

Genetic variation in a newly established population of the Atlantic rock crab *Cancer irroratus* in Iceland

Óskar Sindri Gíslason^{1,2,*}, Snæbjörn Pálsson¹, Niall J. McKeown³,
Halldór P. Halldórsson², Paul W. Shaw³, Jörundur Svavarsson^{1,2}

¹Department of Life and Environmental Sciences, University of Iceland, Sturlugata 7 and Aragata 9, 101 Reykjavík, Iceland

²The University of Iceland's Research Centre in Suðurnes, University of Iceland, Garðvegur 1, 245 Sandgerði, Iceland

³Institute of Biological, Environmental and Rural Sciences (IBERS), Aberystwyth University, Penglais, Aberystwyth, SY23 3FG, UK

ABSTRACT: The Atlantic rock crab *Cancer irroratus* is native along the east coast of North America. The species was first recorded in Iceland in 2006 and has since rapidly spread throughout the country's southwestern and western coastal waters. The transport of larvae in ballast water is the most probable route by which introduction into Iceland occurred. As this species is commercially valuable, it may be possible to establish a viable industry harvesting rock crabs in Iceland; however, to do this, more information on species-wide genetic diversity and demography is required. In this study, genetic variation at 7 microsatellite markers was analyzed in samples from Iceland and 5 sites in North America, capturing most of the known range of this species. Our results divided samples from the native range into 2 groups, divided by a previously proposed barrier to gene flow, compatible with local hydrographic factors restricting larval-mediated gene flow. The Icelandic population was markedly differentiated from all other samples, but exhibited comparable levels of genetic diversity with no evidence of small population effects or genetic bottlenecks. No single population could be identified as a source for the Icelandic population, though the likely origin could be attributed to the 'northern group' in the native range. Genetic data indicate that the number of founders of the Icelandic population was sufficient to retain genetic variation. As the Icelandic population shows evidence of self-recruitment and population expansion, it may represent a potential harvestable resource in Iceland.

KEY WORDS: Crustacea · Decapoda · Biological invasion · Bottleneck · Genetic diversity · Population connectivity · Microsatellite

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INTRODUCTION

Marine biodiversity is subjected to a number of anthropogenic pressures such as exploitation, pollution, climate change and rising sea level (Coll et al. 2010, Floerl et al. 2013). Furthermore, increasing maritime commerce has in many areas led to increased introductions of non-indigenous species which may dramatically change native biodiversity and ecosystem functioning (Cohen & Carlton 1998, Brickman 2006, Seebens et al. 2013). Genetic studies have not

only enhanced our knowledge of the status and evolution of marine biodiversity but are increasingly being used to study the introduction, colonization and spread of invasive taxa (Dupont et al. 2003, Blakeslee et al. 2010, Ghabooli et al. 2011).

Atlantic rock crab *Cancer irroratus* Say, 1817 (Canceridae) is native along the east coast of North America, from South Carolina to Labrador (Sastry 1977). It is a relatively large crab species with a carapace width up to 15 cm (Robichaud & Frail 2006). The crab is of commercial value, originally initiated as a by-

*Email: osg3@hi.is

catch in lobster fisheries in the 1960s, and has supported fisheries in its northern range since 1974 (DFO [Fisheries and Oceans Canada] 1999, 2008). The distribution and migration patterns of adult rock crabs vary seasonally and with water temperatures (Bigford 1979). In the northern part of its natural range, the rock crab is found along the coast all year round; however, frequent offshore migrations are thought to occur in the southern part of the range that spread the population into deeper water over the summer months (Bigford 1979). Individuals generally reach sexual maturity at >50 mm carapace width in the northern part of the distribution (Krouse 1972, Scarratt & Lowe 1972), but at a smaller size in the southern range (Reilly & Saila 1978). Taking into account the high fecundity (up to >500 000 eggs per female; DFO 2001), planktonic larval duration from 3 to 7 wk (Johns 1981), and the general near-surface large scale circulation in the Northwest Atlantic (Chapman & Beardsley 1989, Han et al. 1999, Hannah et al. 2000), there is potential for high levels of dispersal and connectivity among the populations in the Northwest Atlantic.

The first record of the rock crab in Icelandic waters, Northeast Atlantic, was in 2006 (Svavarsson & Dungal 2008) and it is the only record of adult crabs out of its native range. Considering the size of individuals caught in Iceland in 2006, the colonization must have occurred sometime before the year 2000. The species has since spread quickly from inner Faxaflói Bay, SW Iceland, along the southwest and west coasts of Iceland (~20% of the coastline) where it is reproducing successfully (Gíslason 2009, unpubl. data). This rapid spread is thought to be driven by larval drift with the coastal current that runs in a clockwise direction around Iceland (Valdimarsson & Malmberg 1999). The rock crab has most likely been unintentionally introduced as larvae transported in ballast water from North America to SW Iceland (import of live animals is strictly regulated) where most of the maritime traffic in Iceland is operated. Such transport has been shown to take place, as rock crab larvae in transit between the USA and England survived in ballast water for 17 d (Hamer et al. 1998). In addition to irregular cargo and fisheries-related transportation, at least one sailing schedule has been operated for decades between North America and Iceland (Magnússon 1998) that nearly covers the known distribution range of the species (Sastry 1977). Colonization through natural dispersal by currents can be ruled out. A large anti-clockwise sub-polar gyre south of Iceland makes a fast drift from Canada to Iceland impossible (Jakobsen et al. 2003). There is a very

small possibility for larvae to cross the Labrador Current along the Canadian coast, to enter the Atlantic Drift and hence the eastern branch of the gyre, to drift north-eastward and finally northward to Iceland. The length of this path would be around 5000 km and with an average speed of around 10 cm s^{-1} , the transport of the larvae would take around 600 d. Since the pelagic phase of larval development for the rock crab can vary from 17 to 50 d depending on temperature (Johns 1981), this possibility can be ruled out. Migration of adult rock crabs to Iceland can also be excluded because of the distances between Iceland and North America and due to the depth in the intervening waters that far exceeds the known depth limits of the species (Haefner 1976). Geographically, Greenland appears as a likely route for dispersal of American species to Iceland, located midway between Canada and Iceland. However, the climatic conditions for rock crab larval development (Johns 1981) are not favorable in Greenland due to low water temperatures (Ribergaard 2013). No records of this species are known in Greenland (A. D. Burmeister pers. comm.), with commercial crab fisheries practiced in the warmer sea along the western coast of the country (Burmeister 2010).

A previous study by Gíslason et al. (2013) on rock crab mitochondrial (mt)DNA diversity found the population in the Gulf of St. Lawrence to differ from samples from the Canadian Maritimes and Iceland, and that the amount of variation was similar at all locations. However, clear interpretation of the mtDNA variation was confounded by a high frequency of nuclear inserts (numts). The lack of population genetic information for the rock crab means that little is known about its population demography. As rock crab is harvested in Northeast America and interest in future commercial exploitation of rock crab in Iceland is growing, genetic information is of importance for a sustainable management of the resource. The amount of variation in Iceland can provide further information about whether the population is viable and able to form a sustainable resource. The rock crab expansion in Iceland also provides an opportunity to study colonization and range expansion processes which are increasingly important in light of climate change-induced range shifts.

The objectives of this study were to (1) study the population structure within the rock crab native range in Northeast America; (2) infer the origin of the recently founded population in Iceland; and (3) determine if levels of genetic diversity within the Iceland population are likely to be sufficient for the species to persist in the long term.

MATERIALS AND METHODS

Sample collection and DNA isolation

A total of 262 adult rock crabs were collected in Faxaflói Bay, Iceland (I) and at 5 locations in North America: Bay Bulls, Newfoundland (N), Gulf of St. Lawrence, New Brunswick (L), Duncan Cove, Nova Scotia (S), Bay of Fundy, New Brunswick (F) and Nahant Bay, Massachusetts (M) (Fig. 1). Leg muscles were excised and preserved in 96% ethanol. DNA was extracted using 6% Chelex 100 (Bio-Rad) and Proteinase K (1%) (Walsh et al. 1991).

Microsatellite loci

All samples were genotyped for nuclear variation at 7 microsatellite loci that were originally developed for *Cancer pagurus*. These included CPAG-2D7, CPAG-4, CPAG-5D8, CPAG-15, CPAG-4C1 and CPAG-1C8 (McKeown & Shaw 2008), and a new locus CPAG-6C4A (forward primer 5'-AGA GGC ACA GGG GAT ATC AG-3'; reverse primer 5'-GCG AAA TGG TCT GGT TTT AAT C-3'). Each locus was individually PCR amplified in 10 μ l reactions containing 50 ng of template DNA, 1 pmol of each primer, 1 \times buffer, 2.0 mM MgCl₂, 0.2 mM deoxy-

nucleoside triphosphates (dNTPs), and 0.2 U of *Taq* DNA polymerase (Bioline). Amplifications involved an initial denaturation step (95°C for 3 min) followed by 35 cycles of 30 s at 95°C, 30 s at 52°C and 30 s at 72°C. PCR products were size separated using an AB3500 DNA sequencer (Applied Biosystems) with allele inference performed using the GENEMAPPER software (version 4.1, Applied Biosystems).

Statistical analysis

Microsatellite variation was described using variance in allele size s^2 , the number of alleles (A), allelic richness (A_R , the number of alleles estimated by rarefaction if all samples were equal to the smallest sample size in a set of comparisons), observed (H_O) and expected (H_E) heterozygosity using FSTAT version 2.9.3.2 (updated in 2002 from Goudet 1995). The inbreeding coefficient (F_{IS}) was calculated as the difference between observed and expected heterozygosity (Nei & Kumar 2000). Deviation from predictions for Hardy Weinberg equilibrium within loci and linkage disequilibrium between loci were tested with GENEPOP 4.0 (Raymond & Rousset 1995, Rousset 2008).

To test for differences in genetic variation among samples, differences in the ranks of values of A_R , H_E and s^2 within loci for pairs of samples including populations from the native range and the Icelandic sample were compared with Wilcoxon signed-rank tests using R (R Development Core Team 2011). Differences among groups of samples (for A , A_R , H_E , H_O and F_{IS}) were tested using the permutation approach in FSTAT. Evidence for population size bottlenecks affecting genetic diversity within each sample was evaluated using the Wilcoxon signed-rank test implemented in the software BOTTLENECK (Piry et al. 1999), which assumes that the signature of a severe reduction in an effective size of a population results in an excess of heterozygosity (H_E) relative to expected heterozygosity (H_{eq}) at mutation drift equilibrium (Cornuet & Luikart 1996). Tests for such an excess were performed using the Wilcoxon signed-rank test under Infinite Allele (IAM) (Crow & Kimura 1970), Step-Wise Mutation (SMM) (Ohta & Kimura 1973) and Two-

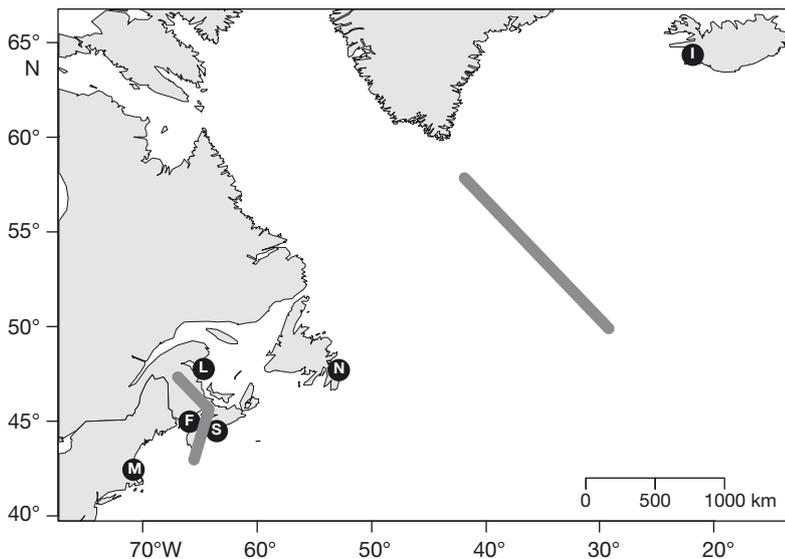


Fig. 1. Sampling locations of the rock crab *Cancer irroratus* Say, 1817: (I) Faxaflói Bay, Iceland (64.392°N, 21.580°W), (N) Bay Bulls, Newfoundland (47.311°N, 52.808°W), (L) Gulf of St. Lawrence, New Brunswick (47.911°N, 64.463°W), (S) Duncan Cove, Nova Scotia (44.580°N, 64.181°W), (F) Bay of Fundy, New Brunswick (44.976°N, 65.908°W) and (M) Nahant Bay, Massachusetts (42.438°N, 70.902°W). Gray lines show separation between clustered populations

Phase Mutation (TPM) (Di Rienzo et al. 1994) models of microsatellite evolution, and also the mode-shift test (Luikart et al. 1998), which may be more sensitive to less severe bottlenecks (Mock et al. 2004). The M -ratio test (Garza & Williamson 2001), based on the ratio of the number of alleles (k) and the range of allele sizes (r) at the same locus was also performed. When populations are reduced in size, genetic drift is intensified and rare alleles are quickly lost. Consequently, the M -ratio will be smaller for bottlenecked populations compared to populations at equilibrium (Garza & Williamson 2001). In comparison to the BOTTLENECK methods that are tailored to detect recent bottlenecks, M -ratio reflects a population size decline over a longer timescale and may be more powerful for detecting ancestral bottlenecks (Garza & Williamson 2001).

Genetic differences between pairs of samples were tested using exact tests of allele frequency homogeneity performed in GENEPOP with default Markov chain parameters. Genetic differentiation between sample pairs was quantified using the unbiased F_{ST} estimator, θ (Weir & Cockerham 1984), calculated using FSTAT, with the significance of estimates tested by 10 000 permutations of genotypes among samples.

Isolation by distance (IBD), which describes the tendency of individuals to find mates from nearby populations rather than distant populations (Wright 1943), was tested by the association of pairwise F_{ST} values with logarithm of pairwise geographic distances using the Mantel test (Mantel 1967), and a partial Mantel test (Legendre & Legendre 1998) considering the geographic barriers to gene flow indicated by the cluster analyses (see below), with 1000 permutations, using the R package vegan (Oksanen et al. 2012). Geographic distances were measured as shortest sea distances in km between localities using Google Earth.

Population structuring was also investigated using clustering methods implemented in BAPS 5.4 (Corander et al. 2008), STRUCTURE 2.3.3 (Hubisz et al. 2009) and discriminant analysis of principal components (DAPC) in the adegenet 1.3-4 package (Jombart et al. 2010) in R (R Development Core Team 2011). These methods partition individuals into a number of genetic clusters (K) and determine their respective assignment probabilities to these clusters. BAPS and STRUCTURE perform a Bayesian analysis to identify hidden population structure by clustering individuals into genetically distinguishable groups on the basis of allele frequencies and linkage disequilibrium. The maximum number of clusters (K) in BAPS was initially set to 6, and then the K -value

exhibiting the minimum log-likelihood was selected in order to obtain the optimal partition of populations. Three different models were run with STRUCTURE, an admixture model with and without making use of geographic prior information, and a non-admixture model without priors. The 2 admixture models and 1 non-admixture model were run for a burn-in of 10^5 steps and followed by 5×10^5 steps. Ten replicates of each run were simulated to estimate the probability of the observed data X given the number of K -clusters $p(X|K)$. Evanno et al.'s (2005) method for testing K was applied to detect the strength of the genetic structure detected by the software. DAPC, on the other hand, is a multivariate method designed to identify and describe clusters of genetically related individuals (Jombart et al. 2010). This method seeks linear combinations of the original variables (alleles) which maximizes the ratio of between-group differences and within-group variation. Based on the retained discriminant functions, the analysis derives probabilities of membership (or assignment values) of each individual to each of the different groups (Jombart et al. 2010). As DAPC requires construction of prior groups, a sequential K -means clustering algorithm was first run for $K = 1$ to $K = 6$. The DAPC was performed for $K = 3$, which gave the lowest Bayesian Information Criterion (BIC), retaining 25 principal component analysis (PCA) components (the 'optimal' value following the a-score optimization procedure proposed in adegenet).

To further investigate genetic relationships, gene flow and dispersal between native range populations and the Icelandic population, Bayesian population assignment and exclusion tests were conducted using the method of Rannala & Mountain (1997) implemented in GENECLASS 2.0 software (Piry et al. 2004), with simulations performed following Paetkau et al. (2004). The ratio of the likelihood of sampling individuals from the population where they were sampled (L_{home}) and the highest likelihood value among all available populations ($L = L_{\text{home}}/L_{\text{max}}$) was used to detect first-generation migrants (F_0) (Paetkau et al. 2004). The individual likelihood scores were summarized with average scores (Piry et al. 2004) and unidirectional D_{LR} distances (D_{LRXY}) between all samples. The D_{LR} distance developed by Paetkau et al. (1997) is an average of 2 distances, D_{LRXY} and D_{LRYX} , where each is an average log ratio of the likelihoods of sampling individual i from population X in population X (L_{iXX}) and population Y (L_{iXY}). The unidirectional D_{LRXY} distance allows evaluation of asymmetrical gene flow between the pairs compared.

RESULTS

The mean number of alleles per microsatellite locus ranged from 7.7 in Iceland to 9.6 in New Brunswick (F), allelic richness values were similar across all samples (Table 1). Multi-locus heterozygosity was similar in all locations, with H_E ranging from 0.64 in New Brunswick (L) to 0.71 in Massachusetts (Table 1), and also when each locus was considered separately (see Table S1 in the Supplement at www.int-res.com/articles/suppl/m494p219_supp.pdf). The 2 most southern samples (Massachusetts and Bay of Fundy, New Brunswick) exhibited significantly higher allele numbers and allele richness than all other samples. Diversity indices for Iceland were not significantly different from the 3 northernmost native range samples (Table 1). Genotype proportions for all populations at all loci conformed to Hardy-Weinberg equilibrium expectations ($p > 0.05$; Table 1) after correcting for multiple testing with the Bonferroni adjustment (see Table S2 in the Supplement). No significant linkage disequilibrium between markers was found after Bonferroni adjustment.

The Icelandic and the Nova Scotia samples reported significant Wilcoxon signed rank test results under IAM but not SMM and TPM (Table 1), however the IAM values were only marginally significant ($p = 0.048$) with Bonferroni adjustment. The mode-shift tests all conformed to expectations for stable populations and the allele frequency distributions of alleles of all samples were similar (Fig. 2), although the Icelandic sample showed a slight indication of shift in allele frequencies from alleles with frequencies less than 10% to frequencies in the 10 to 20% class. Multi-locus average M values (as well as values obtained for each locus, ranging from

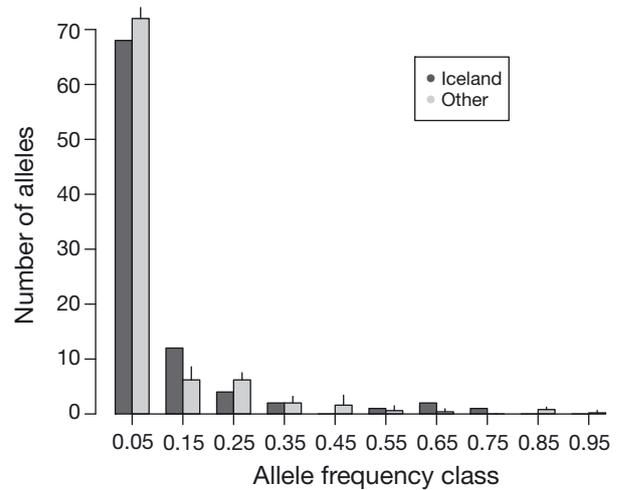


Fig. 2. *Cancer irroratus*. Allele frequency distributions of the microsatellite loci for the Icelandic sample (black) and the samples from North America (grey). Variation for the American samples is 1 SD

0.012 to 0.321) obtained for the 6 populations were well below the suggested values of $M \leq 0.68$ (Garza & Williamson 2001) (see Table S3 in the Supplement) that might reflect a bottleneck in an historic population.

Iceland is most differentiated from the other samples, as revealed by pairwise F_{ST} , $p < 0.001$ for all comparisons (see Table S4 in the Supplement). Both Bay of Fundy and Massachusetts were significantly different from all other samples, $p < 0.001$, but were not significantly different from each other. The pairwise F_{ST} values were strongly correlated with the shortest sea distances between samples (Mantel test, $p = 0.005$, with $r = 0.88$ from linear regression; Fig. 3). While geographic distance explained a large amount

Table 1. *Cancer irroratus*. Genetic variation in 6 samples averaged over 7 microsatellite loci: number of individuals screened (N), number of alleles per locus (A), allelic richness (A_R), observed heterozygosity (H_O), expected heterozygosity (H_E), inbreeding coefficient (F_{IS}), variance (s^2), p -values for the genetic bottleneck detection using Wilcoxon signed-rank test under Infinite Allele (IAM), Step-Wise Mutation (SMM) and Two-Phase Mutation (TPM) models, and mean values for the M -ratio test (M). Underlined values indicate significance after Bonferroni adjustment (Sokal & Rohlf 1995). * $p < 0.05$ in pairwise comparison with the Icelandic sample using Wilcoxon signed-rank test. See Fig. 1 for site codes

Site	N	Microsatellite									
		A	A_R	H_O	H_E	F_{IS}	s^2	IAM	SMM	TPM	M
I	55	7.714	7.294	0.651	0.659	0.018	0.740	<u>0.008</u>	0.297	0.688	0.178
N	40	8.714	8.459	0.629	0.679	0.059	0.741	0.016	0.109	0.469	0.064
L	50	8.286	8.218	0.635	0.642	-0.001	0.600	0.109	0.469	1.000	0.076
S	41	8.429	7.550	0.646	0.684	0.048	0.775	<u>0.008</u>	0.016	0.297	0.166
F	36	9.571*	9.523*	0.699	0.697	-0.014	0.846	0.016	0.023	0.688	0.129
M	40	9.286*	9.057*	0.656	0.706	0.086	0.880	0.078	0.039	0.938	0.151

of the variation in pairwise F_{ST} , inclusion of a barrier between the northern and southern groups in the native range greatly improved the model (partial Mantel test, $p < 0.005$, with $r = 0.94$). It is noteworthy that despite the clear relationship between genetic and geographic distance, the values for F_{ST} between the Icelandic sample and the different North American samples are similar (Fig. 3, Table S4). Excluding Iceland from the comparison resulted in a weaker relationship that nevertheless remained significant ($p = 0.035$).

In agreement with the results of pairwise tests, individuals were clustered into 3 genetic groups by the DAPC and STRUCTURE clustering methods: Cluster 1 = Iceland (I); Cluster 2 = Newfoundland (N), New Brunswick (L) and Nova Scotia (S); and Cluster 3 = New Brunswick (F) and Massachusetts (M) (Figs. 4 & 5, and see Table S5 in the Supplement). The most likely outcome with the BAPS analyses clustered individuals into 2 groups, Iceland vs. North America (Table S5), with the second most likely outcome generating the same 3 clusters as detected by STRUCTURE and DAPC. Pairwise comparisons of genetic variation among the 3 clusters revealed significantly lower allelic richness within Iceland compared to the southern group (Cluster 3) ($p = 0.019$), and lower observed heterozygosity in the northern group (Cluster 2) than in the southern group ($p = 0.036$). All other indices of genetic variation were not significantly different among the 3 groups. Overall, the differentiation statistics and clustering approaches reveal a clear pattern whereby Iceland is the sample most distinct from the others, while the North American samples are less differentiated from each other.

The individual assignment probabilities, obtained with GENECLASS, point to clear differentiation among all samples; the likelihood of self-assignment was 100% for all collection sites and null when assigned to other populations. Only a single individual in the Icelandic sample was detected as a possible first generation migrant (originated from Nova Scotia) and 2 in Nova Scotia (originated from Iceland and Massachusetts). The log likelihoods (Table 2) also clearly show this and the direction of gene flow (past or present) between any pairs of populations. The negative log likelihoods of samples

assigned to sample location were in the range of 43.2 to 48.3, generally much lower than assignments to other locations (ranging from 68.5 to 119.3), reflecting the frequency differences. An asymmetry in assignment values was clearly observed when comparing the southern samples Massachusetts and

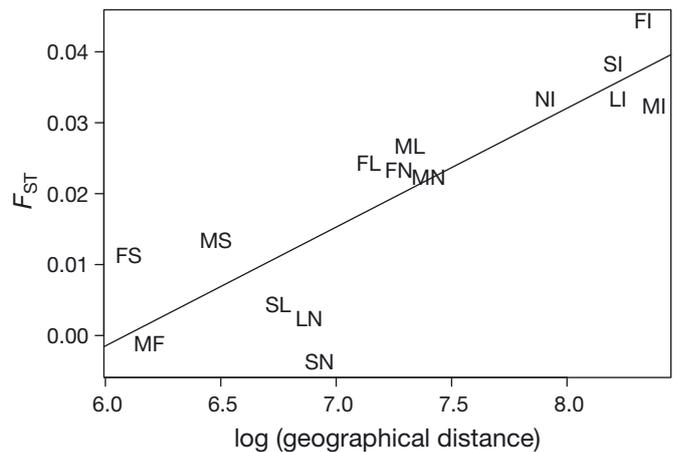


Fig. 3. *Cancer irroratus*. Isolation by distance (IBD) pattern comparing logarithms of shortest sea distance in km (in Google Earth) and genetic differentiation (F_{ST}) between sampling locations: Iceland (I), Newfoundland (N), Gulf of St. Lawrence, New Brunswick (L), Nova Scotia (S), Bay of Fundy, New Brunswick (F) and Massachusetts (M)

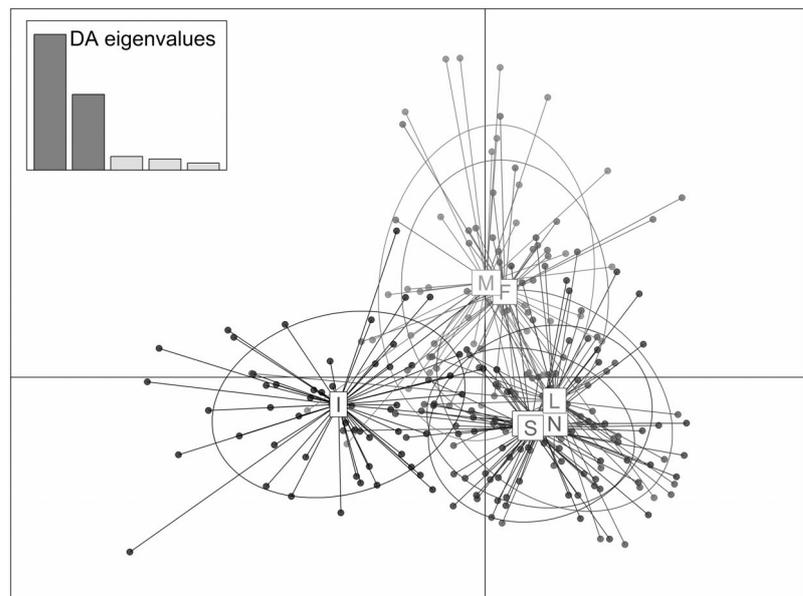


Fig. 4. *Cancer irroratus*. Discriminant analysis of principal components (DAPC) based on multi-locus microsatellite data from the 6 locations defines 3 clusters. Cluster 1: Iceland (I); Cluster 2: Newfoundland (N), Gulf of St. Lawrence, New Brunswick (L) and Nova Scotia (S); and Cluster 3: Bay of Fundy, New Brunswick (F) and Massachusetts (M). The first 2 principal axes are shown, with the first on the vertical axis. Relative contribution of the first 5 axes are shown in the panel

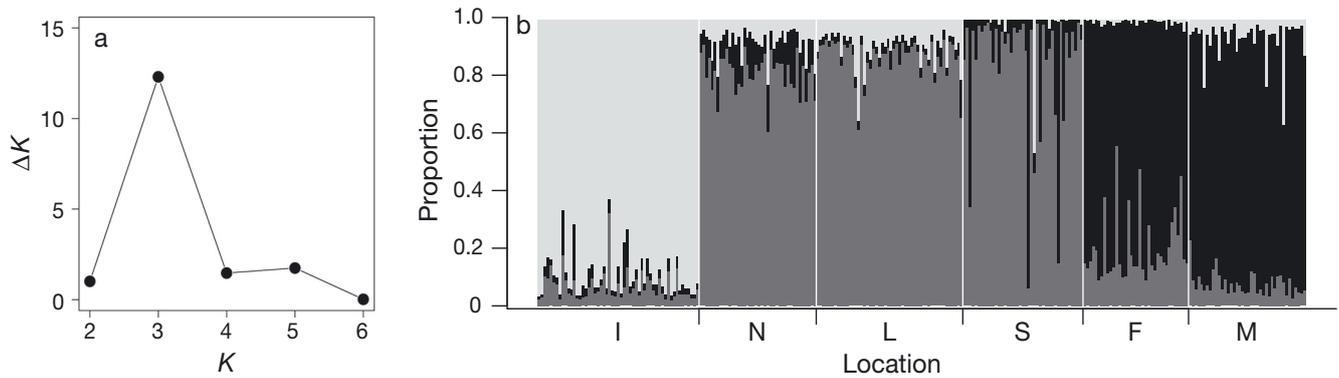


Fig. 5. *Cancer irroratus*. Results of Bayesian clustering analyses. (a) Plot of ΔK for different numbers of genetic clusters, $K = 3$ are best supported. Simulated using STRUCTURE. (b) Bar plot of inferred population assignment using STRUCTURE. Individual assignment sampled using admixture model with prior information. Bar shading indicates probability of being assigned to 3 genetic clusters corresponding to sample locations I (light grey), N + L + S (dark grey) or F + M (black). Sampled locations: Iceland (I), Newfoundland (N), Gulf of St. Lawrence, New Brunswick (L), Nova Scotia (S), Bay of Fundy, New Brunswick (F), and Massachusetts (M)

Table 2. *Cancer irroratus*. Pairwise assignment likelihood ($-\log L$ values) of samples obtained with GENECLASS. See Fig. 1 for location codes

	I	N	L	S	F	M
I	43.207	94.020	98.737	106.171	109.767	97.811
N	95.966	44.035	69.532	71.917	81.376	88.572
L	99.651	68.501	43.401	74.116	84.227	88.549
S	106.178	69.979	73.209	43.590	83.084	83.673
F	119.295	88.958	92.841	92.605	48.227	80.722
M	107.658	96.474	97.482	93.512	81.042	48.295

New Brunswick (F) with the other samples. The difference in the log likelihood ranged from 7.6 to 9.8, indicating that the southern samples were less similar to the northern samples than the northern samples were to the southern samples. The relationships were symmetric within the clusters (1 to 3) described above and, interestingly, a symmetry between Iceland and the northern samples can be observed. A similar result was observed when comparing differences in unidirectional D_{LR} distances between samples (see Table S6 in the Supplement) based on individual likelihoods obtained from GENECLASS (Mantel test, $p = 0.015$, $r = 0.91$); overall, despite the barrier, individuals in the southern populations (M and F) were less likely to be assigned to the northern populations than the individuals sampled in the north to the southern populations. The Icelandic sample had the highest likelihood of being assigned to Newfoundland (96, Table 2) and the number of individuals in Iceland that were found to have originated in one of the sample populations was as follows: 23 from Newfoundland, 14 from

Massachusetts, 5 from New Brunswick (F) and only 1 from New Brunswick (L) and Nova Scotia. These affinities of the Icelandic samples were different from the mean (\pm SD) scores obtained from the likelihoods of all individual assignments, which were highest in Massachusetts (27.1 ± 4.28), New Brunswick (L) (25.7 ± 3.87) and New Brunswick (F) (24.6 ± 3.14) and lower in Newfoundland (12.1 ± 2.54) and Nova Scotia (10.5 ± 1.65). The average score may be less reliable as it will pool low and high likelihood scores and it may thus not reflect the individual cases of high likelihood which are of most interest.

DISCUSSION

The sampled Atlantic rock crab populations from the east coast of North America and Iceland cluster into 3 genetic groups based on microsatellite genotypes. This clustering was supported by pairwise tests of genetic differentiation and followed a clear geographic pattern. The samples collected within the native range separated into a southern and a northern group. The Icelandic sample constituted the third and the most genetically distinct group, and was more similar to the northern group than the southern one. The genetic distances increased with geographic distances, both among all samples and when taking the structure into account, indicating isolation by distance (Wright 1943) within the clusters but also

a reduced gene flow across their boundary in North America.

Genetic analyses of decapod crustaceans have revealed complex patterns of genetic differentiation at various geographic scales, from low genetic divergence (e.g. American lobster *Homarus americanus*, Harding et al. 1997; European green crab *Carcinus maenas*, Tepolt et al. 2009; brown crab *Cancer pagurus*, Ungfors et al. 2009) to high genetic divergence (e.g. spider crabs *Inachus dorsettensis* and *Hyas coarctatus*, Weber et al. 2000; brown shrimp *Crangon crangon*, Weetman et al. 2007; Harris mud crab *Rhithropanopeus harrisi*, Projecto-Garcia et al. 2010). Despite the potential for high connectivity, the native range rock crab samples partitioned into 2 geographic groups; one distributed south of Nova Scotia to Massachusetts; the other stretching northward from Nova Scotia to Labrador, including the Gulf of St. Lawrence and Newfoundland. The genetic similarities between samples from the 2 clusters were largest between the southern samples and the neighboring population in Nova Scotia (S), indicating some gene flow across the barrier. Similar differentiation in the Canadian Maritimes has also been reported for 2 other species. The European green crab *Carcinus maenas* shows a steep genetic cline from Halifax to the Bay of Fundy due to invasion of different lineages (Roman 2006), and the variation among populations of American lobster *Homarus americanus* have been attributed to post-Pleistocene colonization events (Kenchington et al. 2009).

Several marine boreal species, including the rock crab, are likely to have had a compressed distribution to the south of their present range during the last glacial maximum (LGM) due to temperature limitations. Recent reconstructions of the ice sheet, however, suggest that Maritime Canada (e.g. Newfoundland and Nova Scotia) might have been ice-free habitats during the LGM (Charbit et al. 2007), and some species are believed to have survived the LGM in these refugia (Panova et al. 2011). These putative refugia are not likely to have been used by the rock crab due to temperature limitations of its planktonic stages (Johns 1981). It is much likelier, as Kenchington et al. (2009) suggested for the American lobster, that the rock crab persisted and expanded from a southern refuge. *M*-ratio tests indicated historical bottlenecks for all samples, which may reflect signatures of shared Pleistocene population contractions. Furthermore, although levels of genetic diversity were highly similar among samples, certain indices were reported to be significantly higher for southern samples, compatible with 'southern richness' (Hewitt 1996).

While range expansion from a southern refuge may have contributed to the association of genetic and geographic distances (e.g. Hutchison & Templeton 1999) within the native range, clustering analysis supported a north–south barrier to gene flow located between the 2 closest samples. There is a major oceanographic boundary off southwest Nova Scotia that acts as a barrier between the Scotian Shelf and the Gulf of Maine (Hannah et al. 2000, 2001). Fronts associated with this boundary could reduce gene flow via planktonic larvae of the rock crab and other species such as the European green crab and the American lobster. Furthermore, the genetic breakpoint at Nova Scotia coincides with an area of patchy distribution (Tremblay et al. 2007), which may reflect an area of habitat that is unsuitable for settling larvae and/or a breakdown in dispersal for adults. Interestingly, the barrier effect appears to be asymmetric, with more gene flow from north to south.

Rock crab in Iceland provides a model of a recently founded marine population. For several crab species, populations that are believed to have been founded by larvae transported in ballast water are characterized by reduced levels of genetic variation compared to native populations. Such species include the Harris mud crab *Rhithropanopeus harrisi* (Projecto-Garcia et al. 2010) and the Chinese mitten crab *Eriocheir sinensis* in Europe (Hänfling et al. 2002, Dittel & Epifanio 2009), and the European green crab *Carcinus maenas* outside Europe (Carlton & Cohen 2003, Yamada et al. 2005, Tepolt et al. 2009). However, levels of genetic variation in the Icelandic rock crab were comparable to those in the native range and similar variation within the native American populations and the Icelandic population was also observed in mtDNA sequences (Gíslason et al. 2013). A significant bottleneck effect was detected for Iceland under an IAM model but was not evident under the SMM and TPM models, or in the mode-shift test. Furthermore, a bottleneck under IAM was also detected for the Nova Scotia sample. Although the statistical power to detect a recent reduction in population size may be low (Peery et al. 2012), this suggests that the significant bottleneck results may reflect the greater propensity of Type I errors under this mutation model (Luikart & Cornuet 1998). A number of studies have indicated that genetic diversity of invading populations may be increased through admixture from multiple source populations (e.g. Roman 2006, Simon-Bouhet et al. 2006, Roman & Darling 2007, Wattier et al. 2007). Such a scenario for rock crab would be expected to generate Wahlund-like effects in the recently founded Icelandic population. These were

not detected, thus indicating that genetic admixture has occurred for 1 or more generations in Iceland. Both the individual clustering and the assignment tests report high 'self-assignment' of Icelandic individuals and low numbers of migrants; indicating that the population is largely self-recruiting. The rapid population expansion of the rock crab in Iceland may also have reduced the possible bottleneck effect, as it will favor the maintenance of rare alleles. The absence of migrants indicates that migration from the native range to Iceland is limited and that the colonization might have been a rare successful event. No changes have occurred on the shipping routes between the east coast of North America and Iceland but a new legislation of ballast water exchange in Icelandic waters, aimed to reduce transport of non-indigenous species, was passed in 2010.

The exact origin of the Icelandic population remains unknown. The newly colonized population shares the same alleles found in the North American native samples, and is thus genetically similar, but the frequencies of the alleles differ. Although the Icelandic population showed more similarity to the northern populations, the evidence is only weak and dependent on the method applied, i.e. certain assignment tests indicate a greater affinity to southern samples. A genetic origin from unsampled northern source populations is unlikely, given the known species distribution and information about shipping routes. A more extensive sampling in North America might elucidate the source(s) of the Icelandic population, whether it comes from a single unsampled source population or whether the frequencies have been shaped by admixture of different source populations and then for more than 1 generation. This study shows that an 'unnatural' establishment of species in a new environment can appear as a native population, and should be a caution to other researchers studying whether a species has recently invaded a new region.

The reason for the successful colonization of rock crab in Icelandic waters might lie in the large-scale changes observed in the North Atlantic Ocean in recent years (ICES 2004). Since 1996, Icelandic waters have become warmer, which has led to noticeable changes in the Icelandic marine ecosystem, especially with regard to the distribution of some fish species (Astthorsson & Pálsson 2006, Astthorsson et al. 2007, Gíslason et al. 2009, Stefansdóttir et al. 2010). The marine climate of Iceland is similar to that of the Canadian Maritimes, though sea surface temperatures in winter are lower and summer temperatures higher in most areas of the Maritimes (Ingólfsson

1992, DFO 2012). The size, aggression and broad diet of the rock crab are also factors that are likely to be aiding the colonization of the species. The rock crab is bigger than the native decapod crab species, the European green crab *Carcinus maenas* and the spider crab *Hyas araneus*, which inhabit the same areas and are presumably the main competitors. However, nothing is yet known about the predator-prey interactions and effects of the rock crab on the marine ecosystem in Iceland. The apparent lack of founder effects, high genetic variation and the spreading of the species along the southwest and west coast of Iceland indicates that the population is healthy and could thrive in Icelandic waters.

CONCLUSION

A clear split is found among Atlantic rock crab populations along the eastern coast of North America, coinciding with patterns observed in other species and indicating an oceanographic boundary to gene flow. The genetic variation in the newly founded population in Iceland is similar to that observed in the North American samples. The apparent lack of founder effects, high genetic variation and the spreading of the species along the southwest and west coast of Iceland indicates that the population is healthy and could thrive in Icelandic waters.

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