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Evidence for a residual postglacial founder effect in a highly dispersive freshwater invertebrate

Abstract—In the northern hemisphere, glacial periods have had profound and lasting effects on the population genetics of numerous species, with founder effects often persisting for many generations in formerly glaciated regions. We found an unusual example of this in the freshwater bryozoan *Cristatella mucedo*, which showed regional differences in haplotype diversity with relatively low levels of haplotype diversity in northern Europe compared to central/southern Europe despite previous evidence for frequent dispersal between *C. mucedo* populations. Such contradictions between high dispersal and low gene flow have now been reported in several other freshwater taxa and may be attributed to persistent founder effects following colonization of sites by a few individuals whose efficient reproduction leads to rapid population growth. Alternatively, selection may determine which genotypes can thrive in northerly locations, or it may be that *C. mucedo* has undergone cryptic speciation. Future work on adaptive genomic regions is required before we can understand how gene flow, local adaptation, and speciation influence the current distribution patterns of bryozoans and other freshwater invertebrates.

Regional levels of genetic variation within species are influenced to some degree by relatively recent processes, such as ongoing gene flow, local selection pressures, and reproductive strategies. The demographic history of a region, which may be influenced by past events such as glaciation, will also impact current levels of genetic diversity. In both North America and Europe, multiple taxa including vertebrates, invertebrates, and plants have revealed relatively high levels of genetic variation in or near sites that were refugia during the last glacial period, compared to sites that were more recently colonized once the ice had retreated (e.g., Hewitt 1996; Bernatchez and Wilson 1998; Vogel et al. 1999). These regional discrepancies have been attributed to the inability of some refugial genotypes to colonize new areas following glacial retreat, either because of limited dispersal or because they were unable to invade previously established populations.

Facultatively clonal species are particularly suited for colonization of new sites since populations can be established and maintained through clonal reproduction. Many freshwater invertebrates fall into this category, including the bryozoan *Cristatella mucedo* (Bryozoa: Phylactolaemata). Populations of *C. mucedo* have the potential to expand rapidly within the lakes and ponds that they inhabit because

they have several methods of reproduction. Although populations can undergo a brief period of sexual reproduction near the start of the growing season, reproduction in *C. mucedo* is predominantly asexual and includes budding, colony fission, and the production of large numbers of seed-like overwintering stages known as statoblasts (Okamura and Hatton-Ellis 1995). These statoblasts can survive throughout the winter, and their buoyancy allows wide dispersal within a site. In addition, their highly resistant nature allows them to survive hostile conditions during overland dispersal to more distant sites (Okamura and Hatton-Ellis 1995; Freeland et al. 2000a), and, because statoblasts are produced in such large numbers, colonization of neighboring sites appears to be rapid. For instance, populations were present in gravel pits that ranged from 15–47 yr in age in the Thames Valley region of southern England (Okamura 1994). Statoblasts may also facilitate the long-term survival of populations by forming viable propagule banks (Freeland et al. 2001).

In this study we have compared regional patterns of *C. mucedo* genetic diversity based on mitochondrial DNA. In particular, we compared the haplotypes of colonies collected from northern Europe with those collected from central/southern Europe (Table 1). Europe at the peak of the last glaciation can be roughly divided into three regions: the northerly ice-covered region, the central tundra-covered region, and the southerly warmer region (Hewitt 1999). Previous studies on the effects of glaciation on patterns of genetic diversity have tended to concentrate on comparisons between southern Europe and central/northern Europe. This is because the taxa investigated could not survive in the central regions of tundra and inhabited only the glacial refugia in the southern region such as the peninsulas of Iberia, Italy, the Balkans, and Greece. When the ice retreated, these taxa dispersed north beyond mountain ranges such as the Alps and the Pyrenees. These expansions often involved a series of founder effects, the genetic signatures of which can still be seen today in terms of reduced levels of genetic diversity in northern Europe compared to southern Europe (e.g., Hewitt 1996, 1999; Vogel et al. 1999).

In order to identify any persistent effects of postglacial dispersal on the genetic variation of *C. mucedo*, we have adopted an approach appropriate to the biology of *C. mucedo*, which entails a comparison between the northern region (sites located in the formerly ice-covered areas) and a

Table 1. Location of populations and identity of haplotypes found at each location followed by number of colonies with that haplotype in brackets. Population ID refers to numbers used in Fig. 1. Haplotype identity corresponds to numbers used in Fig. 2. Haplotype data from locations in bold were obtained specifically for this study. Haplotype data from other locations were obtained in previous studies (Freeland et al. 2000b).

Population ID	Location	Latitude, longitude	Haplotypes present (and number of colonies)
Southern Europe			
1	Lago di Piediluco, Italy	42°31'N, 12°46'E	H6(1), H9(2), H12(1)
2	River Sile, Italy	45°39'N, 12°10'E	H1(1), H10(1)
3	Le Lac de Grand Lieu, France	47°3'N, 1°35'W	H1(1), H5(2)
4	Etang du Gros Caillou, France	47°7'N, 2°5'W	H5(3)
5	Etang de la Thévinère, France	47°10'N, 1°6'W	H5(1)
6	Barrage de la Cantache, France	48°6'N, 1°11'W	H4(1), H5(1), H8(1)
7	Lake Hallwiler, Switzerland	47°19'N, 8°11'E	H11(2)
8	Bonnerweiher, Germany	48°1'N, 10°47'E	H7(1)
9	Hochstadt, Germany	48°36'N, 10°35'E	H3(1), H6(1)
10	Nationaal Park de Biesbosch, Netherlands	51°40'N, 4°53'E	H1(1), H5(2)
11	Herpen Lake, Netherlands	51°48'N, 5°47'E	H5(3)
12	Bussloo, Netherlands	52°12'N, 6°7'E	H5(1)
Northern Europe			
13	Ry Mølle Sø, Denmark	56°5'N, 9°45'E	H1(1), H5(2)
14	Hald Sø, Denmark	56°23'N, 9°23'E	H5(1)
15	Søndersø Norresø, Denmark	56°27'N, 9°23'E	H1(1), H5(2)
16	Tassjö, Sweden	56°20'N, 13°5'E	H1(1)
17	Rössjön, Sweden	56°23'N, 13°5'E	H2(1), H5(2)
18	Sommen, Sweden	58°0'N, 15°15'E	H5(3)
19	Hunn, Sweden	58°49'N, 15°57'E	H5(1)
20	Enärjärvi, Finland	60°28'N, 22°55'E	H5(3)
21	Littoistenjärvi, Finland	60°30'N, 22°19'E	H5(3)
22	Konnevesi, Finland	62°40'N, 26°34'E	H2(1), H5(2)
23	Keitele, Finland	62°53'N, 25°58'E	H1(1), H5(3)

composite central/southern region (sites located in the formerly unglaciated areas, which may or may not have been covered by tundra). This approach was chosen for two reasons. First, *C. mucedo* conforms to a Holarctic distribution with such small overlap with southern regions that a comparison solely between southern and northern regions is of little interest. Second, it is likely that *C. mucedo* could have persisted to some extent in the tundra that covered central Europe during the last glaciation. Colonies have been found in water as cold as 4°C (Ricciardi and Reiswig 1993) and at northerly locations including the Northwest Territories (Rawson 1953), Iceland (Heding 1938), and Norway north of the Arctic circle at 71°09'N, which is the most northerly part of the European continent (Økland and Økland 2000). *C. mucedo* has also been found at altitudes up to 2293 m in the Alps (Wiebach 1960) and 1116 m in Norway (Økland and Økland 2000). This species must be tolerant of relatively cold environmental conditions and, therefore, is unlikely to have been restricted to the southern warm region of Europe during the last period of glaciation.

The ecology of *C. mucedo* means that a phylogeographic comparison of populations from northern and central/southern Europe should also be a comparison of relatively new and relatively old populations. In this study we were specifically interested in the question of whether or not the high dispersal abilities of *C. mucedo* had eradicated the genetic signals, such as depleted genetic diversity, that we may oth-

erwise expect to be associated with a postglacial founder effect in northern Europe.

Methods—We compared 478–479 bp of 16S rDNA mitochondrial sequence data from 28 colonies collected from 11 sites in northern Europe and 28 colonies collected from 12 sites in central/southern Europe (regions defined as those outlined in Fig. 1; see also Table 1). Haplotypes of up to four colonies per site were obtained. Colonies were collected at each site from separate substrata in order to avoid sampling from local patches of identical genotypes that could have arisen through colony fission and subsequent movement. Methods for amplifying and sequencing the region of 16S rDNA can be found in Freeland et al. (2000b). Sequences were aligned using Clustal X (Thompson et al. 1997), and, since only a single gap was required, the alignment was unambiguous. All sequences have been submitted to GenBank (accession numbers AF260067–AF260119, AY283929–AY283940). Sequences were then imported into DnaSP version 3 (Rozas and Rozas 1997). Genetic diversity was calculated in three ways: (1) the proportion of individuals with novel genotypes; (2) nucleotide diversity (π), which is the average number of nucleotide differences per site between two sequences (Nei 1987); and (3) haplotype diversity (h), which describes the number and frequency of different haplotypes in the sample (Nei 1987). Nucleotide composition was calculated in Mega v. 1.02 (Kumar et al.

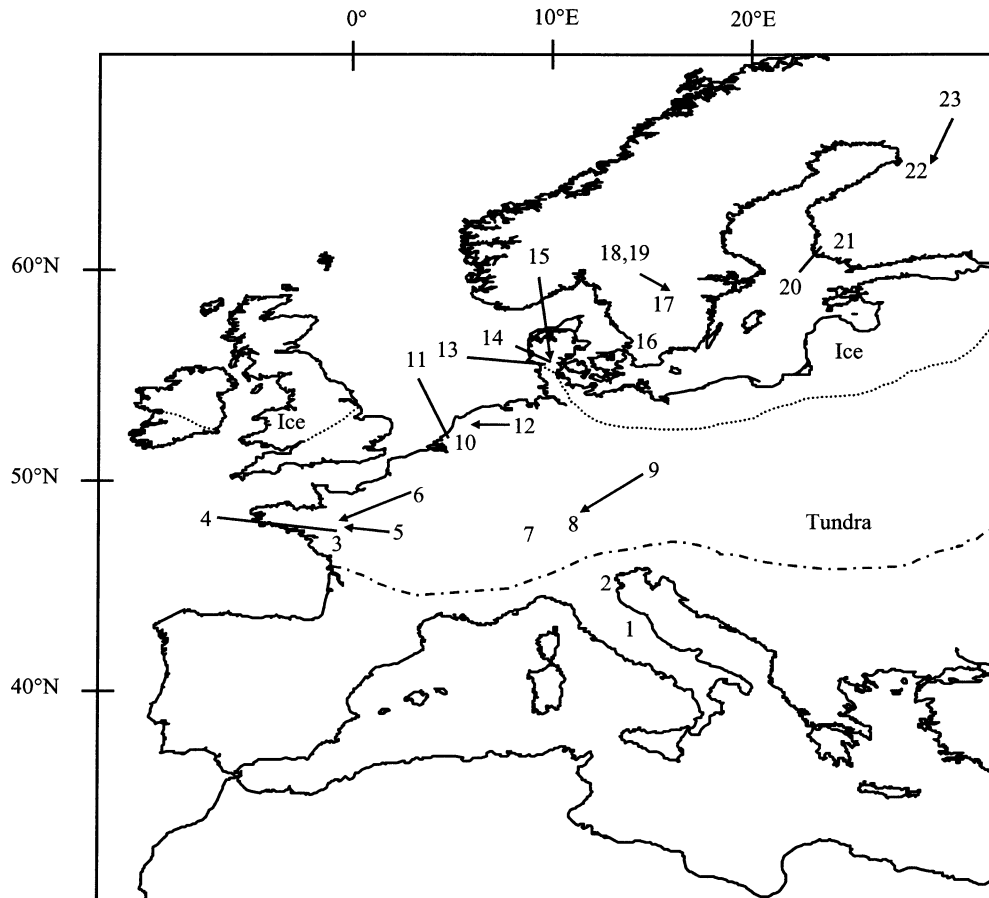


Fig. 1. Map showing the sites from which colonies were collected. The dotted line shows the approximate southern extent of ice cover during the last glaciation, and the dashed line shows the approximate southern extent of tundra.

pers. comm.). The phylogeny was reconstructed in Mega using the neighbor-joining method based on Kimura's two-parameter model of the number of nucleotide substitutions per site (Kimura 1980; Kumar et al. pers. comm.).

Results—The 56 European colonies revealed 12 different haplotypes. The overall nucleotide composition was 30.1% A, 24.7% T, 21.2% C, and 24.0% G. There was a total of 37 variable sites, 24 of which were phylogenetically informative. Figure 2 shows a neighbor-joining tree based on all haplotypes. The distances between haplotypes ranged from 0.0021 to 0.0569. The most common haplotype, H5, was found in 13 colonies in central Europe and in 22 colonies in northern Europe, for a total of 35 colonies (62.5%; Table 1). H1 was found in seven colonies spread across southern, central, and northern Europe, and the remaining haplotypes were found in only one or two colonies. In central/southern Europe there were fewer haplotypes in the area near the coast (populations 3–6, 10–12) compared to those farther inland (populations 1, 2, 7–9), although the difference was not significant ($z = 1.717$, $P = 0.086$). We therefore adhered to our original pooling of populations that was based on latitude, which left us with a comparison of populations north versus populations south of the formerly ice-covered region.

There was a significantly greater proportion of individuals

with novel genotypes in central/southern Europe (11/28 = 39.3%) compared to northern Europe (3/28 = 10.7%; $z = 2.111$, $P = 0.035$). Similarly, haplotype diversity was lower in northern Europe (0.370 ± 0.105) compared to central/southern Europe (0.778 ± 0.078 ; $z = 2.026$, $P = 0.043$). Nucleotide diversity was also lower in northern Europe (0.00110 ± 0.00038) compared to central/southern Europe (0.01766 ± 0.00384), although the difference was not significant ($z = 0.187$, $P = 0.852$).

Discussion—The significantly higher levels of genetic diversity in *C. mucedo* collected from central/southern Europe versus those from northern Europe are consistent with numerous previous studies that have found an inverse relationship between genetic diversity and distance from glacial refugia (e.g., Armbruster et al. 1998; Hewitt 1999; Schmitt and Seitz 2001). This may be attributed to leading edge colonization, in which individuals from the northerly limits of the refugia are the first to colonize recently unglaciated regions, and later immigrants cannot easily invade these established populations. The majority of genetic lineages thereafter remain in the regions of glacial refugia because they are unable to invade the expanding northerly populations (Vogel et al. 1999).

The extent to which leading edge colonization can influ-

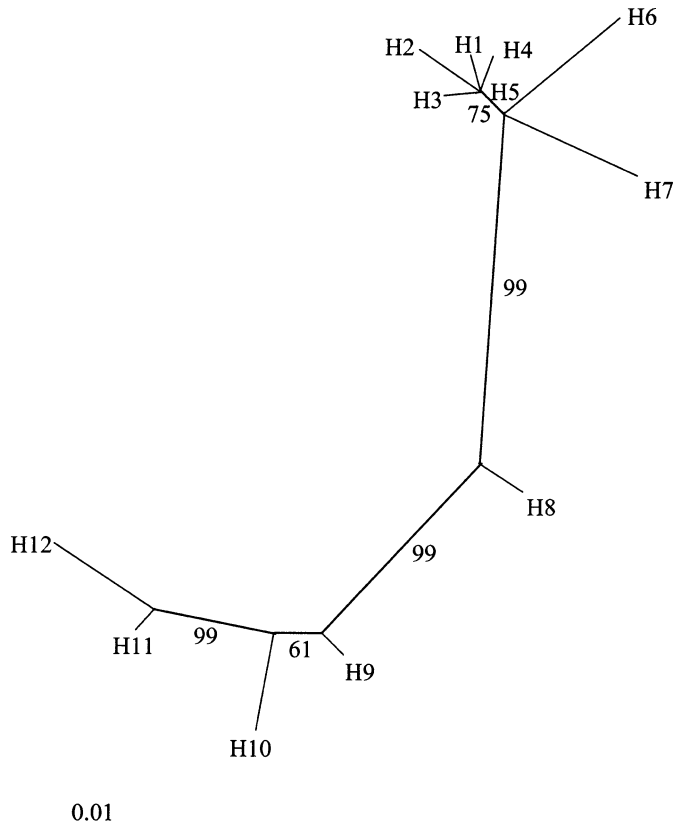


Fig. 2. Neighbor-joining tree based on Kimura's two-parameter model of the number of nucleotide substitutions per site. Distances are drawn to scale.

ence the overall distribution of genetic diversity will depend to a large extent on the speed with which species can disperse (Ibrahim et al. 1996; Haase et al. 2003). As mentioned in the introduction, *C. mucedo* can rapidly colonize neighboring sites. Freshwater bryozoans may also be dispersed over long distances if their statoblasts become attached to feathers or fur (Freeland et al. 2000a). In addition, statoblasts can remain viable after passing through the digestive tracts of ducks and amphibians (Chiralambidou et al. 2003). The discovery of *C. mucedo* statoblasts in Norwegian lake sediment dating approximately from 9500 to 9300 years B.P. reflects early postglacial colonization (Solem et al. 1997) and is testimony to the rapidity with which *C. mucedo* can colonize newly available sites. Furthermore, apparent extinction-recolonization events, combined with low ongoing levels of gene flow between some European *C. mucedo* populations inferred from microsatellite data, previously suggested a metapopulation structure (Freeland et al. 2000a). These data, in combination with the lack of phylogeographic structuring of haplotypes in northwestern Europe (Freeland et al. 2000b), provide further evidence of widescale and rapid dispersal in *C. mucedo*.

Despite the dispersal abilities of *C. mucedo* and its inferred metapopulation structure, relatively few haplotypes from central/southern Europe have been found in northern Europe. Poor correlations between the high dispersal abilities of species and accompanying low estimates of gene flow

based on genetic data have been found in several other freshwater taxa including cladocerans (Boileau et al. 1992) and dragonflies (Freeland et al. 2003). There are at least two possible explanations for this. First, simulations have shown that rapidly dispersing species, particularly leptokurtic dispersers (many short-distance, few intermediate, and more long-distance dispersers), should be more likely than slowly dispersing species to experience a reduction in genetic diversity in newly colonized areas (Ibrahim et al. 1996). This is because there will be a time lag between the arrival of long-distance dispersers and the other representatives of the gene pool, and this delay will allow the early arrivals to establish themselves and colonize neighboring demes before the short- and intermediate-distance dispersers have arrived. This effect was used by Boileau et al. (1992) to explain the apparent paradox of high dispersal coupled with low levels of gene flow in freshwater cladocerans. They showed that when populations are founded by a few individuals and subsequently undergo rapid growth before receiving additional immigrants, the associated founder effect may persist for thousands of generations. If a similar process can be attributed to *C. mucedo*, the initial long-distance dispersers would have included H1, H2, and H5. Two of these (H1 and H5) were also found in the source region of southern/central Europe, and it is likely that H2 would also be found if a greater number of colonies were genotyped.

The second explanation for relatively low genetic diversity in formerly glaciated regions is local adaptation and selection. The temperature and photoperiod regimes at northerly latitudes may limit the number of genotypes that can thrive there (e.g., Davis and Shaw 2001). Selection may be strong enough on its own to reduce genetic diversity in relatively harsh climates. The three haplotypes found in northern Europe (H1, H2, and H5) are in the same phylogenetic cluster and are separated by low levels of genetic differentiation. This may lend some support to the idea of local adaptation if their genetic similarity means that individuals with these haplotypes share a selectively advantageous genome.

A third possible explanation for the current distribution of *C. mucedo* haplotypes is the presence of more than one cryptic species of *Cristatella* in Europe. A diversity of haplotypes was apparently maintained in glacial refugia in southern/central Europe, but the reason for the large genetic disjunction between H5 and H8 (Fig. 2) remains unclear. Evidence for cryptic speciation of *C. mucedo* in North America was previously suggested by combined mitochondrial and microsatellite data identifying two distinct genetic lineages and possible hybridization (Freeland et al. 2000b) and also by the detection of two different genome sizes of *C. mucedo* in North America (Potter 1979). In addition, an earlier comparison of North American and European haplotypes suggested the possibility of a past dispersal event between the two continents (Freeland et al. 2000b). This would have preceded the most recent glaciation by many thousands of years (Freeland et al. 2000b) and therefore would not affect our interpretation of European postglacial dispersal. Such dispersal could, however, be relevant to questions of local adaptation and cryptic species. It is clear that future work on adaptive genomic regions is needed before we can fully appreciate the relative importance of historical and ongoing processes, such as dispersal, gene flow, and local ad-

aptation, to the distribution patterns of bryozoans and other freshwater invertebrates.

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