Molecular Ecology (2006) **15**, 3641–3653 doi: 10.1111/j.1365-294X.2006.03012.x

Contrasting patterns in genetic diversity following multiple invasions of fresh and brackish waters

DAVID W. KELLY, JAMES R. MUIRHEAD, DANIEL D. HEATH and HUGH J. MACISAAC
Great Lakes Institute for Environmental Research, University of Windsor, 401 Sunset Avenue, Windsor, Ontario, Canada N9B 3P4

Abstract

Biological invasions may combine the genetic effects of population bottlenecks and selection and thus provide valuable insight into the role of such processes during novel environmental colonizations. However, these processes are also influenced by multiple invasions, the number of individuals introduced and the degree of similarity between source and receiving habitats. The amphipod *Gammarus tigrinus* provides a useful model to assess these factors, as its invasion history has involved major environmental transitions. This species is native to the northwest Atlantic Ocean, although it invaded both brackish and freshwater habitats in the British Isles after introduction more than 65 years ago. It has also spread to similar habitats in Western Europe and, most recently, to Eastern Europe, the Baltic Sea, and the Laurentian Great Lakes. To examine sources of invasion and patterns of genetic change, we sampled populations from 13 native estuaries and 19 invaded sites and sequenced 542 bp of the mitochondrial COI gene. Strong native phylogeographical structure allowed us to unambiguously identify three allopatrically evolved clades (2.3–3.1% divergent) in invading populations, indicative of multiple introductions. The most divergent clades occurred in the British Isles and mainland Europe and were sourced from the St Lawrence and Chesapeake/Delaware Bay estuaries. A third clade was found in the Great Lakes and sourced to the Hudson River estuary. Despite extensive sampling, *G. tigrinus* did not occur in freshwater at putative source sites. Some European populations showed reduced genetic diversity consistent with bottlenecks, although selection effects cannot be excluded. The habitat distribution of clades in Europe was congruent with the known invasion history of secondary spread from the British Isles. Differences in salinity tolerance among lineages were suggested by patterns of habitat colonization by different native COI clades. Populations consisting of admixtures of the two invading clades were found principally at recently invaded fresh and brackish water sites in Eastern Europe, and were characterized by higher genetic diversity than putative source populations. Further studies are required to determine if these represent novel genotypes. Our results confirm that biological invasions need not result in diminished genetic diversity, particularly if multiple source populations, each with distinctive genetic composition, contribute to the founding populations.

Keywords: amphipod, bottlenecks, freshwater adaptation, genetic diversity, invasion, phylogeography

Received 29 January 2006; revision accepted 2 May 2006

Introduction

Population bottlenecks associated with colonizations and range expansions are expected to lead to stochastic loss of genetic variation (Nei *et al*. 1975) which can modify ecological and evolutionary responses to environmental challenges (e.g. see Parsons 1983; Hawley *et al*. 2006; but see Lee 2002). Species invasions are ideal natural experiments in which to investigate these responses as they may involve both stochastic processes and the selection effects associated with small founding populations and rapid and novel

Correspondence: David W. Kelly, Fax: (519) 971 3616; E-mail: dwkelly@uwindsor.ca

environmental transitions (Lee & Bell 1999; Sakai *et al*. 2001; Wares *et al*. 2005). Low genetic diversity in some invasion studies is consistent with bottleneck and drift effects (Barrett & Shore 1989; Tsutsui *et al*. 2000; Sakai *et al*. 2001) but the effect on establishment success and long-term persistence is difficult to predict. For example, bottlenecks may create opportunities for new allelic combinations, converting genetic variation due to epistatic or dominance interactions into additive genetic variation (Lee 2002; Barton & Turelli 2004). Increased additive genetic variance during invasion would increase heritable phenotypic variation, providing fuel for evolutionary adaptation in novel environments (Lee 2002). The likelihood of seeding a population also may be greater for single, self-compatible propagules than for multiple propagules that are self-incompatible (Baker 1967). Furthermore, the apparent loss of diversity may be actually a consequence of native population differentiation across habitats, such that only specific genotypes survive new selective regimes (Bastrop *et al*. 1998; Reznick & Ghalambor 2001; Lee 2002). For example, Vasquez *et al*. (2005) identified salinity-dependent invasion success of different genotypes in the common reed *Phragmites australis*.

Surprisingly, a number of contemporary reviews of both animal and plant species find little support for a loss of genetic diversity associated with successful invasions (see reviews in Lee *et al*. 2004; Novak & Mack 2005; Wares *et al*. 2005). These patterns have been explained by high propagule pressure, that is, the introduction of a large number of individuals, as the effects of random genetic drift are inversely related to the effective size of the founding population size. High propagule pressure can be achieved either through frequent introductions or by a single introduction of a large number of individuals. Multiple introductions reduce bottleneck effects as they provide gene flow between source and destinations. Multiple introductions from divergent source populations can lead to admixture zones, which are characterized by higher within-population genetic diversity compared to source populations (Kolbe *et al*. 2004; Novak & Mack 2005; Voisin *et al*. 2005). Admixture zones may lead to novel genotypes that are more fit than parental genotypes in the novel environment, thereby promoting accelerated rates of evolution and range expansion (Ellestrand & Schierenbeck 2000; Wares *et al*. 2005).

The invasion of freshwater by estuarine and saltwater species requires a major physiological transition that few taxa have made (Lee & Bell 1999). Success of those species that have made this transition over the past 200 years has been attributed to new invasion vectors and pathways, disturbance and pollution, and the absence of competition (Lee & Bell 1999; Ricciardi & MacIsaac 2000; Reznick & Ghalambor 2001). Invasions of freshwater by estuarine and saltwater species should be accompanied by strong selection; thus, genetic surveys in these systems provide an excellent opportunity with which to identify mechanisms underlying evolutionary change (Lee & Bell 1999; Lee 2002).

The objectives of this study were to use a molecular phylogeographical approach to deduce invasion pathways between source and introduced populations of the amphipod *Gammarus tigrinus*, and to explore mechanisms underlying ecological changes associated with transition between divergent source and destination habitats.

Invasion history and expectations

Gammarus tigrinus, native to tidal estuaries of the northwest Atlantic Ocean, is widely distributed from the St Lawrence River in Quebec to the east coast of Florida, and occurs in salinities up to 25 PSU (practical salinity units; Bousfield 1973; D. Kelly, personal observation). The amphipod also has an extensive invasion history. *G. tigrinus* was first reported in 1931 in England in fresh waters contaminated by natural brine seepage (Sexton & Cooper 1939). However, it was reported thereafter in purely freshwater sites in Northern Ireland, leading Hynes (1955) to ascribe broad physiological plasticity for its invasion success. In 1957, 1000 individuals were introduced from a brackish brine lake in England to the salt-polluted River Werra, Germany, ostensibly to replace native species that declined as a consequence of pollution (Schmitz 1960; Fig. 1). The population established and spread downstream to the River Weser estuary in 1967, west to the Ems River estuary, and east to the oligohaline Kiel Canal and western Baltic Sea between 1975 and 1979 (Bulnheim 1980, 1985; Jaźdźewski 1980). In 1960, a few dozen individuals from freshwater Lough Neagh in Northern Ireland were reportedly introduced to the freshwater Ijsselmeer, the Netherlands to supplement fish feeding (Nijssen & Stock 1966; Fig. 1) after which, dispersal to inland fresh waters, including the Rhine River system and east to the Ems River, followed (Nijssen & Stock 1966; Pinkster *et al*. 1992). An allozyme study showed differentiation between Dutch and Weser-Werra populations, suggesting that these secondary introduction waves resulted from populations of different origins (Bulnheim 1985). However, close to the German-Dutch border, the Ems River estuary population was genetically intermediate between these groups, suggesting the occurrence of gene flow (Bulnheim 1985). More recently, *G. tigrinus* dispersed over a much greater spatial scale, including throughout the freshwater Elbe and Oder Rivers, and in the Baltic Sea in the Vistula Lagoon and along the northern Finnish coast (Jaźdźewski *et al.* 2002; Pienimaki *et al.* 2004). Finally, *G. tigrinus* invaded the Laurentian Great Lakes, first reported in sample collections from 2002.

A study of native North American *G. tigrinus* populations identified two cryptic species, highly divergent

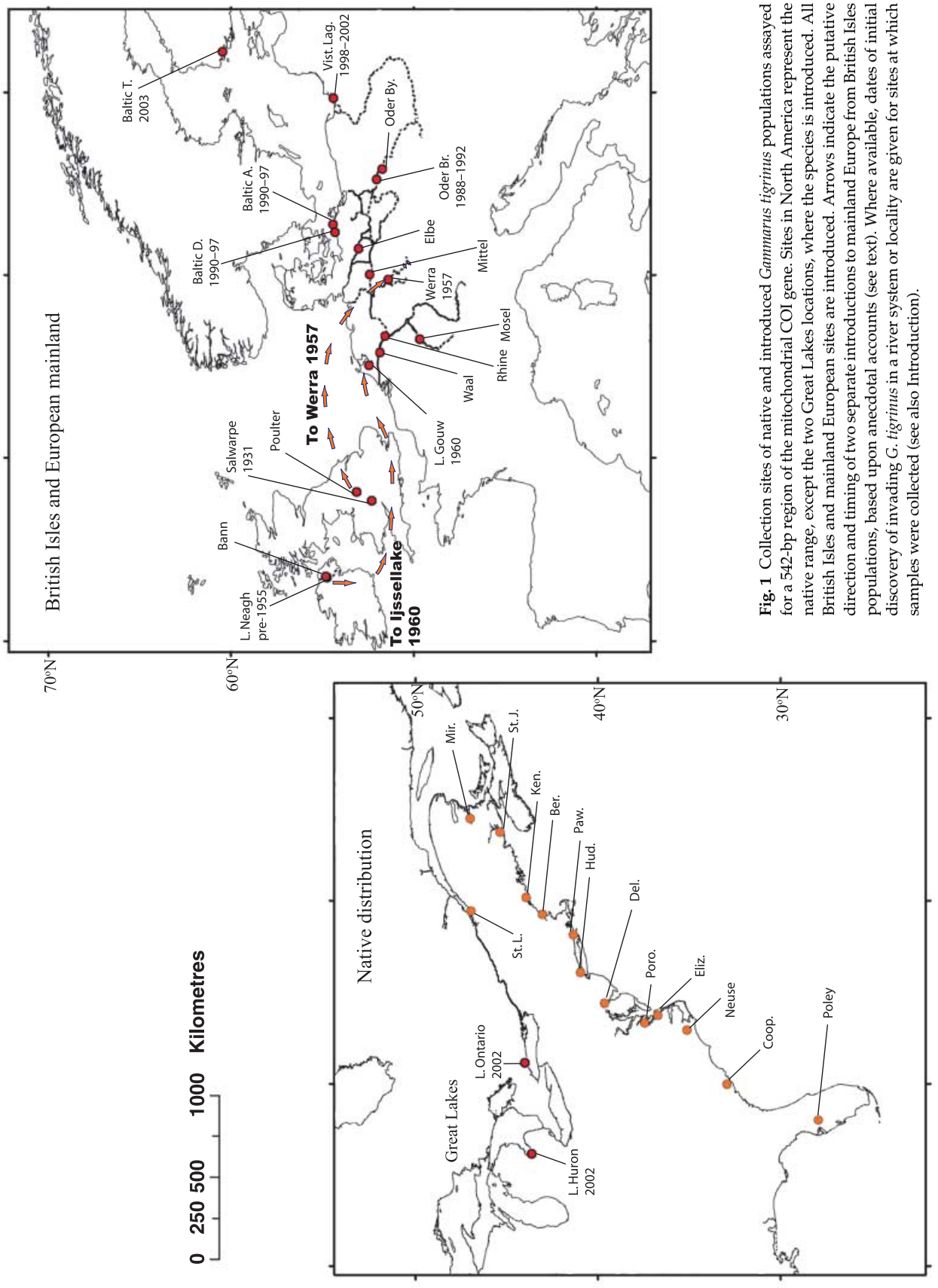

Fig. 1 Collection sites of native and introduced *Gammarus tigrinus* populations assayed for a 542-bp region of the mitochondrial COI gene. Sites in North America represent the native range, except the two Great Lakes locations, where the species is introduced. All British Isles and mainland European sites are introduced. Arrows indicate the putative direction and timing of two separate introductions to mainland Europe from British Isles populations, based upon anecdotal accounts (see text). Where available, dates of initial discovery of invading *G. tigrinus* in a river system or locality are given for sites at which samples were collected (see also Introduction).

clades within the cryptic species, and largely allopatric distributions in estuaries, thereby providing evidence for strong barriers to dispersal (Kelly *et al*. 2006). Thus, we expect that analysis of introduced populations should reveal which source population and cryptic species contributed to invasions and the presence of multiple introductions if they involved several source populations. With genetic drift and strong selection likely during invasion and the transition to freshwater, we expect low within-population genetic diversity in invading as compared to source populations. However, this pattern may be influenced by the size of founding populations and by the occurrence of multiple introductions. Finally, putative English and Irish source populations for secondary introduction to mainland Europe originated from brackish and freshwater habitats, respectively. They were then introduced and spread throughout different salinity environments in Europe, raising the possibility of distinct habitat-dependent dispersal in these lineages.

Materials and methods

The identification of source populations is hampered in many studies by low sampling effort and/or insufficient genetic structure in the native range. However, source population identification is essential when establishing a baseline for evolutionary change and for making comparisons with genetic diversity in invading populations (Wares *et al*. 2005). Therefore, it is essential that genetic surveys should encompass the full native and introduced range of a species. *Gammarus tigrinus* was collected from 19 sites throughout its entire invaded range in Europe and the North American Great Lakes, and 13 estuarine sites spanning its native range in North America (Table 1; Fig. 1). The total number of individuals collected at each site exceeded 30. A broad range of salinity habitats were represented across sampled European sites (Table 1; values > 0.5 PSU are brackish, Lee & Petersen 2002). Habitats were defined as brackish if subject to natural brine seepage, salt pollution, or if occurring in the low-salinity Baltic Sea. All sites in the native range occurred in estuaries subject to tidal fluctuation. All specimens were preserved in 95% ethanol and genomic DNA was extracted as in Kelly *et al*. (2006). A 542-bp fragment of the cytochrome *c* oxidase subunit I gene (COI) was amplified using polymerase chain reaction (PCR) with species-specific primers (forward primer 5′-TGCTTGAGCAAGTGCCTTAG-3′, reverse primer 5′-CTCTAGGGTCAAAGAAGGAAG-3′) under conditions described in Kelly *et al*. (2006). PCR products from 123 individuals from the introduced populations were sequenced using the DTCS Quick Start cycle sequencing kit (Beckman Coulter) and CEQ8000 automated sequencer, following the manufacturer's instructions. Sequence data for 143 native individuals were obtained from Kelly *et al*. (2006). All sequences were aligned by eye using OMIGA 1.2 (Oxford Molecular) and no insertions/deletions were found. Using the same protocol, outgroup sequences were obtained for another North Atlantic species, *Gammarus daiberi*, from the Delaware estuary.

We assessed patterns of phylogeographical divergence to identify source populations, the likelihood of multiple independent introductions, and to determine the relationship between the distribution of invading populations and habitat salinity. Divergence was assessed using the neighbour-joining algorithm, with genetic distance calculated as pairwise sequence divergence (Kimura 2-parameter distance model) in MEGA 2.1 (Kumar *et al*. 2004). Nodal support was calculated using 10 000 bootstrap pseudoreplicates. An additional test of phylogeny employed maximum parsimony heuristic searches conducted in PAUP* 4.0 (version 4.0b10; Swofford 2001) on all unique haplotypes using the branch-swapping algorithm, tree-bisection–reconnection (TBR) with 100 random stepwise additions. Branch support was obtained with 1000 bootstrap replicates.

We examined the genetic structure among and within regions, habitat types and populations, using analyses of molecular variance (AMOVA; Excoffier *et al*. 1992), as implemented in ARLEQUIN 2.0 (Schneider *et al*. 2000). Pairwise sequence divergences were tested by partitioning total variance into components analogous to *F*-statistics, the significance of which were tested using 10 000 permutations of haplotypes between populations and an alpha value of 0.05 (Schneider *et al*. 2000). We initially conducted separate AMOVAs on populations in the native range (northern species only; see Results) and for the introduced European populations to characterize among- and within-population genetic structure in each region. Two additional AMOVAs were conducted on invading European populations using an additional hierarchical grouping level. In the first, the effect on genetic structure of presumed invasion history and secondary dispersal in Europe was assessed by grouping populations according to 'invaded region' (British Isles populations vs. mainland European populations; see Table 1). The second test assessed genetic structure in relation to habitat salinity (freshwater vs. brackish) for all invading European populations.

We used four estimates of genetic diversity to assess the presence of population bottlenecks after introduction. First, we assessed whether significant differences existed in mean pairwise sequence divergence (p-distance; in MEGA) within each population in Europe as compared to its putative source population. In order to make valid comparisons, we accounted for two different putative source regions. For example, European populations were characterized by two highly divergent clades (N1 and N4, see Results) that sourced to geographically disjunct native regions. Some European populations were monomorphic,

Table 1 *Gammarus tigrinus* collection sites in North America and Europe with the number of individuals sequenced per population for the COI gene. Brackets indicate final sample sizes after a preliminary analysis identified several putative source estuaries (see text). Abbreviations denote populations in Figs 1 and 2. For habitat: E, estuarine/tidal; B, brackish; F, freshwater. For status: N, native; I, invaded; SI, secondary invaded. Salinity values are those recorded at the time of collection

Location	Habitat	Status	Salinity (PSU)	Abbreviation	*N*	Latitude	Longitude
St Lawrence estuary, Montmagny, Quebec	E	N	6.5	St L	25*	46.9	–70.5
Miramichi estuary, New Brunswick	E	N	16.0	Mir.	12	47.0	–65.5
St John estuary, New Brunswick	E	N	4.1	St J	9	45.3	–66.2
Kennebac estuary, Maine	E	N	8.5	Ken.	5	43.9	43.9
Berrys Creek, New Hampshire	E	N	16.0	Ber.	11	43.0	–70.7
Pawcatuck estuary, Rhode Island	E	N	11.2	Paw.	10	41.3	–71.8
Hudson estuary, New York	E	N	7.8	Hud.	24*	40.9	–73.8
Delaware estuary, Deemers beach, Delaware	E	N	5.0	Del.	30*	39.6	–75.5
Poropotank estuary, Virginia	E	N	10.0	Poro.	16*	37.4	–76.6
Elizabeth estuary, canal locks, Virginia	E	N	10.2	Eliz.	40*	36.7	–76.2
Neuse estuary, New Bern, N. Carolina	E	N	14.2	Neuse	11	35.1	–77.0
Cooper River, u/s Charleston; S. Carolina	E	N	20.0	Coop.	7	32.9	–80.0
Poley Creek, Florida	E	N	4.0	Poley	11	27.9	–81.9
Great Lakes							
Lake Ontario, Frenchman's Bay, Ontario	F	I	< 0.5	L.Ontario	5	43.8	–79.0
Lake Huron, Saginaw Bay, Michigan	F	I	< 0.5	L. Huron	8	43.6	–83.8
British Isles							
Lough Neagh, Northern Ireland	F	I	< 0.5	L. Neagh	10	54.7	–6.5
Bann River, Northern Ireland	F	I	< 0.5	Bann	10	54.8	–6.4
Salwarpe River, England	B	I	1.7	Salwarpe	2	52.2	–2.1
Poulter River, England	B	I	1.0	Poulter	5	53.2	–1.0
Mainland Europe							
Lake Gouwzee, the Netherlands	F	SI	< 0.5	L. Gouw	10	52.4	5.0
R. Waal, Rhine, the Netherlands,	F	SI	< 0.5	Waal	10	51.8	5.8
Rhine River, Germany	F	SI	< 0.5	Rhine	2	51.5	6.6
Mosel, nr Zell, Germany	F	SI	< 0.5	Mosel	2	50.1	7.2
Elbe River, Germany	F	SI	< 0.5	Elbe	4	53.0	11.4
Mittlelandkanal, Germany	F	SI	< 0.5	Mittel.	2	52.0	10.0
Oder River, Bytom, Poland	F	SI	< 0.5	Oder By	10	51.7	15.8
Oder River, Brody, Poland	F	SI	< 0.5	Oder Br	10	52.0	15.4
Werra River, Germany	B	SI	3.4	Werra	10	51.3	9.7
Baltic Sea, Vistula Lagoon, Poland	B	SI	4.5	Vist. Lag.	10	54.3	19.7
Baltic Sea, Dierhagen Lagoon, Germany	B	SI	1.5	Baltic D	10	54.2	12.3
Baltic Sea, Anleger Lagoon, Germany	B	SI	5.0	Baltic A	10	54.4	12.7
Baltic Sea, Turku, Finland	B	SI	4.4	Baltic T	10	60.4	22.2

*denotes putative source populations where sample size was increased (see text).

that is, all individuals sequenced belonged either to clade N1 or clade N4. The remaining populations comprised sympatric admixture zones of individuals representing both clades (see Results). In each European population where N1 was monomorphic, genetic diversity was compared with that in the St Lawrence River estuary, the putative source of N1 (see Results). For each European population wherein N4 was monomorphic, genetic diversity was compared with the populations in the Delaware, Poropotank and Elizabeth estuaries, as any of these may have served as a source of N4. Sample size in several introduced populations was small ($n \leq 5$), and thus, only those populations of $n = 10$ were used in these comparisons. For putative sources, we standardized n to 10 when calculating mean p-distances, as their sample sizes were much higher. For example, n in the St Lawrence, Delaware, Poropotank and Elizabeth estuaries was $n = 25$, 30, 16, and 40, respectively. Each source-introduced population comparison involved a Monte Carlo-based rarefaction approach, in which mean p-distance was recalculated for 5000 iterations using 10 randomly chosen individuals from the appropriate source pool. A Monte Carlo approach was warranted as there is no theoretical distribution of p-distances for standard statistical analyses (Sokal & Rohlf 2003). Furthermore, rarefaction and Monte Carlo sampling has been widely employed in ecological studies of species diversity

(Gotelli & Colwell 2001 and in genetic studies of allelic diversity (e.g. see Kalinowski 2004; Petit *et al*. 2005). In each comparison, we tested the null hypothesis that within population genetic diversity (mean p-distance) in the introduced population was not significantly different than that in its putative source (s). We assessed significance of the contrast at alpha = 0.05 by calculating the proportion of iterations in which the mean p-distance of the resampled source population was greater than or equal to the p-distance from the introduced population. For each introduced population, we employed a Bonferroni adjustment to account for contrasts involving multiple sources (Sokal & Rohlf 2003). For example, four contrasts of genetic diversity were made for introduced populations that were admixtures of the two clades (see Results), one with the St Lawrence estuary (N1 putative source) and three involving comparisons with the Delaware, Poropotank and Elizabeth estuaries (N4 putative sources).

The remaining three indices of genetic diversity for the same populations involved qualitative contrasts, and these were (i) haplotype diversity (*H*), (ii) the number of segregating sites, that is, the number of nucleotide differences [theta θ(*S*); Tajima 1983], and (iii) the average number of pairwise differences [theta θ(δ); Tajima 1983]. Haplotype diversity was standardized for putative source populations to $n = 10$ using the program RAREFAC (Petit *et al*. 1998). Theta (*S*) and theta (δ) were calculated in ARLEQUIN.

Results

While our analysis used sequence data for native *Gammarus tigrinus* populations from our previous study (see Kelly *et al*. 2006), we sequenced a further 85 individuals from a subset of these native estuaries (see Table 1). We did this because sample size can limit identification of source regions in molecular studies of invading species (Wares *et al*. 2005), and because a preliminary phylogenetic analysis showed that a few haplotypes of shared ancestry in introduced European and Great Lakes' populations were found in more than one native estuary (see below).

Of the 542-bp region of the COI gene aligned for 351 assayed individuals, we identified 64 haplotypes and 127 polymorphic sites, of which 117 were parsimoniously informative. A novel haplotype in the Great Lakes, and two that were widely distributed in Europe, were not found in any individuals from the native region. In the neighbour-joining analysis, inclusion of additional sequences from native populations and those from introduced populations did not change the phylogenetic relationships detected in our previous study of native populations (Kelly *et al*. 2006). That is, there were cryptic northern and southern species with the former composed of four largely geographically separate clades (Fig. 2). Parsimony analysis identified a similar overall topology but with higher branch support for the separation of clades N1/2 from N3 (100%, not shown). The distribution of haplotypes in introduced populations in Europe and the Great Lakes matched exactly or shared similar ancestry to three clades (N1, 3 and 4) drawn only from the 'northern' species in the native range (Fig. 2). These clades were highly divergent, with the most divergent and geographically disjunct being N1 and N4; both of these clades also occurred in the British Isles and Europe (Figs 2 and 3). The NI clade was represented by nine haplotypes which occurred in five estuaries in the native range, although none of the haplotypes were shared among estuaries (Fig. 2). Only a single haplotype from the N1 clade was present in invading populations. This haplotype was found in both English sites and in eastern mainland Europe. This haplotype was found only at the northern extent of our native survey in the St Lawrence estuary, and is referred to hereafter as N1 (Figs 2 and 3).

Five haplotypes (N4a–e) represented the N4 clade in its invaded range throughout Ireland and mainland Europe (Figs 2 and 3). Only three of these haplotypes (N4a–c) were found in the native range, and they were sourced to the Delaware, Poropotank and Elizabeth estuaries. However, additional sequencing of individuals from these sites ($n = 30$, 16 and 40, respectively) failed to recover the two unique introduced haplotypes N4d and N4e.

Clades N1 and N4 appeared to have different habitat distributions in Europe (see also AMOVA below). Clade N1 occurred in allopatry only in four brackish water habitats: two in England, the River Werra, and in Anleger Lagoon of the Baltic Sea (Table 1; Fig. 3). Clade N4 occurred in allopatry in seven freshwater sites: two in Northern Ireland, one in Lake Gouzee (part of the IJsselmeer, the Netherlands), and four throughout the Rhine system and Mittelandkanal (Germany). Both clades were present in sympatric admixture zones at the remaining six sites, which spanned both fresh and brackish water habitats (Table 1; Fig. 3). Populations at these sites had very high mean sequence divergence (3.4%). The North American Great Lakes populations were represented by a third divergent clade (N3). A single haplotype was dominant in this system and was sourced to the Hudson and St Lawrence River estuaries (Figs 2 and 3). Additional sequencing ($n = 25$ St Lawrence estuary; $n = 24$ Hudson estuary) failed to recover a unique N3 haplotype in an individual from Lake Huron that differed by a single base substitution.

As only clades from the northern *G. tigrinus* species were introduced, all AMOVAs excluded the southern species. In the native region, 67.5% ($P < 0.0001$) of the total genetic variation occurred among populations, with the remainder within populations. In contrast, invading populations of British Isles/European regions exhibited lower among-population genetic variation (54.1%, $P < 0.0001$) and higher within-population variation (45.9%). This difference was

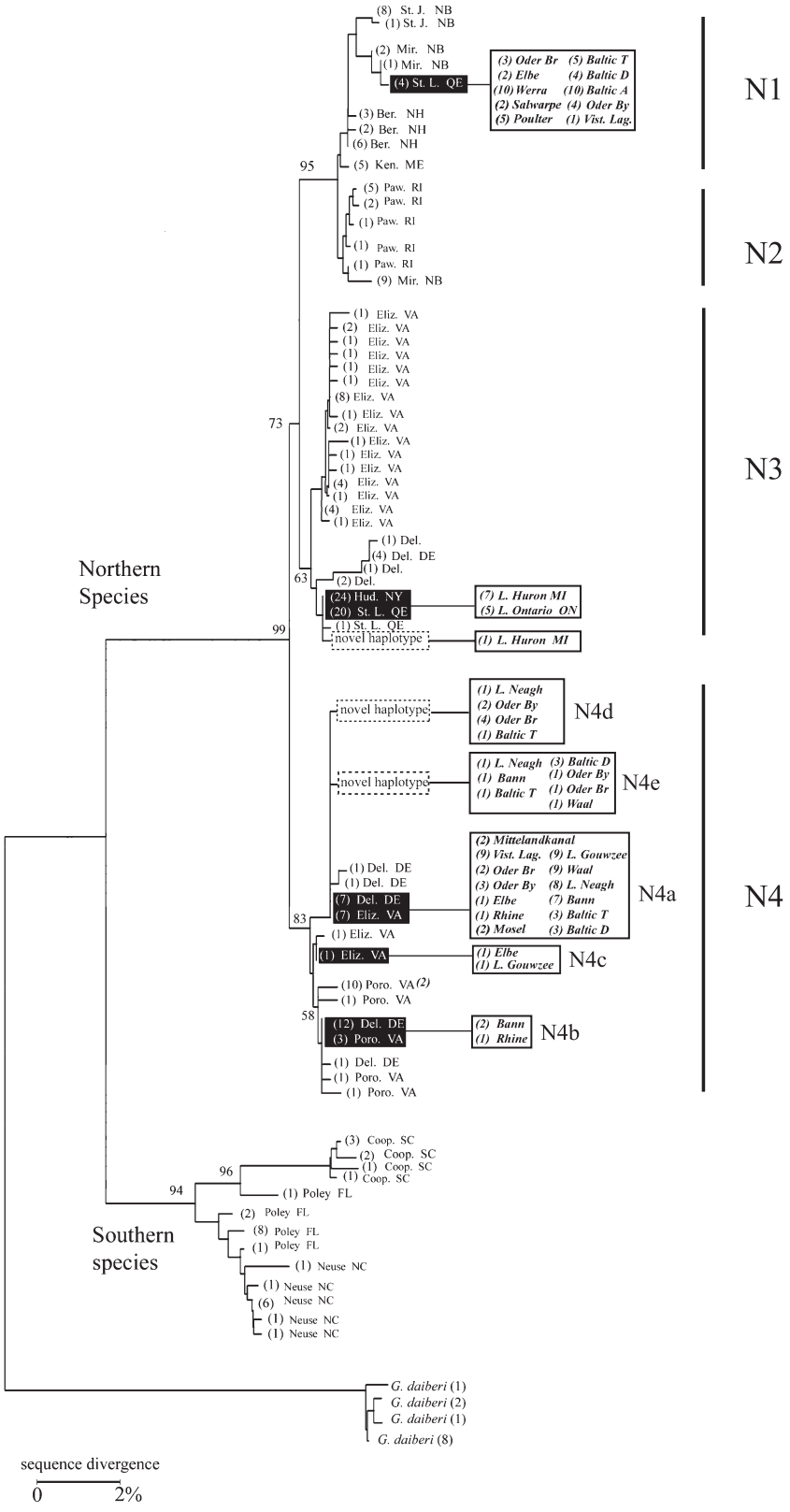

Fig. 2 Neighbour-joining tree of *Gammarus tigrinus* COI haplotypes from populations across the northwest Atlantic coast (nodal support with 10 000 bootstrap pseudo-replicates). Black boxes designate native haplotypes which are identical to those in introduced populations (white boxes) and, thus, putative source sites. Introduced populations in Europe comprise two deeply divergent clades (N1 and N4a, b, c) of the four that occur in the northern species (i.e. N1–4). Of the N4 clade, haplotypes N4d and N4e are unique to several European populations but cluster with N4a (dashed boxes). Introduced populations from the Great Lakes derive from a single haplotype in a separate divergent clade (N3). One individual in Lake Huron is unique but clusters with the putative source populations (dashed box).

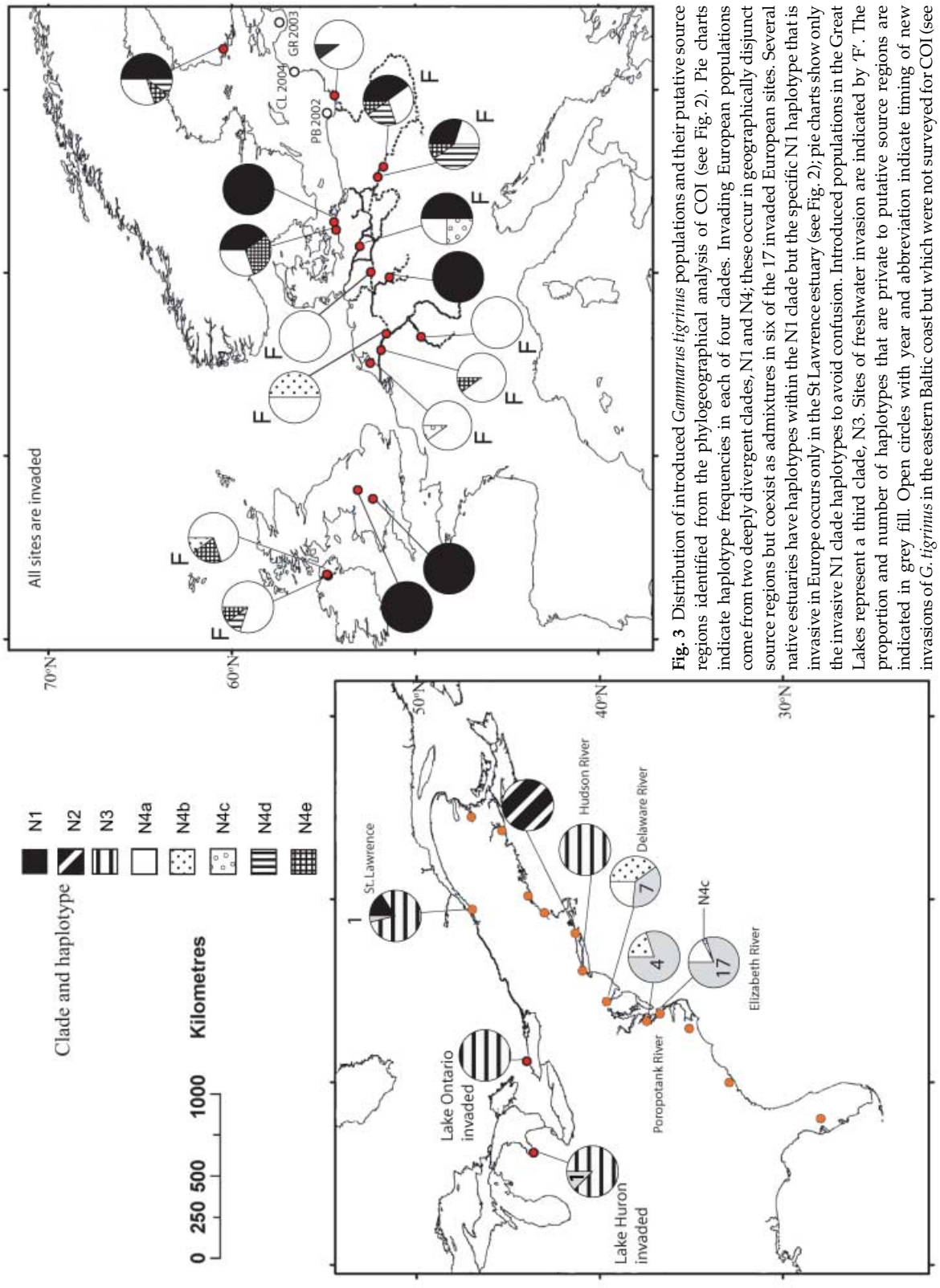

Fig. 3 Distribution of introduced *Gammarus tigrinus* populations and their putative source regions identified from the phylogeographical analysis of COI (see Fig. 2). Pie charts indicate haplotype frequencies in each of four clades. Invading European populations come from two deeply divergent clades, N1 and N4; these occur in geographically disjunct source regions but coexist as admixtures in six of the 17 invaded European sites. Several native estuaries have haplotypes within the N1 clade but the specific N1 haplotype that is invasive in Europe occurs only in the St Lawrence estuary (see Fig. 2); pie charts show only the invasive N1 clade haplotypes to avoid confusion. Introduced populations in the Great Lakes represent a third clade, N3. Sites of freshwater invasion are indicated by 'F'. The proportion and number of haplotypes that are private to putative source regions are indicated in grey fill. Open circles with year and abbreviation indicate timing of new invasions of *G. tigrinus* in the eastern Baltic coast but which were not surveyed for COI (see text). PB, Puck Bay; CL, Curonian Lagoon; GR, Gulf of Riga.

Table 2 Contrasts of genetic diversity (Monte Carlo rarefaction estimate, see text) between putative source and introduced European populations (n = 10 per population). Values in bold and underline indicate contrasts where genetic diversity is significantly lower or higher, respectively, in native regions as compared to invading populations. Significance was assessed after Bonferroni adjustment (see text)

		Mean sequence divergence				
Introduced population (n = 10)	Clade(s) present	Introduced population	N1 source	N4 source (s)		
			St Lawrence	Elizabeth	Delaware	Poropotank
Werra	N1	0.0000	0.00723***	—	—	—
Baltic, Anleger	N1	0.0000	0.00715***	—	—	—
L. Neagh	N4	0.0007	—	0.0087***	0.0120***	0.0035**
R. Bann	N4	0.0037	—	0.0087*	0.0120***	0.0035 NS
L. Gouwzee	N4	0.0011	—	0.0087***	0.0120***	0.0035**
Waal	N4	0.0004	—	0.0086***	0.0119***	0.0034**
Baltic, Dierhagen	N1 & N4	0.0197	**0.00720*****	**0.0087*****	**0.0120*****	**0.0034*****
Oder, Bytom	N1 & N4	0.0199	**0.00700*****	**0.0087*****	**0.0120*****	**0.0034*****
Oder, Brody	N1 & N4	0.0179	**0.00712*****	**0.0086*****	**0.0120*****	**0.0034*****
Vistula Lagoon	N1 & N4	0.0070	0.00712 NS	0.0087 NS	*0.0120**	**0.0034*****
Baltic, Turku	N1 & N4	0.0204	**0.00710*****	**0.0086*****	**0.0120*****	**0.0034*****

***P < 0.001, **P < 0.01, *P < 0.05; NS, not significant.

likely driven by the highly divergent clade admixture populations; thus, AMOVA was repeated using only monomorphic populations. As expected, a much larger proportion of genetic variation was then explained among populations (94.5%, P < 0.0001). In the AMOVA to assess a regional effect between the British Isles and mainland Europe, a negative variance component and the percentage variation explained (–10.28%, P = 0.66) indicated that genetic structure was absent at this level (ARLEQUIN webpage: http://lgb.unige.ch/arlequin/software/2.000/doc/faq/faqlist.htm). However, the AMOVA to assess the effect of habitat salinity in Europe revealed significant genetic structuring (38.2%, P < 0.01) between freshwater and brackish sites.

Two main patterns arose in contrasts of genetic diversity between putative sources and introduced European populations, and not surprisingly, were dependent on whether clades N1 or N4 occurred in allopatry or as admixtures. With one exception (Poropotank vs. River Bann), in introduced populations in which N1 or N4 clades were monomorphic, mean sequence divergence was significantly lower than that in either putative source region (Table 2). A similar pattern was evident in haplotype diversity, the number of segregating sites, and the average number of pairwise differences (Table 3). Given that clades N1 and N4 were highly divergent, it was not surprising that admixture populations were significantly more diverse (mean p-distance) than either of their putative sources. An exception was the Vistula Lagoon, ostensibly due to the low frequency of N1 haplotypes (Fig. 3). Invading populations that were admixtures of N1 and N4 generally had higher haplotype diversities, numbers of segregating sites, and average number of pairwise differences as compared to the putative St Lawrence source. However, comparisons of admixture populations with each of the putative source estuaries for the N4 clade had mixed results. Haplotype diversity in invading admixture populations was higher than in the Poropotank estuary, similar to that in the Delaware estuary, and lower than that in the Elizabeth estuary (Table 3). The high number of private haplotypes

Table 3 Population diversity indices (see text) for invading and putative native source areas for the two divergent clades N1 and N4

Site	Invaded (I) or native (N) source population	Clade	H	θ (S)	θ (δ)
Elizabeth	N	N4	0.919	4.54	6.43
Delaware	N	N4	0.784	5.87	4.66
Poropotank	N	N4	0.600	1.86	1.85
L. Neagh	I	N4	0.378	0.70	0.40
R. Bann	I	N4	0.511	2.12	1.97
L. Gouwzee	I	N4	0.200	1.06	0.58
Waal	I	N4	0.200	0.35	0.20
St Lawrence	N	N1	0.347	3.70	3.72
Werra	I	N1	0.000	0.00	0.00
Baltic Anleger	I	N1	0.000	0.00	0.00
Baltic Dierhagen	I	N1 & N4	0.733	6.71	10.06
Oder Bytom	I	N1 & N4	0.778	7.07	10.15
Oder Brody	I	N1 & N4	0.778	7.06	10.15
Vistula Lagoon	I	N1 & N4	0.200	6.36	3.60
Baltic Turku	I	N1 & N4	0.711	7.07	10.40

present in the latter two estuaries may explain this pattern (see Fig. 3). Nevertheless, additional measures of population genetic diversity [$\theta(S)$; $\theta(\delta)$] were largely higher in admixture populations as compared to each of the putative N4 source populations.

Discussion

Sources and pathways of independent invasions

Genetic differences among allopatric, native populations should provide opportunities to discern the source of introduced populations, as well as whether founding populations have been derived from single or multiple sources. Our study identified at least three independent invasions, sourced from geographically and genetically distinct populations. The NI clade occurred in England and in Eastern Europe, and was sourced to the St Lawrence estuary. This pattern is consistent with a pre-1931 ballast water transfer to brackish habitats of western England (see Hynes 1955), with subsequent dispersal throughout brackish and tidal waters after transfer to the German Werra system (Schmitz 1960; Fig. 1). The N4 clade was distributed in Ireland and Europe, and was sourced to the Delaware/Chesapeake Bay region. The co-occurrence of haplotypes N4a and N4b in Ireland and in the Delaware estuary supports the latter as the most parsimonious source of this introduction, although the Elizabeth and Poropotank estuaries cannot be excluded (Fig. 3). Additionally, haplotypes N4d and N4e — which were found in Ireland but not in any native estuaries — grouped close to those from the Delaware estuary, differing by a single base-pair substitution from N4a and providing further support of a Delaware source (Fig. 2). The four N4 haplotypes that were found in Ireland also occurred throughout Europe, corroborating the species' spread from Ireland after their introduction to Dutch fresh waters (Nijssen & Stock 1966; Pinkster *et al.* 1992; Fig. 1). The third clade N3, which occurred in the Great Lakes, was sourced to the Hudson and St Lawrence estuaries (Figs 2,3). However, in the St Lawrence estuary, haplotypes from the N3 clade co-occurred with those of the N1 clade. Kelly *et al.* (2006) showed that this distribution was geographically disjunct since several native populations that grouped within the N3 clade occurred in the mid-Atlantic region. This supported a probable introduction to the St Lawrence estuary from the Hudson (Kelly *et al.* 2006). Considering that the St Lawrence River is the main navigational corridor to the Great Lakes, it is likely that the St Lawrence River served as the donor region in a secondary invasion from the Hudson (see Holeck *et al.* 2004).

The co-occurrence in Europe of haplotypes from geographically and genetically distinct North American clades indicates multiple introductions. These admixture zones are consistent with reports of intentional seeding of the species in the Dutch IJsselmeer and the German River Werra from Ireland and England, respectively, and their subsequent spread from these sites (Schmitz 1960; Nijssen & Stock 1966).

Genetic bottlenecks with invasion?

We found significantly lower genetic diversity in invading, monomorphic N1 and N4 populations of *Gammarus tigrinus* as compared to their putative sources (Tables 2,3). Genetic bottlenecks are commonly reported in studies of colonizing species, either because low founding population size predisposes the species to drift effects or because of intense selection in the novel environment (e.g. Tsutsui *et al.* 2000; Sakai *et al.* 2001; Hawley *et al.* 2006). It should be noted, however, that low neutral genetic diversity commonly reported in invasion genetic studies may not be indicative of the high additive genetic variation that underlies adaptive phenotypic traits (Lee 2002; Barton & Turelli 2004). For example, Lindholm *et al.* (2005) showed that in introduced guppy *Poecilia reticulata* populations, low genetic diversity in mitochondrial and microsatellite markers was contrasted by high additive genetic variance. Similarly, Loh & Bitner-Mathe (2005) found that, despite a population bottleneck, a large amount of additive genetic variation for wing size and shape was retained in the fruit fly *Zaprionus indianus* after invasion of Brazil from Africa. Wing morphology–latitude correlations are considered adaptive in fruit flies; thus, Loh & Bitner-Mathe (2005) suggested that such high additive variation facilitated the species rapid and broad latitudinal distribution in South America.

Despite a loss of neutral genetic diversity in the invading *G. tigrinus* in Europe, we also detected population admixtures which displayed elevated genetic diversity relative to source estuaries. For example, while haplotype diversity for admixture populations at Dierhagen, Germany, Turku, Finland, and two sites in the Oder River, Poland, were similar to those in the Delaware and higher than those in the St Lawrence River, other measures of diversity were consistently higher in the introduced populations (Tables 2 and 3). These findings are similar to the increased neutral genetic diversity in invading populations of brown anole lizards *Anolis sagrei* (Kolbe *et al.* 2004) and of brown algae *Undaria pinnatifida* (Voisin *et al.* 2005). Both studies suggested that admixture and the subsequent redistribution of genetic variation from among populations in the native range to within populations in the introduced range was important to invasion success. However, correlating neutral genetic diversity with a population's ability to adapt to future environmental change is tenuous at best (Lee 2002).

Detailed historical, ecological and genetic data for the *G. tigrinus* invasion of Europe support the hypothesis that

increased genetic diversity associated with multiple introductions from genetically divergent sources has accelerated their range expansion and habitat distribution (see Ellestrand & Schierenbeck 2000; Lee 2002). For example, N1 and N4 admixture populations were distributed across both brackish and freshwater habitats, unlike monomorphic populations of either clade. Historical data also show that prior to contact, lineages N1 and N4 each had more restricted ranges. Dutch and German populations were allopatric and highly divergent at allozyme markers (presumably clades N4 and N1) during 1975 through 1983 (Dieleman & Pinkster 1977; Bulnheim 1985). In 1981, allozyme markers showed an admixture in the Ems estuary near the Dutch–German border (see Bulnheim 1985) providing the first evidence that contact and gene flow may have occurred. By 1987, *G. tigrinus* had spread east of the Werra River and of the western Baltic Sea (Tittizer *et al*. 2000). Within 15 years, it occurred from the Oder River to the Gulf of Riga and northern Finnish Baltic region (Zettler 1998; Szaniawska *et al*. 2003; Pienimaki *et al*. 2004; J. Kotta, personal communication; M. Zettler, personal communication; see also Fig. 3). This dispersal rate was over twofold greater than that attained in the previous 27–30 years, during which the clades were strictly allopatric. The observed increase in dispersal rate is unlikely to be due to increased dispersal routes as most canals were constructed long before the species established in mainland Europe (see Jaźdźewski 1980). Indeed, other studies suggest that evolutionary change and resulting range expansion may be dependent on gene flow through multiple introductions of individuals from genetically diverse sources (Villablanca *et al*. 1998; Kolbe *et al*. 2004; Voisin *et al*. 2005). However, in these situations it is unclear whether multiple introductions have stimulated invasiveness after colonization or merely represent additional introductions in new areas (see Ellestrand & Schierenbeck 2000). Genetically divergent, introduced European lineages were restricted to fresh or brackish water habitats in allopatry but in contact zones they occurred in both habitat types. It will be interesting to determine whether this process has generated novel genotypes and hence novel adaptations. This could be achieved through assessment of cytonuclear disequilibrium indicative of reproductive isolation between the mitochondrial lineages (*sensu* Asmussen *et al*. 1987). Crossbreeding experiments between the two parental clades, together with common garden experiments, could then assess if the salinity distribution resulted from adaptive changes.

Environmental transitions during invasion

Unlike many invasions in which species move between similar habitats in native and introduced regions, the transition experienced by *G. tigrinus* moving from estuarine, native habitats to freshwater habitats should have predisposed individuals to withstand substantial osmotic stress. Such acclimation may have resulted in a stronger selection pressure than that in other invasions in which the environmental change may be much less extreme (see Lee & Bell 1999; Reznick & Ghalambor 2001). Furthermore, the loss of genetic diversity during the first invasion (North America to Ireland) followed by a second invasion (Ireland to Europe) associated with no loss in genetic diversity is perhaps best explained by a founder effect compounded by selection effects during the first colonization event. As Lee (1999) demonstrated, low survival and strong directional selection for freshwater tolerance accompany estuarine to freshwater transitions. Freshwater invasion in Ireland was probably facilitated by a large initial propagule supply (from ships' ballast; see Hynes 1955) providing sufficient numbers and additive genetic variation to allow severe selection losses in the newly established population. The possibility that mainland European populations were sourced directly from North America is discounted by documented introduction of *G. tigrinus* from Ireland to Europe, its subsequent spread, and the observed molecular patterns in this study.

Our data suggest that several independent lineages have breached freshwater during invasion. However, native populations of euryhaline species may vary in salinity distribution or even occur as cryptic freshwater populations (e.g. Bastrop *et al*. 1998; Lee & Petersen 2002). To assess this possibility, we collected additional data on the salinity distribution of putative source estuarine populations. We sampled across a salinity gradient in the Delaware estuary (N4 clade) in June and December 2004, and found *G. tigrinus* only in waters between 3.6 and 18.0 PSU, and never at freshwater sites (salinity < 0.5 PSU). Likewise, Palmer & Ricciardi (2004) showed that *G. tigrinus* (N1 clade) was absent from freshwater sections of the St Lawrence River. The species was also absent in archived Great Lakes' samples (Barton & Hynes 1976) which we have examined. Furthermore, all individuals except one in introduced populations in the Great Lakes had the same N3 haplotype as native populations, a pattern inconsistent with deeper levels of divergence expected in a more ancient colonization (see Lee 1999). Collectively, these data support recent freshwater invasions, similar to the independent invasions of freshwater in the estuarine copepod *Eurytemora affinis*, though they involve different mechanisms (Lee 1999). For example, the range expansion of *E. affinis* into fresh water occurred either within the same drainage, or during acclimation when formerly saline habitats became impounded (see Lee & Bell 1999; Lee 1999). With the exception of the Great Lakes, *G. tigrinus* invasions occurred between continents and likely involved a more intense selective regime after ballast water discharge. However, the extent of selection effects on the evolution of populations may be lineage and habitat-dependent (e.g. Lee 1999; Vasquez *et al*. 2005).

This is partially supported in the *G. tigrinus* invasion of Europe where populations monomorphic for clades N1 and N4 had different habitat salinity associations. Furthermore, populations that were presumably monomorphic for the N1 clade were not observed in freshwater, and despite its rapid spread throughout the salt-polluted Werra River, populations declined under low-salinity regimes (see Ruoff 1968; Jaźdźewski 1980). Although this suggests lineage variation in adaptation, the history of introduction alone is congruent with the allopatric distribution of clades N1 and N4 between the two habitat types. That is, N1 was intentionally spread to a brackish site in mainland Europe from a similar habitat in England, whereas N4 was intentionally transferred to a freshwater site in mainland Europe from a similar habitat in Ireland. To test this, salinity-tolerance experiments are needed for populations from each clade.

Our study highlights a need to incorporate ecological and genetic analyses at the population level and over the full native range of a species when examining the processes associated with novel environmental transitions. As well, recognition of ecological differences among populations (e.g. Lee & Bell 1999) should be an important consideration in predicting future spread of introduced species, particularly for studies that utilize environmental niche modelling.

Acknowledgements

We thank the following people for providing specimens: Jan Ciborowski, Mike Grabowski, Dirk Platvoet, Mariel van Rhiel, Marjo Pienimaki, Misun Kang, Lee Knight, Gesche Winkler, Jaimie Dick, Franz Scholl, Bruce Cowell, David Knott, Geoff Smith, Michael Zettler, Peter Zwick. Thanks also to Derek Gray for assistance with field collections and Dave Barton and Anthony Ricciardi for providing archival samples and data. We acknowledge financial support to D.W.K. from a GLIER postdoctoral fellowship, to J.R.M. from an Ontario Graduate Scholarship, to D.D.H. from NSERC Discovery and Canada Research Chair programs, and to H.J.M. an NSERC CRO and DFO Invasive Species Research Chair.

References

Asmussen MA, Arnold J, Avise JC (1987) Definition and properties of disequilibrium statistics for associations between nuclear and cytoplasmic genotypes. *Genetics*, **115**, 755–768.

Baker HG (1967) Support for Baker's Law—as a rule. *Evolution*, **21**, 853–856.

Barrett SCH, Shore JS (1989) Isozyme variation in colonising plants. In: *Isozymes in Plant Biology* (eds Soltis DE, Soltis PS), pp. 106–126. Dioscorides Press, Portland, Oregon, USA.

Barton DR, Hynes HBN (1976) The distribution of amphipoda and isopoda on the exposed shores of the Great Lakes. *Journal of Great Lakes Research*, **2**, 207–214.

Barton NH, Turelli M (2004) Effects of genetic drift on variance components under a general model of epistasis. *Evolution*, **58**, 2111–2132.

Bastrop R, Jurss K, Sturmbacher C (1998) Cryptic species in a marine polychaete and their independent introduction from North America to Europe. *Molecular Biology and Evolution*, **15**, 97–103.

Bousfield EL (1973) *Shallow-Water Gammaridean Amphipoda of New England*. Cornell University Press, London.

Bulnheim H-P (1980) Zum Vorkommen von *Gammarus tigrinus* im Nord-Ostsee-Kanal. *Archives Fuer Fischereiwissenschaft*, **30**, 67–73.

Bulnheim H-P (1985) Genetic differentiation between natural populations of *Gammarus tigrinus* (Crustacea, Amphipoda) with reference to its range extension in European continental waters. *Archive für Hydrobiologie*, **102**, 273–290.

Dieleman J, Pinkster S (1977) Further observations on the range extension of the alien amphipod *Gammarus tigrinus* Sexton, 1939, in the Netherlands during the years 1974–76. *Bulletin Zoologisch Museum Universiteit Van Amsterdam*, **6**, 21–29.

Ellestrand NC, Schierenbeck KA (2000) Hybridization as a stimulus for the evolution of invasiveness in plants? *Proceedings of the National Academy of Sciences, USA*, **97**, 7043–7050.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distance among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Gotelli NJ, Colwell RK (2001) Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecology Letters*, **4**, 379–391.

Hawley DM, Hanley D, Dhondt AA, Lovette IJ (2006) Molecular evidence for a founder effect in invasive house finch (*Carpodacus mexicanus*) populations experiencing an emergent disease epidemic. *Molecular Ecology*, **15**, 263–275.

Holeck KT, Mills EL, MacIsaac HJ, Dochoda MR, Colautti RI, Ricciardi A (2004) Bridging troubled waters: understanding links between biological invasions, transoceanic shipping, and other entry vectors in the Laurentian Great Lakes. *Bioscience*, **54**, 919–929.

Hynes HBN (1955) Distribution of some freshwater Amphipoda in Britain. *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie*, **12**, 620–628.

Jaźdźewski K (1980) Range extensions of some Gammaridean species in European inland waters caused by human activity. *Crustaceana, Suppl.*, **6**, 84–107.

Jaźdźewski K, Konopacka A, Grabowski M (2002) Four Ponto-Caspian and one American gammarid species (Crustacea, Amphipoda) recently invading Polish waters. *Contributions to Zoology*, **71**, 115–122.

Kalinowski ST (2004) Counting alleles with rarefaction: private alleles and hierarchical sampling designs. *Conservation Genetics*, **5**, 539–543.

Kelly DW, MacIsaac HJ, Heath DD (2006) Vicariance and dispersal effects on phylogeographic structure and speciation in a widespread estuarine invertebrate. *Evolution*, **60** (2), 257–267.

Kolbe JJ, Glor RE, Schettino LRG, Lara AC, Larson A, Losos JB (2004) Genetic variation increases during biological invasion by a Cuban lizard. *Nature*, **431**, 177–181.

Kumar S, Tamura K, Nei M (2004) MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics*, **5**, 150–163.

Lee CE (1999) Rapid and repeated invasions of fresh water by the copepod *Eurytemora affinis*. *Evolution*, **53**, 1423–1434.

Lee CE (2002) Evolutionary genetics of invasive species. *Trends in Ecology & Evolution*, **17**, 386–391.

Lee CE, Bell MA (1999) Causes and consequences of recent freshwater invasions by saltwater animals. *Trends in Ecology & Evolution*, **14**, 284–288.

Lee PLM, Patel RM, Conlon RS, Wainright SJ, Hipkin CR (2004) Comparison of genetic diversities in native and alien populations of hoary mustard (*Hirschfeldia Incana* (L.) Lagreze-Fossat). *International Journal of Plant Sciences*, **165**, 833–843.

Lee CE, Petersen CH (2002) Genotype–by–environment interaction for salinity tolerance in the freshwater-invading copepod *Eurytemora affinis*. *Physiological and Biochemical Zoology*, **75**, 335–344.

Lindholm AK, Breden F, Alexander HJ, Chan W-K, Thakurt SG, Brooks R (2005) Invasion success and genetic diversity of introduced populations of guppies *Poecilia reticulata* in Australia. *Molecular Ecology*, **14**, 3671–3682.

Loh R, Bitner-Mathe B (2005) Variability of wing size and shape in three populations of a recent Brazilian invader, *Zaprionus indianus* (Diptera: Drosophilidae), from different habitats. *Genetica*, **125**, 271–281.

Nei M, Maruyama T, Chakraborty R (1975) The bottleneck effect and genetic variability in natural populations. *Evolution*, **29**, 1–10.

Nijssen H, Stock JH (1966) The amphipod, *Gammarus tigrinus* Sexton, 1939, introduced in the Netherlands (Crustacea). *Series of Miscellaneous Publications*, **13**, 197–206.

Novak SJ, Mack RN (2005) Genetic bottlenecks in alien plant species: influence of mating systems and introduction dynamics. In: *Species Invasions: Insights Into Ecology, Evolution, and Biogeography* (eds Sax DF, Stachowicz JJ, Gaines SD), pp. 201–228. Sinauer Associates, Sunderland, Massachusetts.

Palmer ME, Ricciardi A (2004) Physical factors affecting the relative abundance of native and invasive amphipods in the St Lawrence River. *Canadian Journal of Zoology*, **82**, 1886–1893.

Parsons PA (1983) *Evolutionary Biology of Colonizing Species*. Cambridge University Press, Cambridge.

Petit RJ, Deguilloux MF, Chat J, Grivet D, Garnier-Géré P, Vendramin GG (2005) Standardizing for microsatellite length in comparisons of genetic diversity. *Molecular Ecology*, **14**, 885–890.

Petit RJ, Mousadik E, Pons O (1998) Identifying populations for conservation on the basis of genetic markers. *Conservation Biology*, **12**, 844–855.

Pienimaki M, Helavuori M, Leppakoski E (2004) First finding of the North American amphipod *Gammarus tigrinus* Sexton, 1939 along the Finnish coast. *Memoranda Society Fauna Flora Fennica*, **80**, 17–19.

Pinkster S, Scheepmaker M, Platvoet D, Broodbakker N (1992) Drastic changes in the amphipod fauna (Crustacea) of Dutch inland waters during the last 25 years. *Bijdragen Tot de Dierkunde*, **61**, 193–204.

Reznick DN, Ghalambor CK (2001) The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. *Genetica*, **112–113**, 183–198.

Ricciardi A, MacIsaac HJ (2000) Recent mass invasions of the North American Great Lakes by Ponto-Caspian species. *Trends in Ecology & Evolution*, **15**, 62–65.

Ruoff K (1968) Experimentelle untersuchungen uber den in die Weser eingeburgerten amerikanischen bacflohkrebs *Gammarus tigrinus* Sexton. *Archive für Fischereiwissenschaft*, **19**, 134–158.

Sakai AK, Allendorf FW, Holt JS *et al.* (2001) The population biology of invasive species. *Annual Review of Ecology and Systematics*, **32**, 305–332.

Schmitz W (1960) Die Einburgerung von *Gammurus tigrinus* Sexton auf dem europaischen Kontinent. *Archive Fûr Hydrobiologie*, **57**, 223–225.

Schneider S, Roessli D, Excoffier L (2000) *ARLEQUIN: A Software for Population Genetics Data Analysis*. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva, Switzerland.

Sexton EW, Cooper LHN (1939) On a new species of *Gammarus* (*G. tigrinus*) from the Droitwich District. *Journal of the Marine Biological Association of the UK*, **23**, 543–551.

Sokal RR, Rohlf FJ (2003) *Biometry: the Principles and Practice of Statistics in Biological Research*. Freeman and Company, New York.

Swofford DL (2001) *PAUP**. Phylogenetic analysis using parsimony (*and other methods). Sinauer Associates, Sunderland, Massachusetts.

Szaniawska A, Lapucki T, Normant M (2003) The invasive amphipod *Gammarus tigrinus* Sexton, 1939, in Puck Bay. *Oceanologia*, **45**, 507–510.

Tajima F (1983) Evolutionary relationships of DNA sequences in finite populations. *Genetics*, **105**, 437–460.

Tittizer TF, Scholl F, Banning M, Haybach A, Schleuter M (2000) Aquatische Neozoen im Makrozoobenthos der Binnenwasserstrasen Deutschlands. *Lauterbornia*, **39**, 1–72.

Tsutsui ND, Suarez AV, Holway DA, Case TJ (2000) Reduced genetic variation and the success of an invasive species. *Proceedings of the National Academy of Sciences, USA*, **97**, 5948–5953.

Vasquez EA, Glenn EP, Brown JJ, Guntenspergen GR, Nelson SG (2005) Salt tolerance underlies the cryptic invasion of North American salt marshes by an introduced haplotype of the common reed *Phragmites australis* (Poaceae). *Marine Ecology Progress Series*, **298** (1–8), 2005.

Villablanca FX, Roderick GK, Palumbi SR (1998) Invasion genetics of the Mediterranean fruit fly: variation in multiple nuclear introns. *Molecular Ecology*, **7**, 547–560.

Voisin M, Engel CR, Viard F (2005) Differential shuffling of native genetic diversity across introduced regions in a brown alga: aquaculture vs. maritime traffic effects. *Proceedings of the National Academy of Sciences, USA*, **102**, 5432–5437.

Wares JP, Hughes AR, Grosberg RK (2005) Mechanisms that drive evolutionary change: insights from species introductions and invasions. In: *Species Invasions: Insights Into Ecology, Evolution, and Biogeography* (eds Sax DF, Stachowicz JJ, Gaines SD), pp. 229–257. Sinauer Associates, Sunderland, Massachusetts.

Zettler M (1998) Distribution of the Malacostraca (Crustacea) in inland and coastal waters of Mecklenburg-Vorpommern/Germany. *Lauterbornia*, **32**, 49–65.

This work was part of David Kelly's postdoctoral research with Hugh MacIsaac at the Great Lakes Institute, University of Windsor, Ontario, Canada. David is interested in invasion ecology, aquatic biology and adaptation to novel habitats in euryhaline species. He is currently developing micro-array techniques for identification of zooplankton species that pose an invasion risk to the Great Lakes. Jim Muirhead is working on his PhD, exploring vector-based models of invasion. Dan Heath holds a Canada Research Chair in Conservation Genetics, and Hugh MacIsaac holds an Invasive Species Research Chair from the Department of Fisheries and Oceans Canada.
