

Diluting the founder effect: cryptic invasions expand a marine invader's range

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Most invasion histories include an estimated arrival time, followed by range expansion. Yet, such linear progression may not tell the entire story. The European green crab (*Carcinus maenas*) was first recorded in the US in 1817, followed by an episodic expansion of range to the north. Its population has recently exploded in the Canadian Maritimes. Although it has been suggested that this northern expansion is the result of warming sea temperatures or cold-water adaptation, Canadian populations have higher genetic diversity than southern populations, indicating that multiple introductions have occurred in the Maritimes since the 1980s. These new genetic lineages, probably from the northern end of the green crab's native range in Europe, persist in areas that were once thought to be too cold for the original southern invasion front. It is well established that ballast water can contain a wide array of nonindigenous species. Ballast discharge can also deliver genetic variation on a level comparable to that of native populations. Such gene flow not only increases the likelihood of persistence of invasive species, but it can also rapidly expand the range of long-established nonindigenous species.

Keywords: *Carcinus maenas*; cline; cryptic invasion; mitochondrial DNA; planktonic dispersal

1. INTRODUCTION

Elton (1958) noted that the transport of exotic species was

gathering momentum every year...gradually breaking down the...distribution that species had even a hundred years ago.

(Elton 1958, p.30)

In the five decades since the publication of his *Ecology of Invasions of Animals and Plants*, this momentum has continued to grow. Although fouled ships and dry ballast have traditionally been important vectors for transporting marine nonindigenous species, it is now recognized that a wide diversity of vectors can transport these invaders, including aquaculture, aquaria, ballast water in ocean-going ships, restoration efforts, even sushi and live seafood (Carlton & Geller 1993; Carlton 2001; Chapman *et al.* 2004). As a result, the rate of marine invasions has risen exponentially in the past two centuries (Ruiz *et al.* 2000). Yet, there are few studies that compare modern and historical invasion processes.

The European green crab (*Carcinus maenas*) is one of the most successful marine invaders. It has high fecundity, a long planktonic larval period, opportunistic diet and tolerance for a wide variety of environmental factors (Yamada 2001). Being large and easily recognizable, this species has a well-established invasion record, spanning two centuries and four continents. The earliest records are from 1817, when the green crab was found in New York and southern Massachusetts (Say 1817). By 1900, these populations had also become established in Australia (Fulton & Grant 1900). In the 1980s, green crabs were discovered in Tasmania, South Africa, western North

America and Japan, elevating the species to the status of a global invader on temperate shores (Yamada 2001).

In eastern North America, it took almost 100 years for the green crab to expand its range from southern Massachusetts to the Gulf of Maine. It is now established from New Jersey to southeastern Prince Edward Island (figure 1), where it is often the most abundant crab in the rocky intertidal zone (Tyrell & Harris 1999). Despite its success in the Gulf of Maine, the green crab was not recovered in northern Nova Scotia until the 1980s (Audet *et al.* 2003). The episodic northern expansion of this invader is hypothesized to be the result of warming trends in surface waters along the Scotian Shelf (Berrill 1982; Jamieson 2000; Yamada 2001; Audet *et al.* 2003), a response consistent with studies linking climate change and the spread of invasive species (Stachowicz *et al.* 2002). It has also been postulated that green crabs may have adapted to cold-temperature regimes since the 1950s, when abundance dropped during severe winter conditions (Audet *et al.* 2003). I used molecular data from contemporary and museum samples to examine this range expansion and colonization pattern.

2. MATERIAL AND METHODS

Samples of *C. maenas* were collected from 25 locations in eastern North America (table 1). In 1999 and 2000, I gathered crabs from beneath cobbles in the rocky intertidal zone and used baited minnow traps in sandy areas. Six individuals were collected in Chester, Nova Scotia, in 1996, and 16 crabs were gathered in Searsport, Maine, in 2001. Eight museum specimens, collected in Nova Scotia between 1959 and 1976, were used to observe the temporal change in genetic diversity in this region.

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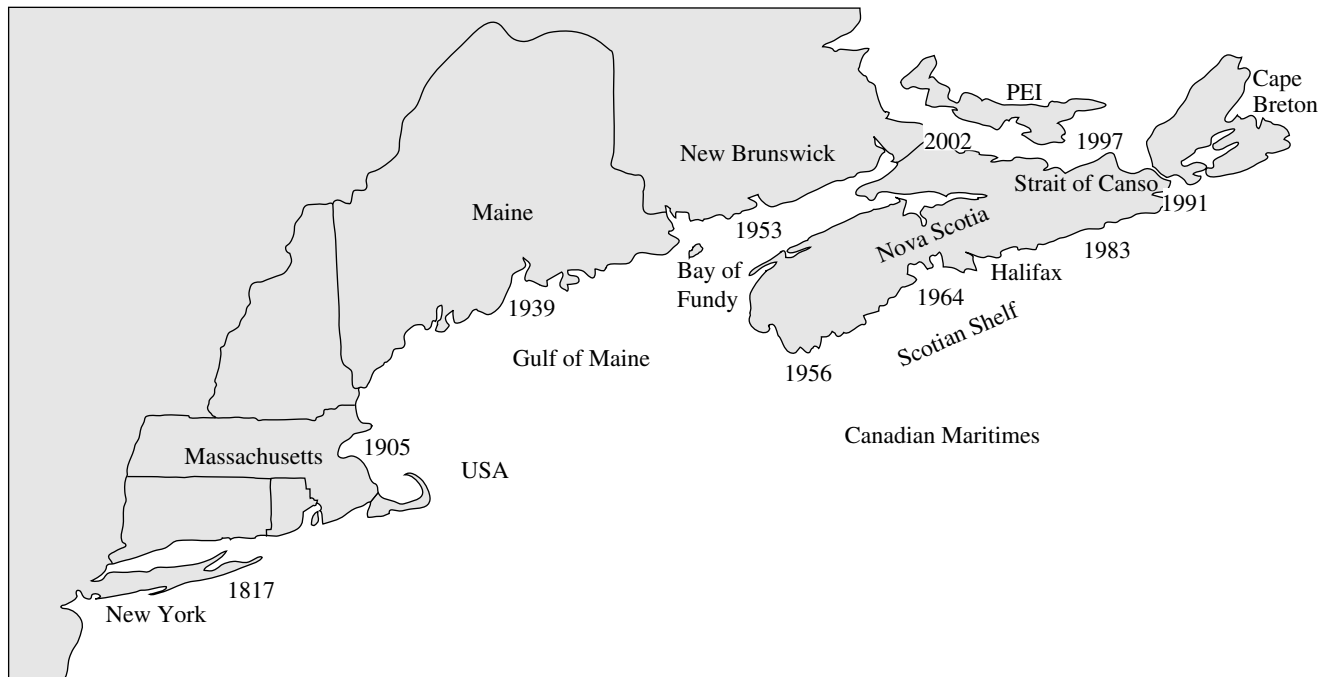


Figure 1. Northern range of the green crab, *Carcinus maenas*, on the east coast of North America. Dates indicate the first confirmed sighting of the species (adapted from Audet *et al.* 2003).

I amplified and sequenced a 502 bp fragment of the mitochondrial cytochrome oxidase I (*COI*) gene for 296 samples using primers designed for *Carcinus* (Roman & Palumbi 2004). DNA was extracted with a 10% Chelex (BioRad) solution (Walsh *et al.* 1991) from gills or muscle tissue of periopods. Although preservation techniques were not recorded for the museum samples, I isolated DNA from these specimens following a protocol for formalin-fixed crustaceans (France & Kocher 1996). PCR amplifications, sequencing and alignment followed the steps described in detail by Roman & Palumbi (2004).

The AMOVA algorithm in ARLEQUIN (Schneider *et al.* 2000) was used to estimate the molecular diversity indices and the levels of population subdivision. For each group, genetic variation is described by nucleotide diversity, π , and haplotype diversity, h (Nei 1987). Pairwise ϕ_{ST} s were employed to measure gene flow between European and North American populations. All European samples of *Carcinus* included in this analysis are from Roman & Palumbi (2004).

3. RESULTS

Eight mitochondrial haplotypes were identified for *C. maenas* in eastern North America (GenBank accessions AY616437–AY616439, DQ523682–DQ523686). These genotypes match, or are closely related to sequences of *C. maenas* collected along the North Atlantic coast of Europe. Sequence divergence between North American haplotypes is less than 2%. All substitutions are silent and occur at third-codon positions.

A steep cline occurs along the Scotian Shelf (figure 2). A single genotype, in red, is shared among most populations in the south of the Bay of Fundy, covering a geographical distance of more than 1000 km and representing 93% of all samples collected in the US and New Brunswick. The southern haplotype occurred at 75% frequency at Gulliver's Cove (GU), Nova Scotia, in the Bay of Fundy but only at 10% frequency in Musquodoboit Harbour (MU) along the Scotian Shelf; it was not found in

the north of this area. The shift between southern and northern genotypes occurs along a 460 km section of the shelf. Northern populations in Nova Scotia contained five haplotypes that are not found in the south. Haplotype diversity was 10 times higher in Nova Scotia ($h=0.77$) than in New York and the western Gulf of Maine ($h=0.06$). Northern genotypes do not form a separate clade from those in New England (figure 3).

Values of ϕ_{ST} were insignificant ($p>0.05$) among populations from Freeport (FR), New York, to Chance Harbour (CH), New Brunswick. Yet, values were significant between these southern groups and all populations along the eastern Scotian Shelf and Cape Breton. I used these values to separate the populations into two groups: Mid-Atlantic and Nova Scotia. Most of the genetic diversity was found within populations (60%). A smaller but strongly significant amount of diversity was found between Nova Scotia and the Mid-Atlantic groups (35%), and less between populations (5%). In contrast to crabs collected in Nova Scotia in 1999–2000, no haplotype diversity was found in eight samples collected along the Scotian Shelf between 1956 and 1979 (table 2). All these museum samples matched the widespread red haplotype (figure 2) and, as a group, they were indistinguishable from contemporary Mid-Atlantic populations ($\phi_{ST}=-0.052$). They were significantly divergent from samples collected in Halifax in 2001 ($\phi_{ST}=0.292$, $p<0.05$).

Five North American haplotypes were recovered in the European North Atlantic. This species has slight population structure along the continental shelf of Atlantic Europe, with a northern clade found in Scandinavia and northern Germany (Roman & Palumbi 2004). Pairwise-distance measures between Nova Scotia and northern Europe were much lower than the distances to western Europe, indicating that gene flow may have occurred between the North Sea and the Canadian Maritimes (figure 4).

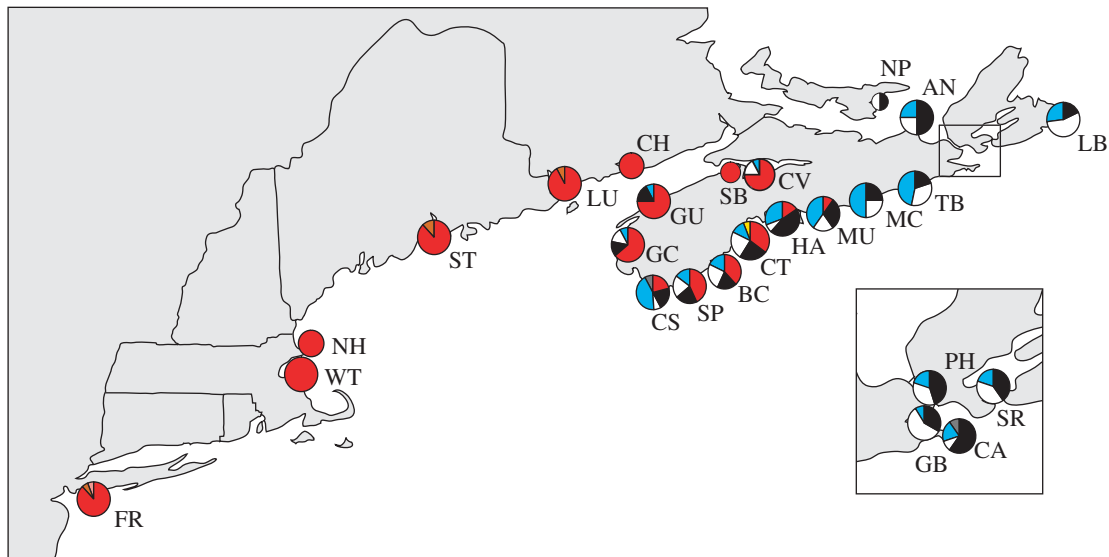


Figure 2. Relative frequencies of green crab haplotypes from 25 locations in North America sampled in 1996–2001. The size of each circle is approximately proportional to the sample size. Inset presents collection locales along the Strait of Canso.

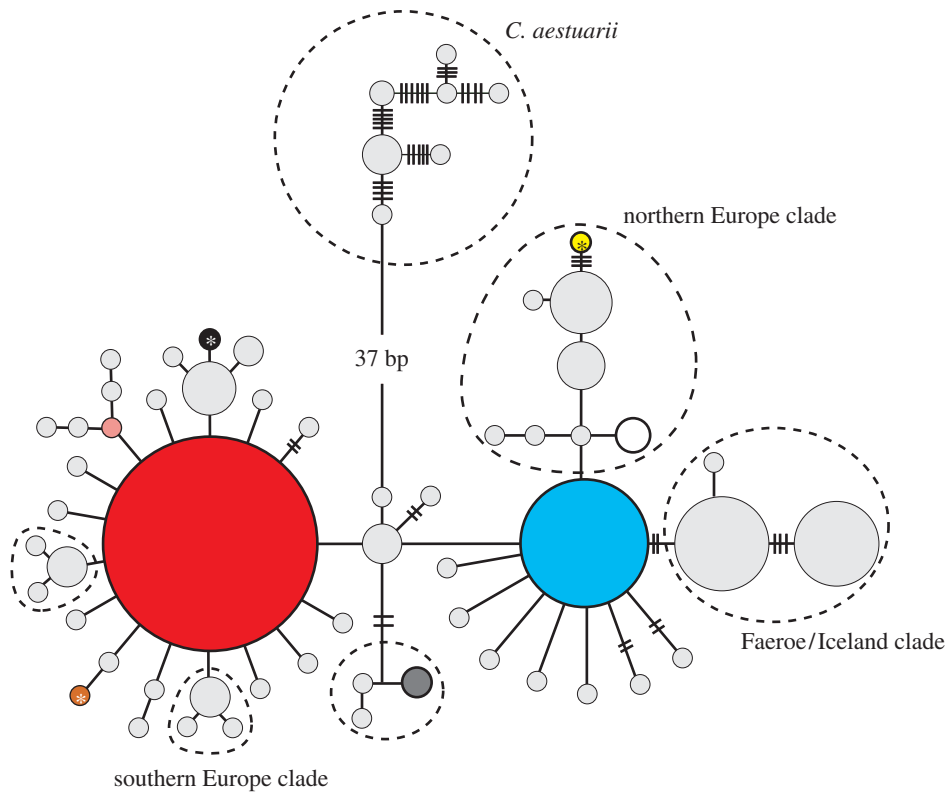


Figure 3. Parsimony network for mtDNA haplotypes of *Carcinus maenas* and *Carcinus aestuarii*. Genotypes marked with an asterisk were recovered in eastern North America but not in Europe. European haplotypes not represented in North America are in light grey. The size of the circles represents haplotype frequencies in Europe. Clades encircled by dashed lines have 100% majority-rule consensus support.

4. DISCUSSION

Contrary to prior expectations, haplotype diversity is much higher in the newly colonized northern regions of North America than in the Mid-Atlantic, where the green crab has resided for almost 200 years. A strong genetic cline exists along the Scotian Shelf, with the Mid-Atlantic haplotype (red in figure 2) diminishing in frequency from south to north. I consider three scenarios that may have caused this finding: (1) contact between an invasion front and a population native to the Canadian Maritimes, (2)

differentiation through selection in the northern part of the range, and (3) contact between two invasion fronts.

(1) The scenario of a native population of green crabs residing in the Maritimes is unlikely. *Carcinus* is a large intertidal species with well-established sighting records: none in northern Nova Scotia until the 1980s (Audet *et al.* 2003). Furthermore, all haplotypes recovered in this area are nested within the European parsimony network for *C. maenas* (figure 3) and show no evidence of a distinct evolutionary history outside of Europe.

Table 1. Collection locales for *Carcinus maenas* in eastern North America (sites without exact coordinates have been rounded to the nearest 10 min), abbreviations for field collection sites, number of individuals per sampling site (*N*) and sampling date.

sampling location	latitude N/longitude W	code	<i>N</i>	sampling date
<i>museum specimens</i>				
Black Rock, Nova Scotia	not available		1	Jul 1959
Prospect, Nova Scotia	not available		3	1966
			1	Sep 1969
			1	Aug 1970
Annapolis River, Nova Scotia	not available		1	Aug 1975
Lawrencetown, Nova Scotia	not available		1	Sep 1976
<i>field collections</i>				
Freeport, New York	40°38'/73°34'	FR	17	Dec 1999
Winthrop, Massachusetts	42°23'/70°58'	WT	11	Jul 2000
Nahant, Massachusetts	42°25'/70°54'	NH	7	Aug 1999
Searsport, Maine	44°27'/68°55'	ST	16	Jul 2001
Lubec, Maine	44°50'/66°59'	LU	13	Sep 1999
Chance Harbour, New Brunswick	45°07'/66°21'	CH	8	Aug 2000
Cheverie, Nova Scotia	45°10'/64°10'	CV	12	Jul 2001
Scots Bay, Nova Scotia	45°20'/64°30'	SB	6	Jul 2001
Gulliver's Cove, Nova Scotia	44°36'/65°55'	GU	13	Aug 2000
Grosses Coques, Nova Scotia	44°22'/65°05'	GC	14	Aug 2000
Cape Sable, Nova Scotia	43°26'/65°35'	CS	14	Aug 2000
Sandy Point, Nova Scotia	43°41'/65°14'	SP	13	Aug 2000
Broad Cove, Nova Scotia	44°10'/64°30'	BC	16	Aug 2000
Chester, Nova Scotia	44°32'/64°35'	CT	12	Aug 2000
	not available		6	Aug 1997
Halifax, Nova Scotia	44°37'/63°34'	HA	14	Aug 2000
Musquodoboit Harbour, Nova Scotia	44°50'/63°10'	MU	10	Aug 2000
Murphy's Cove, Nova Scotia	44°47'/62°45'	MC	16	Aug 2000
Tor Bay, Nova Scotia	45°11'/61°21'	TB	15	Aug 2000
Canso, Nova Scotia	45°19'/60°58'	CA	10	Aug 2000
Guysborough, Nova Scotia	45°23'/61°29'	GB	12	Aug 2000
Port Hawkesbury, Nova Scotia	45°37'/61°22'	PH	7	Aug 2000
St Peter's, Nova Scotia	45°39'/60°52'	SR	6	Aug 2000
Louisburg, Nova Scotia	45°55'/59°57'	LB	11	Aug 2000
Antigonish, Nova Scotia	45°37'/61°58'	AN	7	Aug 2000
Newport, Prince Edward Island	46°10'/62°30'	NP	2	Aug 2000

(2) Given the persistence of *Carcinus* in the Canadian Maritimes, it has been postulated that green crabs may have adapted to cold-temperature regimes since the 1950s, when abundance dropped during severe winter conditions (Audet *et al.* 2003). Yet, differentiation through selection from a single source in the Mid-Atlantic is highly unlikely. All changes are silent and are in the third position, so the mutations in *COI* would presumably have no selective advantage, and the mutation rates would have to be much higher than the estimates of 1.4–2.3% per million years (Knowlton & Weigt 1998; Schubart *et al.* 1998) to explain the genetic diversity in Nova Scotia. Silent variants can hitch-hike along with selective variants in the mitochondrial or nuclear genome, as has been found in *Drosophila melanogaster* (Kilpatrick & Rand 1995). But genetic hitch-hiking along with a nonsynonymous mutation typically leads to fixation or the reduction of variation (Barton 2000; Ballard & Dean 2001) and is unlikely to result in the rapid expansion of genetic diversity found in Nova Scotia. As noted for scenario (1), many of these northern haplotypes can be found in Europe and all cluster within the European parsimony network (figure 3), indicating that they have not been produced locally.

(3) The most likely scenario is the recent invasion of new genetic lineages in Nova Scotia. All eight specimens

Table 2. Summary statistics for genetic variability of the green crab in North America and selected locations in Europe (from Roman & Palumbi 2004).

population	haplotype diversity (<i>H</i>)	nucleotide diversity (π)
Mid-Atlantic, USA	0.06 ± 0.04	0.0001 ± 0.0004
Nova Scotia, 1956–1979	0.00	0.0000
Nova Scotia, 2000	0.77 ± 0.01	0.0048 ± 0.0029
Palmones, Spain	0.93 ± 0.07	0.0049 ± 0.0033
Bilbao, Spain	0.65 ± 0.13	0.0029 ± 0.0021
Mongstadt, Norway	0.90 ± 0.05	0.0048 ± 0.0030
Faeroe Islands	0.56 ± 0.06	0.0033 ± 0.0023

collected in this province between the 1950s and 1970s matched the widespread southern haplotype, indicating that the first Nova Scotian green crabs were identical to those in the Gulf of Maine. Yet, these vanguards of the southern invasion front appear to have been replaced with new lineages. The species was not recovered in surveys around Halifax Harbor in the late 1960s and early 1970s (Davis 1972). When green crabs became established along the northern Scotian Shelf and southern Gulf of Saint Lawrence in the 1980s (Audet *et al.* 2003), the genetics of

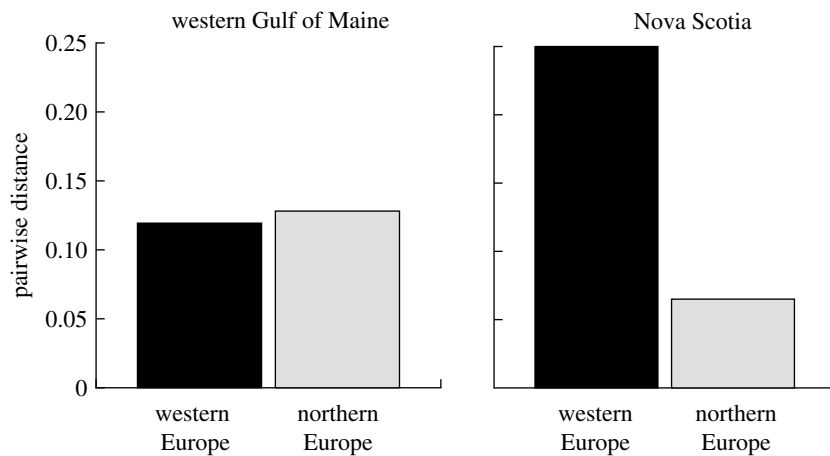


Figure 4. Population pairwise differences (ϕ_{ST}) as an estimate of gene flow in *Carcinus maenas* between western and northern Europe and North America. Western and northern Europe (defined by a break between Hoek van Holland and Bremerhaven, Germany) are approximately at the same distance from the original Mid-Atlantic invasion front. The twentieth-century invasion in Nova Scotia, however, is much closer in genetic distance to northern Europe, indicating recent gene flow from the North Sea.

these invading populations had dramatically changed. Five new lineages were recovered, with multiple mutation steps between them and an absence of intermediate haplotypes.

(a) Modern versus historical invasions

The high nucleotide and haplotype diversities in the Maritimes indicate that modern invasions can dilute the founder effect for nonindigenous marine species. At least five new lineages have been introduced to Nova Scotia in recent decades, and these invasive populations now have levels of diversity more comparable to European locations than to those in the Mid-Atlantic (table 2). Such heightened levels of variation reveal the difference between historical introductions and modern invasions.

The original nineteenth-century introduction, which probably occurred through rock ballast or the hull-fouling community of a wooden ship (Yamada 2001), exhibits reduced genetic diversity—presumably a result of the small number of propagules typically associated with a founder event. (It is also possible that a genetic bottleneck has occurred since this invasion. Yet, given the steady expansion of the species over two centuries, and its persistent population south of Cape Cod, this scenario seems unlikely.) Ballast-water inoculations, in contrast, are not limited to small founding population sizes. Not only can entire planktonic communities be transported in the holds of large ocean-going vessels (Carlton & Geller 1993), but a great deal of the genetic diversity in local populations may be shipped out as well. Perhaps this is not surprising given the size of ballast tanks and the absence of strict controls to marine discharge. Individual tankers docking at Point Tupper, along the Strait of Canso, discharge up to 4×10^7 l of ballast water, with ships arriving from Europe harbouring more than 50 zooplanktonic organisms per litre in their tanks (Carver & Mallet 2002).

(b) The entry of new invaders

If new populations of green crabs arrived along the coast of Nova Scotia in the 1980s, where did they come from? The Strait of Canso is a narrow seaway that connects the Atlantic with the Gulf of Saint Lawrence, defining the southwest coast of Cape Breton Island. In 1955,

a causeway was completed across the strait, creating one of the largest ice-free harbours in northeastern North America and a major shipping port for numerous transoceanic cargo vessels. Green crabs were first sited along this strait in 1991 (Audet *et al.* 2003). The absence of southern haplotypes (inset, figure 2) and the presence of high haplotype diversity in the Canso area support the Strait of Canso Superport as the possible centre for a new crab invasion. In less than 20 years, these lineages appear to have spread north and west around Cape Breton and the Northumberland Strait and south to the Gulf of Maine.

The invasion history of the green crab has been closely watched in North America—so how did this new invasion go undetected? The answer may lie in the episodic expansion of this species. Before the 1980s, green crab populations had stalled along the central Scotian Shelf, around the Halifax area. Then in the 1990s, Cape Breton was rapidly colonized, and, in the absence of genetic tests, this spread appeared to be the result of natural dispersal or a population response to warmer sea temperatures (Jamieson 2000). Although this expansion occurred after the introduction of new propagules, it is important to note that sea temperatures may have also played a role. Stachowicz *et al.* (2002) noted that warming trends in New England in the 1970s and 1980s allowed ascidian propagules to become established. Further warming trends in North America will likely increase the dominance of green crabs and other nonindigenous species in Maritime coastal waters.

The arrival of these lineages may be the result of new shipping routes that have opened up in recent years. The widespread white haplotype (figure 2) and the rare yellow one are derived from a northern European clade restricted to the North Sea (figure 3). In Europe, the white haplotype was recovered only in the Norwegian populations of Mongstad and Trondheim. Frequent shipping between Mongstad, a central port for Norwegian oil companies, and the Strait of Canso supports the possibility of this area as an invasion source, as does the low genetic distance between Nova Scotia and northern European populations (figure 4). Given the slight genetic structure of *COI* in Europe, it is clear that microsatellite data could help resolve the issue. If these new lineages are from the northern end of the range of *Carcinus* in Europe,

then they may have greater tolerance to cold. Future studies on the temperature tolerance of all the invasive lineages would help resolve this issue.

The overlap of these two invasion fronts has produced the cline seen in figure 2. Since coastal surface waters generally flow from the northern Gulf of Maine towards the southwest, the cline may shift anticlockwise towards New Brunswick and the US. This shift, however, could be slow to change, as the relatively stable clines of some native species on the East Coast have exhibited (Gonzalez-Villaseñor & Powers 1990; Karl & Avise 1992). Temporal observations of the *Carcinus* cline may reveal important clues about the dispersal of larvae and the interactions between two fronts along a single coastline.

(c) *Diluting the founder effect: conservation implications*

In the past few years, a number of papers have reported on the increased level of genetic diversity in aquatic invaders, presumably introduced through ballast water or aquaculture (Dillon & Stepien 2001; Hänfling *et al.* 2002; Stepien *et al.* 2002; Dupont *et al.* 2003; Voisin *et al.* 2005; Simon-Bouhet *et al.* 2006). The data for these species indicate that planktonic assemblages entrained by ships are not only species rich (Carlton & Geller 1993) but also genetically diverse. The high levels of variation in these studies have important conservation consequences. Increased diversity is an indication of higher propagule pressure, a function of the size and frequency of inoculations (Grevstad 1999; Minton *et al.* 2005). Large numbers of released individuals increase population growth rates, the probability of establishment (Lockwood *et al.* 2005), and can expand a nonindigenous species's range. Von Holle & Simberloff (2005) have recently shown that this propagule pressure can overwhelm ecological resistance. Prevention strategies will require action against multiple invasion routes and high propagule pressure.

Multiple invasions appear to be widespread and accelerating, even in long-established species such as *C. maenas*. As a result, the genetic winnowing typically associated with the founder effect can disappear. Berg *et al.* (2002) revealed a temporal shift in the genetic structure of North American populations of the invasive cladoceran *Bythotrephes longimanus*: the founder effect, which distinguished North America from Europe in 1989, had disappeared by 1996. The resulting genetic structure of the invading populations was similar to that of native European populations. Similarly, Neilson & Wilson (2005) found that a 1963 invasion of the yellowfin goby, *Acanthogobius flavimanus*, showed evidence of a founder effect, but a subsequent invasion in 1979 exhibited no reduction in genetic diversity. Multiple invasions blend genetic variation from different source populations and allow genetic diversity to increase rapidly over short periods of time.

On the other hand, reduced diversity is not necessarily an obstacle to invasion success. One green crab lineage, the expected genetic signature of a severe bottleneck after a single-event introduction (Allendorf & Lundquist 2003), may have predominated in North America for most of two centuries. The new introductions appear to have promoted the rapid range expansion of this long-established nonindigenous species. The green crab's persistence in areas once thought to be too cold for its survival indicates

that new genetic lineages may deliver novel physiological characteristics, which enhance the ability of invasive species to persist in wider temperature and salinity regimes.

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REFERENCES

- Allendorf, F. W. & Lundquist, L. L. 2003 Introduction: population biology, evolution, and control of invasive species. *Conserv. Biol.* **17**, 24–30. (doi:10.1046/j.1523-1739.2003.02365.x)
- Audet, D., Davis, D. S., Miron, G., Moriyasu, M., Benhalima, K. & Campbell, R. 2003 Geographic expansion of a nonindigenous crab, *Carcinus maenas* (L.), along the Nova Scotian shore into the southeastern Gulf of St Lawrence, Canada. *J. Shellfish Res.* **22**, 255–262.
- Ballard, J. W. & Dean, M. D. 2001 The mitochondrial genome: mutation, selection and recombination. *Curr. Opin. Genet. Dev.* **11**, 667–672. (doi:10.1016/S0959-437X(00)00251-3)
- Barton, N. H. 2000 Genetic hitchhiking. *Phil. Trans. R. Soc. B* **355**, 1553–1562. (doi:10.1098/rstb.2000.0716)
- Berg, D. A., Garton, D. W., Macisaac, H. J., Panov, V. E. & Telesh, I. V. 2002 Changes in genetic structure of North American *Bythotrephes* populations following invasion from Lake Lagoda, Russia. *Freshwater Biol.* **47**, 275–282. (doi:10.1046/j.1365-2427.2002.00805.x)
- Berrill, M. 1982 The life cycle of the green crab *Carcinus maenas* at the northern end of its range. *J. Crustacean Biol.* **2**, 31–39. (doi:10.2307/1548108)
- Carlton, J. T. 2001 *Introduced species in US coastal waters: environmental impacts and management priorities*. Arlington, VA: Pew Oceans Commission.
- Carlton, J. T. & Geller, J. B. 1993 Ecological roulette: the global transport of nonindigenous marine organisms. *Science* **261**, 78–82.
- Carver, C. E. & Mallet, A. L. 2002 *An assessment of the risk of ballast water-mediated introduction of non-indigenous phytoplankton and zooplankton into Atlantic Canadian waters*. Dartmouth, NS: Transport Canada.
- Chapman, J. W., Miller, T. W. & Coan, E. V. 2004 Live seafood as recipes for invasion. *Conserv. Biol.* **17**, 1386–1395. (doi:10.1046/j.1523-1739.2003.01016.x)
- Davis, D. S. 1972 *The Atlantic Shore*. Halifax, NS: Nova Scotia Museum.
- Dillon, A. K. & Stepien, C. A. 2001 Genetic and biogeographic relationships of the invasive round (*Neogobius melanostomus*) and tubenose (*Proterorhinus marmoratus*) gobies in the Great Lakes versus Eurasian populations. *J. Great Lakes Res.* **27**, 267–280.
- Dupont, L., Jollivet, D. & Viard, F. 2003 High genetic diversity and ephemeral drift effects in a recent and successful introduced mollusc (*Crepidula fornicata*: Gastropoda). *Mar. Ecol. Prog. Ser.* **253**, 183–195.
- Elton, C. S. 1958 *The ecology of invasions by animals and plants*. Chicago, IL: University of Chicago.
- France, S. C. & Kocher, T. D. 1996 DNA sequencing of formalin-fixed crustaceans from archival research collections. *Mol. Mar. Biol. Biotechnol.* **5**, 304–313.

- Fulton, S. W. & Grant, F. E. 1900 Note on the occurrence of the European green crab *Carcinus maenas*, Leach, in Port Phillip. *Vic. Nat.* **27**, 147–148.
- Gonzalez-Villaseñor, L. I. & Powers, D. A. 1990 Mitochondrial-DNA restriction-site polymorphisms in the teleost *Fundulus heteroclitus* support secondary integradation. *Evolution* **44**, 27–37. (doi:10.2307/2409522)
- Grevstad, F. S. 1999 Factors influencing the chance of population establishment: implications for release strategies in biocontrol. *Ecol. Appl.* **9**, 1439–1447.
- Hänfling, B., Carvalho, G. R. & Brandl, R. 2002 mtDNA sequences and possible invasion pathways of the Chinese mitten crab. *Mar. Ecol. Prog. Ser.* **238**, 307–310.
- Jamieson, G. S. 2000 European green crab, *Carcinus maenas*, introductions in North America: differences between the Atlantic and Pacific experiences. In *Proc. 10th Int. Aquatic Nuisance Species and Zebra Mussel Conference*, pp. 301–316.
- Karl, S. A. & Avise, J. C. 1992 Balancing selection at allozyme loci in oysters: implications from nuclear RFLPs. *Science* **256**, 100–102.
- Kilpatrick, S. T. & Rand, D. M. 1995 Conditional hitchhiking of mitochondrial DNA: frequency shifts of *Drosophila melanogaster* mtDNA variants depend on nuclear genetic background. *Genetics* **141**, 1113–1124.
- Knowlton, N. & Weigt, L. A. 1998 New dates and new rates for divergence across the Isthmus of Panama. *Proc. R. Soc. B* **265**, 2257–2263. (doi:10.1098/rspb.1998.0568)
- Lockwood, J. L., Cassey, P. & Blackburn, T. 2005 The role of propagule pressure in explaining species invasions. *Trends Ecol. Evol.* **20**, 223–228. (doi:10.1016/j.tree.2005.02.004)
- Minton, M. S., Verling, S., Miller, A. W. & Ruiz, G. M. 2005 Reducing propagule supply and coastal invasions via ships: effects of emerging strategies. *Front. Ecol. Environ.* **3**, 304–308.
- Nei, M. 1987 *Molecular evolutionary genetics*. New York, NY: Columbia University Press.
- Neilson, M. E. & Wilson, R. R. 2005 mtDNA singletons as evidence of a post-invasion genetic bottleneck in yellowfin goby *Acanthogobius flavimanus* from San Francisco Bay, California. *Mar. Ecol. Prog. Ser.* **296**, 197–208.
- Roman, J. & Palumbi, S. R. 2004 A global invader at home: population structure of the green crab, *Carcinus maenas*, in Europe. *Mol. Ecol.* **13**, 2891–2898. (doi:10.1111/j.1365-294X.2004.02255.x)
- Ruiz, G. M., Fofonoff, P. W., Carlton, J. T., Wonham, M. J. & Hines, A. H. 2000 Invasion of coastal marine communities in North America: apparent patterns, processes, and biases. *Annu. Rev. Ecol. Syst.* **31**, 481–531. (doi:10.1146/annurev.ecolsys.31.1.481)
- Say, T. 1817 An account of the Crustacea of the United States. *J. Acad. Nat. Sci. Phila.* **1**, 57–63.
- Schneider, S., Roessli, D. & Excoffier, L. 2000 *ARLEQUIN: a software for population genetics data analysis, version 2.000*. Geneva, Switzerland: Genetics and Biometry Laboratory, University of Geneva.
- Schubart, C. D., Diesel, R. & Hedges, S. B. 1998 Rapid evolution to terrestrial life in Jamaican crabs. *Nature* **393**, 363–365. (doi:10.1038/30724)
- Simon-Bouhet, B., Garcia-Meunier, P. & Viard, F. 2006 Multiple introductions promote range expansion of the mollusc *Cyclope neritea* (Nassariidae) in France: evidence from mitochondrial sequence data. *Mol. Ecol.* **15**, 1699–1711. (doi:10.1111/j.1365-294X.2006.02881.x)
- Stachowicz, J. J., Terwin, J. R., Whitlatch, R. B. & Osman, R. W. 2002 Linking climate change and biological invasions: ocean warming facilitates non-indigenous species invasion. *Proc. Natl Acad. Sci. USA* **99**, 15 497–15 500.
- Stepien, C. A., Taylor, C. D. & Dabrowska, K. A. 2002 Genetic variability and phylogeographical patterns of a nonindigenous species invasion: a comparison of exotic vs. native zebra and quagga mussel populations. *J. Evol. Biol.* **15**, 314–328. (doi:10.1046/j.1420-9101.2002.00385.x)
- Tyrell, M. C. & Harris, L. G. 1999 Potential impact of the introduced Asian shore crab, *Hemigrapsis sanguineus*, in northern New England: diet, feeding preferences, and overlap with the green crab, *Carcinus maenas*. In *Marine bioinvasions: Proceedings of a Conference, January 24–27, 1999* (ed. J. Pederson), pp. 208–220. Cambridge, MA: MIT Sea Grant.
- Voisin, M., Engel, C. & Viard, F. 2005 Differential shuffling of native genetic diversity across introduced regions in a brown alga: aquaculture vs. maritime traffic effects. *Proc. Natl Acad. Sci. USA* **102**, 5432–5437. (doi:10.1073/pnas.0501754102)
- Von Holle, B. & Simberloff, D. 2005 Ecological resistance to biological invasion overwhelmed by propagule pressure. *Ecology* **86**, 3212–3218.
- Walsh, P. S., Metzger, D. A. & Higuchi, R. 1991 Chelex-100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* **10**, 506–513.
- Yamada, S. 2001 *A global invader: the European green crab*. Corvallis, OR: Oregon State University.