Canal construction destroys the barrier between major European invasion lineages of the zebra mussel

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Since the mid-1980s the zebra mussel, *Dreissena polymorpha*, Pallas 1771, has become the protagonist of a spectacular freshwater invasion in North America due to its large economic and biological impact. Several genetic studies on American populations have failed to detect any large-scale geographical patterns. In western Europe, where *D. polymorpha* has been a classical invader from the Pontocaspian since the early 19th century, the situation is strikingly different. Here, we show with genetic markers that two major western European invasion lineages with lowered genetic variability within and among populations can be discriminated. These two invasion lineages correspond with two separate navigable waterways to western Europe. We found a rapid and asymmetrical genetic interchange of the two invasion lines after the construction of the Main–Danube canal in 1992, which interconnected the two waterways across the main watershed.

Keywords: *Dreissena polymorpha*; biological invasion; microsatellites; genetic interchange

1. INTRODUCTION

The recent freshwater invasion of the zebra mussel, *Dreissena polymorpha*, Pallas 1771, in North America holds a central place in public interest because of its high-rated economic and ecological damage (Nalepa & Schloesser 1993; Mooney & Cleland 2001). Several genetic studies on American populations of the zebra mussel have attempted to reveal its invasion history and to determine the invasion source (Marsden *et al.* 1995, 1996; Stepien *et al.* 1999; Lewis *et al.* 2000), but have failed to detect any large-scale geographical patterns. It may be surmised that the situation is different in western Europe, where *D. polymorpha* has, since the early 19th century, proved to be a ‘classical’ invader from the Pontocaspian region (Kinzelbach 1992, 1995). Dates of first records and details of the invasion in western Europe are summarized by Van Martens (1865); Haas (1955) and Thienemann (1950). The navigable waterways of the interconnected rivers Dnieper, Prypet, Bug, Vistula, Oder, Rhine and German canals (Midland canal etc) constitute a northern invasion route, and the Danube a southern route to western Europe. Ship traffic in the Baltic Sea and North Sea have further facilitated transport to the west (Thienemann 1950). By 1992, animals from these two main invasion routes had been given the opportunity to mix extensively across the main watershed by means of the newly constructed Main–Danube canal. The first record of the zebra mussel on both sides of this watershed appeared in 1855 in the river Main—a tributary of the Rhine—and in 1868 in the upper Danube (Thienemann 1950).

The comparison between the American and the European invasion patterns may provide a better understanding of the invasion process of the zebra mussel. In this study, we have investigated the large-scale European population

structure of the zebra mussel, for the first time, to our knowledge, with highly polymorphic genetic markers, considering the information of potential invasion routes and interchange opportunities. The objective of this study was to test whether the invasion history has left genetic marks in the population structure of the zebra mussel.

2. MATERIAL AND METHODS

(a) Large-scale analysis

Population samples collected in 1999 and 2000 over a large geographical scale from the original area (Black Sea area; populations Dni, Mos and Br) and along the two major invasion routes, including the Main–Danube canal, were analysed (figure 1). Samples Wlo, Bs, Wa, M9, Gin, Tre, R1 and M1 are located along the northern route, the populations D0D1 and KaJo belong to the southern route and K1, K3 and K5 are canal populations. All samples were derived from rivers, canals and reservoirs, except two that were taken from isolated dredge lakes near the main river (Gin and Tre). On average, 43 ± 12 individuals from each of 16 populations were genotyped by standard PCR methods at four trinucleotide microsatellite loci (Naish & Boulding 2000). Fragment lengths of 224–389 bp (locus A6), 275–371 bp (B6), 167–308 bp (B8) and 190–343 bp (B9) were revealed on an ABI 377 sequencer with internal length standards. The number of alleles per locus ranged between 30 and 35, in total.

Gene diversities were calculated with GENEPOP (Raymond & Rousset 1995) according to Weir (1996) and arcsin root-transformed prior to *t*-tests. Conventional F_{ST} values (overall and pairwise) were estimated with GENEPOP and ARLEQUIN (Schneider *et al.* 2000) according to Weir & Cockerham (1984) and Weir (1996). Their significance was estimated by a non-parametric permutation approach described in Excoffier *et al.* (1992), with 2000 permutations. To construct the tree of genetic relationship, pairwise F_{ST} s were transformed into Reynolds distances (Reynolds *et al.* 1983) and used according to the tree

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Figure 1. Sampling locations. The different symbols indicate the genetic clusters of figure 3; relevant canals are indicated by bars.

method of Fitch & Margoliash (1967) with PHYLIP (Felsenstein 1993).

(b) *Small-scale analysis*

At a relatively small geographical scale across the Main–Danube canal, 18 regularly spaced population samples were taken between M9 and KaJo (figure 1). This was done during three periods (1994/1995, 1997, 1999/2000) after the canal was opened in 1992. About 50 individuals of each population were genotyped at eight polymorphic allozyme loci (Müller *et al.* 2001). Two to three alleles per locus were distinguished. Allele frequencies of the spatially and temporally most-informative loci (FUM-II, GAPD, GPI) were standardized to obtain a comparable range between 0.05 and 0.95 with the same spatial polarity. These standardized frequencies were averaged and plotted against waterway distance from the edge population KaJo (figure 4). Deviation from a homogeneous regression between years was tested with an analysis of covariance, with geographical distance as the covariate. Prior to the analysis, the standardized allele frequencies of each locus were logit transformed, because the expected allele frequency cline of an introgression zone should follow a logistic curve (Barton & Gale 1993).

3. RESULTS

(a) *Genetic structure of invasion routes*

In the large-scale analysis, gene diversities ranged between 0.79 ± 0.05 up to 0.94 ± 0.02 (figure 2). The Black Sea area (populations Dni, Mos, Br) showed significantly higher values than the invaded area lacking isolated lakes (populations D0D1, K1, K3, K5, M1, M9, R1, Wa, Bs, Wlo) (two-sided *t*-test, $p = 0.011$). Isolated lake populations of the invaded area (Gin, Tre) had the lowest values, which were significantly lower than the other colonized populations (two-sided *t*-test, $p = 0.001$).

Figure 2. Genetic diversity within populations (left side of dotted vertical line, rivers, canals or reservoirs; right side of dotted vertical line, lakes).

Comparatively high gene diversities for the invaded range were found in the zone of interchange between the invasion lines (populations K3, K5, M1, M9).

Although most variation was detected within populations, a significant among-population structure was clearly evident. All F_{ST} values of the following population subsets were significantly different from zero (permutation-tests, $p < 0.001$). Genetic differentiation among populations was highest in the Black Sea area ($F_{ST} = 0.051$), lower for the isolated lakes group ($F_{ST} = 0.032$) and lowest for both the southern invasion group ($F_{ST} = 0.021$) and the northern invasion group ($F_{ST} = 0.018$) without isolated lakes. The total population structure is shown in a tree of genetic relationships among all populations (figure 3). Clearly, populations of the two invasion routes belong to different genetic clusters embed-

Figure 3. Tree of genetic relationships among populations. The two genetic clusters comprising the different invasion routes are indicated.

Figure 4. Allele frequency clines across the Main–Danube canal in three sampling periods. Solid line, 1994/1995; dashed line, 1997; dotted line, 1999/2000.

ded within the range of genetic variability of the original area. The canal population K1, which lies very close to the Danube, is assigned to the southern group. Only the two canal populations K3 and K5 are exchanged in relation to their geographical positions, reflecting irregularities in the zone of interchange. The two isolated lake populations are correctly assigned to the northern route. Further, most pairwise F_{ST} values indicated a significant differentiation among population pairs (permutation tests, $p < 0.05$) except for the following pairs: KaJo–D0D1, KaJo–K1, KaJo–K5, KaJo–Wa, D0D1–K1, K3–Wa, K3–R1, K3–Bs, K3–M1, K3–M9, Wa–R1, Wa–Bs, Wa–M1, R1–M1, R1–Gin, Bs–M1 and M1–M9. All of these non-significant pairs except one (KaJo–Wa) are within the two clusters of figure 3, further corroborating the weak population structure within the invasion groups.

(b) Genetic interchange at the canal

Allele frequencies of the three most informative allozyme loci showed significant clines across the Main–Danube canal between the Danube and Main river systems (figure 4). The cline degraded rapidly over the inves-

tigated time period of ca. 8 years. The regression coefficients (slopes) declined from -0.0078 over -0.0034 to -0.0029 in the years 1994/1995, 1997 and 1999/2000, respectively. The difference in regression slopes was significant ($F_{2,101} = 4.63$, $p = 0.012$). The area of interchange covers a stretch of over 500 km. A true genetic mixing between individuals of the two invasion groups is indicated, as populations were in overall Hardy–Weinberg proportions and hybrid genotypes were found (Müller *et al.* 2001). Moreover, the interchange appears highly asymmetrical, with the Main populations strongly influenced by Danube populations, but not vice versa. This pattern reflects the overall water management across the canal from the wetter south to the drier north.

4. DISCUSSION

Our results clearly show a significant genetic structuring in European *D. polymorpha* populations, which can be attributed to the available invasion routes. This result is a striking contrast to North America, where no large-scale differentiation has been reported, i.e. no isolation by distance or clustering patterns (Marsden *et al.* 1995; Stepien *et al.* 1999; Wilson *et al.* 1999). Significant variability was found there only locally, which led to the hypothesis that cohorts spawn and settle separately (Lewis *et al.* 2000). What causes the difference between the continents? The modern invasion in North America may have started from one or more genetically coherent initial populations. If more than one inoculation took place, then mixing among the first colonies would be strong prior to further spread. The classical European invasion, however, started simultaneously from genetically distinct sources. The invasion process in former times was probably slower and less frequent, if we assume that human vectors like rafts, on which adult zebra mussels can attach by Byssus threads, were involved. This may have maintained the different invasion lines in western Europe for about 150 years. The observed genetic differentiation between the invasion groups demonstrates that different genetic backgrounds can equally lead to the ability to invade successfully. Only when the invasion routes became interconnected by a new canal did the differentiation start to break down, as was clearly shown for a period of 8 years across the Main–Danube canal. Isolation breakdown may be a characteristic of increasing human trade activities, in particular across watersheds.

Invasion groups show less genetic diversity than potential source populations, which can probably be attributed to a historical founder effect (Nei *et al.* 1975; Stone & Sunnucks 1993). This loss of overall genetic variability and the fact that populations of an invasion group are interconnected by linear structures like rivers, canals and reservoirs, explain the erosion of between-population structure. Long-distance transportation by boat traffic and massive downstream dispersal of free-swimming *Dreissena* larvae are assumed to have a significant effect (Koutnik & Padilla 1994; Horvath *et al.* 1996; Schneider *et al.* 1998). The significance and speed of the larval swamping was shown at the Main–Danube canal, where the overall water movement reflected the direction of genetic interchange. Larval dispersal, which can homogenize riverine populations, is, however, significantly hindered between iso-

lated lakes, which exhibit low genetic diversity within and strong differentiation between populations.

The comparison of Europe and North America has shown how different environmental settings and time-frames can lead to different invasion patterns, even within one species. We think that the genetic comparison of large river populations will offer the best chance to describe large-scale invasion patterns and will eventually lead to the detection of still unknown invasion origins, including the North American origin.

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