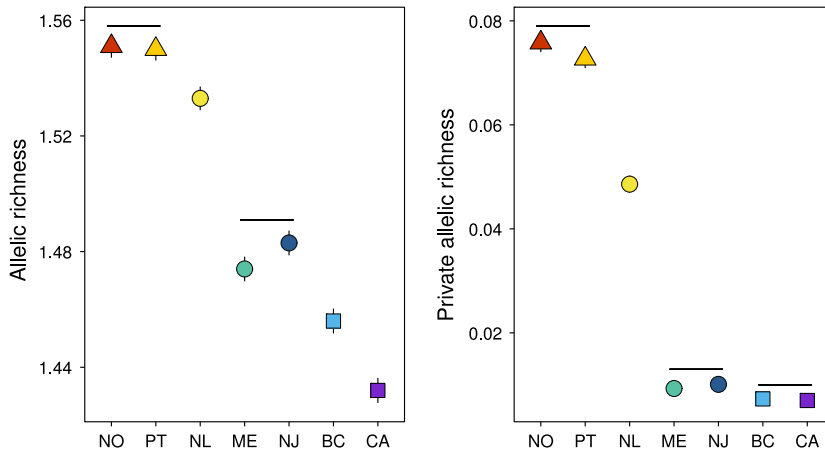


Fig. 5 Allelic richness and private allelic richness by location (mean \pm standard error). Horizontal lines link locations that are not significantly different ($P \geq 0.05$).

2008). In the native European range, the SNP panel shows that the northern and southern populations are significantly different. This result is consistent with a distinct mtDNA cline concurrent with a wide-scale biogeographic break between northern and southern Europe (Roman & Palumbi 2004), characterized by differences in temperature regime and species composition (Adey & Steneck 2001). Populations along the East Coast of North America were also highly structured, reflecting a history of multiple introductions. Newfoundland was diverged from both Maine and New Jersey and showed extensive admixture between the 200-year-old initial introduction and a recently arrived introduction from the native range (Fig. 3). In contrast, Maine and New Jersey were the least differentiated of all the population pairs we studied, and despite the detection of northern mtDNA as far south as New York (Blakeslee *et al.* 2010), it appears that our site in Maine has little to no nuclear genetic influence from the second introduction.

In marked contrast to previous work (Darling *et al.* 2008), we demonstrate clear and significant differentiation between the East and West Coast invasive populations. The spread of *C. maenas* to the U.S. West Coast in the 1980s is a classic example of a secondary introduction (Darling *et al.* 2008), and genetic diversity levels in the SNP panel reflected that serial bottlenecking: high allelic richness in native range populations, a significant drop in diversity after the initial introduction to the East Coast and another significant drop after secondary introduction to the West Coast.

We also found significant structure within the West Coast invasive range, despite its very recent founding *circa* 1989. The California population, which was colonized early and is in the 'core' of the West Coast range, appears more differentiated from all other locations than does the more recently founded British Columbia population, located at the periphery of the species' West Coast

range. This increased divergence of the California population is surprising because the dynamics associated with range expansion frequently favour higher differentiation of peripheral, recently colonized sites relative to more established sites in the centre of a species' range (Excoffier *et al.* 2009). In the case of West Coast *C. maenas*, this discrepancy is potentially resolved by considering the specifics of the sample locations. While the British Columbia population was sampled in a near-pristine natural environment in Barkley Sound, the California population was sampled in Seadrift Lagoon, a small artificial lagoon created in the 1960s by the dredging out of a wide sandbar running in front of the estuarine Bolinas Lagoon (Ritter 1970). This lagoon is shallow, heavily modified and relatively isolated from the open ocean (C.K. Tepolt, personal observation), suggesting that its population may experience idiosyncratic conditions and low genetic exchange with neighbouring embayments. The California population also had significantly lower allelic richness than its northern counterpart in British Columbia, supporting the hypothesis of increased isolation.

Genetic diversity was highest in both native range sites, dropping sharply in Maine and New Jersey. In Newfoundland, we observed an increase in diversity caused by new alleles from the second introduction from northern Europe. However, this bump was not enough to bring Newfoundland up to levels seen in the native range. Intriguingly, Newfoundland remains intermediate between Europe and the East Coast when private allelic richness is concerned, indicating that Newfoundland possesses a number of SNPs not found in either of our native range populations. This suggests that the second introduction to the East Coast derived from an unsampled European source containing private alleles, and hints at the possibility that, with additional sampling, SNPs may be able to detect much more fine-grained population structure in the native range.

Selection and drift in invasion

Population structure due to selection in the native range is quite relevant to the pace and pattern of species invasions. Local adaptation may influence the probability of invasion success, dependent on the quality of matching between source and invaded environment. Once a species has invaded, further adaptation in the invasive range may enhance a species' ability to persist and thrive by rapidly shifting its ecological niche to better match its new home (Sultan *et al.* 2013; Vandepitte *et al.* 2014). In several well-documented examples in insects and plants, native range latitudinal clines were rapidly recapitulated postinvasion (Balanyà *et al.* 2003; Maron *et al.* 2004; Balanyà *et al.* 2006). This dynamic has also been documented at range edges, where rapid adaptation may facilitate the spread of species beyond their previous limits (Phillips *et al.* 2006; Preisser *et al.* 2008; Krehenwinkel & Tautz 2013).

In many high geneflow marine species, where structure may be subtle and largely driven by adaptive differences (Sanford & Kelly 2011), SNPs are beginning to highlight patterns that are not apparent using traditional sequencing approaches such as microsatellites (Lamichhaney *et al.* 2012). Several recent genome-scale studies have suggested that intraspecific adaptation may be widespread in the sea, and include species such as Atlantic cod (Nielsen *et al.* 2009), purple sea urchin (Pespeni & Palumbi 2013) and Atlantic herring (Limborg *et al.* 2012). Studies suggesting adaptation in invasive marine species are much more limited, but suggest that adaptation may play as important a role in the sea as it does on land (Tepolt 2015). Studies within the native range of the invasive snail *Littorina saxatilis* strongly suggest adaptation in response to environmental factors related to tidal height (Grahame *et al.* 2006; Wood *et al.* 2008; Galindo *et al.* 2009, 2010). An AFLP- and microsatellite-based outlier scan of the slipper shell *Crepidula fornicata* suggested local adaptation within the native range, but no evidence for outliers within the invasive range or between the native and invasive ranges, although resolution was limited by a relatively small number of markers (Riquet *et al.* 2013). A similar study in the Pacific oyster *Crassostrea gigas* suggested local adaptation in the invasive range, although this result was confounded by invasion history (Rohfritsch *et al.* 2013).

In *C. maenas*, it has been hypothesized that the second introduction to the East Coast may have introduced cold-adapted genotypes to the range, allowing the species to expand into waters from which they had previously been thermally excluded (Roman 2006). While this pattern of expansion is also consistent with current-mediated dispersal (Pringle *et al.* 2011), physiological

work suggests that crabs in Norway and in Newfoundland are more cold tolerant than their southern counterparts (Tepolt & Somero 2014). Our work supports this by showing that much of the differentiation between Norway and Portugal is driven by non-neutral processes (Tables 2 and 3; Fig. 4). Thus, much of the difference between Norway and Portugal may be driven by local adaptation, operating across a low level of differentiation maintained due to neutral processes such as dispersal limitation.

Further, the difference in between neutral SNPs and all SNPs was much higher in Europe than in any other pairwise comparison, suggesting that the native range harbours more adaptive alleles remaining in place than do recently invaded populations. While this study sampled only two native populations, these populations were chosen because they span much of the species' latitudinal range, and possess significantly different mitochondrial lineages and thermal physiology (Roman & Palumbi 2004; Tepolt & Somero 2014). Thus, they are likely to capture extremes of adaptation in the native range. Sampling from additional, intermediate native range populations may be able to determine whether adaptation plays as important a role in shaping structure across smaller distances.

In contrast to the native range, neutral processes appear to dominate in shaping invasive range structure. In the invasive range, Maine and New Jersey were not significantly different when only the neutral SNP panel was considered (Table 2). It is possible that this difference between neutral markers and all markers reflects some adaptation between these sites, located nearly 5° apart in latitude, but given the low level of initial differentiation, this loss of significance could also be due to the effect of removing high- F_{ST} loci from the data set. However, it is important to note that this apparent dominance of neutral processes does not rule out adaptation. Our estimates of structure are based on F_{ST} , which assumes that populations are in mutation-drift equilibrium (Wright 1978). While the assumption of equilibrium may be sound for the native range populations, it is probably violated in the invasive populations, which have had no more than 200 years (ME–NJ) and as few as 15 (CA–BC) to reach equilibrium (Whitlock & McCauley 1999; Grosberg & Cunningham 2001). Thus, our invasive populations may indeed be experiencing selection, but at a level that is swamped by the signal of recent founding and rapid population expansion, as opposed to the more extensive adaptation that appears to be operating between the 'stable' native populations.

On the West Coast, the neutral SNP panel recapitulated the pattern of California being more differentiated than British Columbia from all other sites (Tables 2 and 3; Fig. 2C, D). As discussed above, this surprising drop

in the core of the range may be related to idiosyncratic conditions in the specific study site. Interestingly, this population also had a dramatic loss of heat tolerance relative to all other studied populations (Tepolt & Somero 2014). While in no way conclusive, it is possible that significant bottlenecks may have negatively influenced the potential future fitness of this population. The fact that California remains more differentiated than British Columbia with both the full and neutral SNP panels supports this hypothesis, suggesting that neutral processes – such as bottlenecks in this enclosed environment – may primarily contribute to divergence.

Comparing neutral and overall structure was able to shed light on the dynamics of invasion in *C. maenas*, and in doing so, it improves our understanding of marine invasions more generally. In the native range, adaptation appeared to be the primary driver of structure. This fits with previous data, which showed no structure for putatively neutral microsatellites, but did indicate a clear break in potentially adaptive mitochondrial lineages. If populations of invasive species are locally adapted, this suggests that there may be a benefit to preventing future introductions to already-invaded areas, in an effort to curb further spread. In the oldest invasive population pair, Maine and New Jersey, weak overall structure was not captured by neutral markers, suggesting high gene flow but potentially incipient adaptive differences. In contrast, neutral structure remained strong between the West Coast sites, reinforcing the potential isolation and divergent physiology of the California site. Because these newly founded populations are not likely to have reached mutation-drift equilibrium, we expect that these patterns – and the dominance of neutral structure – are likely to shift over time. Further studies will focus on identifying specific genes underlying these overarching patterns, and their relationship with potential environmental drivers such as temperature.

Conclusions

Understanding genetic structure plays a crucial role in many conservation and management programmes. In the case of many invasive species, invasion history and pathways are poorly understood, yet this information can aid in designing effective management strategies to prevent further introduction and spread (Estoup & Guillemaud 2010). While these panels are more typically used to identify specific candidate genes, large panels of genomewide markers delivered by high-throughput sequencing permit researchers to begin untangling the relative importance of neutral and adaptive structure in shaping and facilitating marine inva-

sions (Savolainen *et al.* 2013). In the case of our study, these data support the hypothesis that local adaptation in the native range may have played an important role in permitting the spread of the invasion via multiple introductions. At the same time, neutral forces of bottlenecks and genetic drift appear to differentiate many of the populations within the invasive North American range, suggesting that even a highly bottlenecked population may retain an exceptional capacity to thrive and spread without invoking extensive adaptation. We suggest that the ability of population genomics to elucidate fine-scale population structure, and the relative contributions of neutral and selective forces on shaping that structure, holds great promise for understanding the complicated dynamics of marine invasions.

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C.K.T. was responsible for project planning, tissue collection, sample preparation, data analysis and manuscript writing. S.R.P. was responsible for project planning, manuscript writing and partially funding the sequencing.

Data accessibility

The *de novo* *Carcinus maenas* cardiac transcriptome, SNP positions and individual genotypes have been deposited in Dryad at doi:10.5061/dryad.g8b96. The raw sequencing reads have been archived at NCBI's Sequence Read Archive under BioProject ID PRJNA283611, BioSample numbers SAMN03653390–SAMN03653473.