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Ecological, morphological, and genetic differentiation of *Daphnia* (*Hyalodaphnia*) from the Finnish and Russian subarctic

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Abstract

We studied genetic differentiation of two subarctic *Daphnia* species (subgenus *Hyalodaphnia;* Cladocera: Anomopoda) in relation to ecological and morphological diversification. *Daphnia longispina* and the recently discovered species *Daphnia umbra* are genetically differentiated based on mitochondrial 12S rDNA and internal transcribed spacer (ITS) regions. Genetic differentiation of 12S rDNA among the two sister taxa is in the range of differentiation among other *Hyalodaphnia* species (uncorrected genetic distance = 0.11). Despite frequent interspecific hybridization among *Daphnia* (*Hyalodaphnia*) species, we found no interspecific hybrids of *D. umbra* and *D. longispina*. *D. umbra* is for the first time recorded to occur in Northern Finland and Russia (Pechora Delta), and to cooccur in neighboring sympatry with *Daphnia longispina* across a subarctic region in northern Finland. Species are ecologically differentiated: *D. umbra* occurred at higher elevations, in larger and deeper water bodies than *D. longispina.* Species did not differ significantly in levels of ultraviolet-protective melanin pigmentation but varied with regard to environmental preferences, such as fish predation and levels of total dissolved organic carbon (DOC). These findings argue that ecological differentiation and divergent selection might have caused speciation or at least are responsible for the maintenance of reproductive isolation among subarctic *Daphnia* (*Hyalodaphnia*) species.

Arctic and subarctic flora and fauna have been greatly influenced by the repeated episodes of glaciation and deglaciation during the Pleistocene, which created ample opportunity for succession, fragmentation, and secondary contact of species (Hewitt 2001). Distributional variation of populations determines changes in population dynamics and genetics, which may result in founder effects, local adaptation, reproductive isolation, and speciation. Many recent studies of populations from previously glaciated and unglaciated areas have provided insights into species colonization, genetic subdivision, secondary contact of species, genetic structure of populations and species, and community changes over time (Hewitt 2000). In addition, several refugia and postglacial colonization routes have been proposed for a number of organisms across the Holarctic (Hewitt 2001). In particular, arctic and subarctic freshwater animals, such as fishes (Nesbo et al. 1999), ostracods (Little and Hebert 1997), copepods (Boileau and Hebert 1991), *Sida* (Cox and Hebert 2001), amphipods (Witt and Hebert 2000), and *Daphnia* (Colbourne et al. 1998; Weider et al. 1999*a*,*b*) have been used as model systems to reconstruct the impact of glaciations for phylogeographic and speciation processes.

Recent studies on speciation mechanisms have revealed that reproductive isolation may evolve from the same forces that cause phenotypic changes (Schluter 2001). Resource competition and the exploitation of alternative resources can lead to divergent selection. This process is most obvious in remote islands or newly formed lakes (Schluter 1996). In particular, postglacial lakes show accelerated rates of speciation that are associated with large phenotypic shifts. For example, niche differentiation seems to mediate reproductive isolation among whitefish ecotypes, which was found to be the same process driving trophic specialization within lakes (Bernatchez et al. 1996). However, only a few studies have addressed the evolutionary mechanisms of diversification (e.g., speciation, population structure, and gene flow) in relation to environmental gradients that are characteristic for arctic and subarctic freshwater habitats (e.g., habitat size, predation levels, and levels of ultraviolet radiation; Hobæk and Wolf 1991). The transition from subarctic to arctic climate and the resultant change in the characteristics of the

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Table 1. Location (latitude and longitude), lake numbers (LN), and lake names of *Daphnia umbra* (umb) and *D. longispina* (lon) populations analyzed for genetic variation of ITS and 12S mitochondrial rDNA. Numbers under ITS and 12S rDNA represent sample size of screened individuals. Lake characteristics are lake size, altitude (above sea level), average lake depth, and dissolved organic carbon. Missing data are labeled (md).

		ITS		12S rDNA							
LN	Population	umb	lon	umb	lon	Latitude	Longitude	Lake size (km^2)	Altitude (m)	Depth (m)	DOC (mg L^{-1})
1	Saanajärvi	3		3		69.02.54	20.52.50	0.701	679.4	21	1.6
2	Mallajärvi	2		2		69.05.34	20.39.32	0.165	810	8	0.7
3	Somaslompolo	2		4		96.15.50	21.30.37	0.162	765	8	0.5
4	Porevarri	8		4		69.14.17	21.40.36	0.111	794.4	3	1.0
5	Mallalampi A	3		3		69.03.55	20.43.11	0.020	560	2	3.1
6	Mallallampi B	7		3		69.04.03	20.42.44	0.022	560	2	3.1
7	Ristijärvi	md		1		68.48.49	22.02.43	0.106	571.6	2	3.4
8	Pechora Delta, Russia	3		md		68.02.42	53.06.36	md	md	md	md
9	Hillalampi		2		3	69.05.55	20.44.06	0.002	485	0.6	10.0
10	Siilasvuo 3		1		3	69.05.43	20.44.29	0.002	485	0.6	11.3
11	Salmivaaralampi		1		3	69.00.30	20.51.19	0.002	500	1.4	md
12	Laassavaara		7		3	68.55.37	20.57.17	0.0004	720	0.5	md
13	Löysinpä		2		3	69.01.32	20.52.27	0.002	516	1.4	md
14	Muotkantaka		3		3	68.55.09	20.58.19	0.003	530	1.6	md
15	Sierkisvaara		6		2	68.52.08	21.09.57	0.0025	515	1.2	md
16	Tielammikko		1		3	69.01.16	20.51.58	0.002	490	0.9	6.0
17	Tsahkallampi A		md		4	69.01.12	20.54.21	0.002	558	0.7	8.5
18	Tsahkallampi B		3		3	69.01.38	20.53.24	0.001	565	1	9.5
19	Kevo16		2		3	69.45.55	26.58.26	0.0008	218	2	md

catchment vegetation is strongly reflected in the chemical and physical conditions of the respective water bodies. Subarctic lakes below the treeline are small, warm, acidic, and highly colored polyhumic waters, whereas arctic lakes in barren tundra are larger, cold, and clear with higher alkalinity, pH, and calcium (Ca) levels (Blom et al. 2000).

Here we explore the level and rate of genetic, morphological, and ecological diversification for phenotypically variable and taxonomically problematic *Daphnia* (subgenus *Hyalodaphnia*) species, *D. longispina* Müller 1776 and the recently described *D. umbra*, that occur in many lakes and ponds throughout northern Europe (Taylor et al. 1996; Flößner 2000; Schwenk et al. 2000).

In the mountain range of southern Norway, a transparent and a pigmented (melanic) morphotype of *D. longispina* coexist in neighboring sympatry (Wolf and Hobæk 1986). Phenotypic differentiation of individuals was supported by allozyme electrophoresis; however, the two forms could not be assigned to either different clones, locally adapted populations, or species. Ecological genetic studies by Hobæk and Wolf (1991) showed that the two forms are also differentiated in terms of their ecological characteristics. For example, the melanic forms occurred above 900-m altitude in shallow ponds and large lakes, whereas the transparent form occurred in lowland lakes (below 1250 m), but populations at higher elevations were restricted to shallow ponds. Despite the inclusion of potential sister taxa for the genetic analyses, no final conclusion on the species status could be drawn. However, genetic distances among the two forms indicated that melanic and transparent forms belong to different taxa.

So far, several authors have described *Daphnia* (*Hyalodaphnia*) pigmented with melanin (Hebert and Emery 1990; Hessen 1996; Rautio and Korhola 2002*b*); however, it has not been possible to draw conclusions about their species status and their distributional patterns (*see* Hobæk and Wolf 1991). Ecological genetic studies proposed separate species status for pigmented arctic *D. longispina* based on allozyme data (Wolf and Hobæk 1986; Hobæk and Wolf 1991; Taylor and Hebert 1994). Only recent DNA data suggest that melanic forms belong to a previously undescribed taxon: *D. umbra* (Hebert 1995; Colbourne and Hebert 1996; Taylor et al. 1996; Schwenk et al. 2000).

Here we describe the genetic and ecological characteristics of *D. longispina* morphotypes from northern Finland and Russia. We applied genetic markers that differentiate among all *Daphnia* (*Hyalodaphnia*) species in Europe, considering in particular the recently discovered species *D. umbra* (Colbourne and Hebert 1996; Taylor et al. 1996; Schwenk et al. 2000; Billiones et al. in press). In order to address potential hybridization among different species, we used both mitochondrial (12S rDNA) and nuclear DNA (ITS) markers. In addition, genetic information was applied to assess the spatial distribution of taxa across northern Finland and to estimate morphological and ecological differentiation of species.

Methods

Daphnia specimens were collected with vertical and horizontal plankton net (120 μm) tows in 19 populations across northern Finland and in Russia (Pechora Delta; Table 1; Fig. 1). Zooplankton samples were preserved in 70–80% ethanol. Individuals were identified using standard taxonomic keys (e.g., Flößner 2000). Since recent molecular studies have described a previously unrecognized *Daphnia* species, *D.*

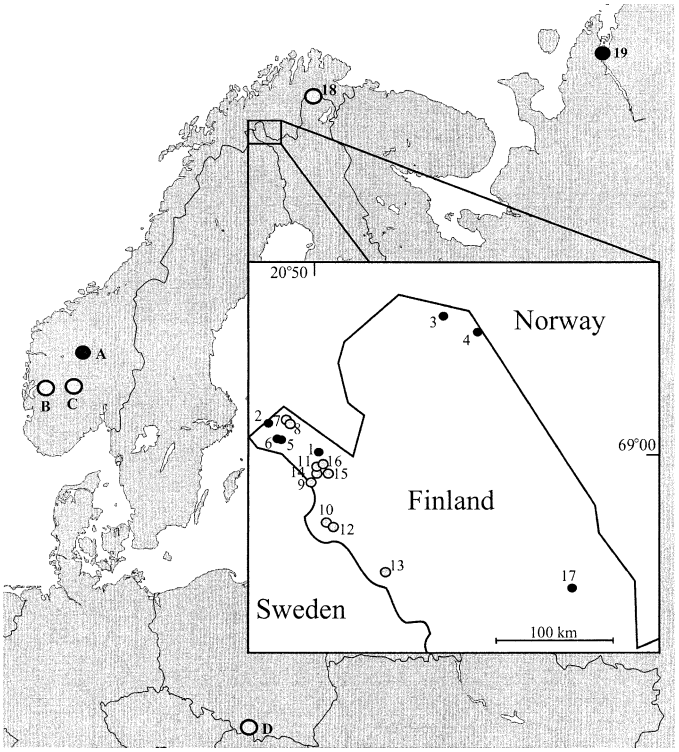

Fig. 1. Location of the northern Finnish and Russian *Daphnia* populations used for the genetic analyses tested for mitochondrial and nuclear DNA polymorphism (1–19, *see also Table 1*). Additional data from Norway and Poland (populations A–D) were either available from previous studies (Taylor et al. 1996; Schwenk et al. 2000) or retrieved from the NCBI Genbank database. Black dots represent *D. umbra* populations, and gray dots represent *D. longispina* populations.

umbra, from the Canadian arctic and Europe (Colbourne and Hebert 1996; Taylor et al. 1996; Schwenk et al. 2000), we also used the digital taxonomic key for Northern American daphniids (Hebert 1995). However, since no taxonomical key includes both species and no detailed species descriptions of *D. umbra* have been published, we applied the following four-level approach: (1) we characterized individuals based on mitochondrial and nuclear DNA (Billiones et al. in press); (2) individuals were assigned to previously described species based on genetic information (National Center for Biotechnology Information, NCBI database); (3) assigned individuals were morphologically (shape) analyzed and (4) characterized by ecological (habitat) parameters.

Individual daphniids were transferred from storage solution (ethanol) to 500 μl Tris-EDTA buffer (TE) and incubated for at least 2 h. Individuals were then subjected to DNA extraction using 75 μl of 10% Chelex (Sigma) and proteinase K (final concentration of 0.1 μg μl^{-1}). Samples were incubated at 55°C in a shaking water bath for at least 1 h, centrifuged for 1 min at 10,000 rpm, and incubated at 95°C for 10 min. Polymerase chain reaction (PCR) concentrations and amplification for both, 12S rDNA and ITS (a short piece of the ITS1 region, 5.8S rDNA, and a large part of ITS2 region) were conducted as described in Schwenk et al. (2000).

ITS-PCR products (10 μl) were cut with the restriction enzymes *Mwo*-I, *Sau96*-I, *Mse*-I, and *Hinc*-II (1 unit) for 3 h according to manufacturers' protocols (New England Biolabs and Invitrogen). RFLP (Restriction Fragment Length Polymorphism) fragments were visualized on 2% agarose gels and stained with ethidium bromide. 12S rDNA PCR fragments were purified (Qiagen), and approximately 20–50 ng per sample were subjected to cycle sequencing and electrophoresis on an ABI 377 automated DNA sequencer. DNA sequences were aligned in ClustalX (Thompson et al. 1997); pairwise sequence divergence estimates (Kimura 2 parameter distances) and phylogenetic reconstruction (maximum parsimony, maximum likelihood, and neighbor-joining trees) were computed using PAUP 4.0 (Swofford 2002).

Morphological shapes (outline) of individuals in lateral view were obtained using a digital image analysis system (Coolsnap Camera, Photometrics). For each image (outline), 80 coordinate points were determined and subjected to an elliptic Fourier transformation (Ferson et al. 1985) using shape information of size, location, rotation, and start position as independent coordinates (Schwenk et al. 2001). Normalized coefficients were arranged as column vectors for a canonical discriminant analysis (Statistica 2000).

For 12 lakes the data of dissolved organic carbon (DOC) in the water column and for nine populations the melanin concentration of *Daphnia* individuals were available from a recently published study (Rautio and Korhola 2002*b*). Although samples for melanin concentration and species composition originate from different years (2000 and 1998/1999, respectively), we argue that neither melanin concentrations (or environmental parameters) nor species composition changed during a 1–2-yr period. Previous studies have shown that the studied sites harbor populations with similar melanin levels between different years (Rautio and Korhola 2002*a*). Moreover, limnological characteristics and zooplankton composition do not differ between years in these sites (pers. obs. from several years; Sorvari et al. 2000; Rautio 2001).

Results and discussion

Mitochondrial 12S rDNA sequence variation of all *Daphnia* individuals was either associated with previous published sequences of *D. longispina* (GenBank accession numbers DLU34638 and AF277279) or *D. umbra* (DUU34640, DUU34639, and AF277276). Independent of phylogenetic methodology (maximum parsimony, maximum likelihood, or neighbor-joining trees based on Kimura 2 parameter distances), we obtained identical topologies. *D. longispina* and *D. umbra* form two monophyletic groups (Fig. 2). Genetic differentiation between *D. longispina* and *D. umbra* was in the range of genetic differentiation among other *Hyalodaphnia* species (Fig. 2). Sequence divergence of 12S rDNA fragments among *D. longispina*, *D. galeata*, *D. hyalina*, and *D. cucullata* was about 9% to 18%, and sequence divergence of *D. umbra* versus *D. longispina*, *D. galeata*, *D. hyalina*, and *D. cucullata* was about 10% to 13%. In addition, sequence divergence within *Hyalodaphnia* species (0–1%) was in the same range as the sequence divergence within *D. um-*

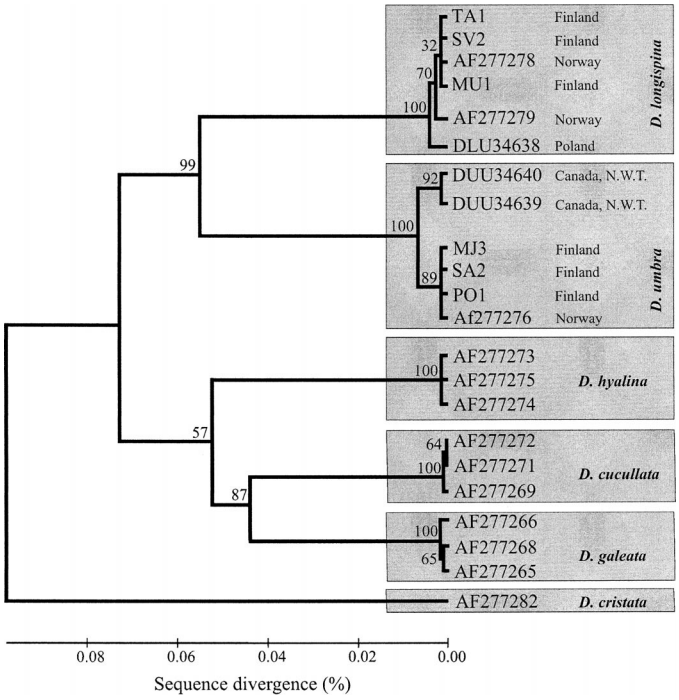

Fig. 2. A neighbor-joining tree of Kimura 2 parameter distances based on mitochondrial 12S rDNA sequences of *D. longispina, D. umbra, D. hyalina, D. cucullata, D. galeata,* and *D. cristata* individuals. Haplotype labels refer to either accession numbers (including the country of sample origin) or to Finnish populations (TA = Tsahkallampia; SV = Salmivaaralampi; MU = Muotkantaka; MJ = Mallajärvi; SA = Saanajärvi; PO = Porovarri). Numbers above branches denote bootstrap values based on 100 permutations.

bra (0–1%). These patterns of genetic differentiation of taxa indicate that *D. umbra* represents a cohesive taxon comparable to all other well-described *Hyalodaphnia* taxa. Our data also verify the sister taxon relationship of *D. umbra* and *D. longispina* (Taylor et al. 1996; Schwenk et al. 2000). In addition, nuclear DNA variation is consistent with mitochondrial DNA variation among species. PCR product length of *D. longispina* species was around 2000 bp, whereas fragment lengths of all other *Hyalodaphnia* species were significantly shorter (around 1400 bp, Billiones et al. in press). ITS segments of *D. longispina* haplotypes were cut into four fragments (180, 260, 280, and 900 bp), and ITS segments of *D. umbra* haplotypes were cut into three fragments (240, 550, and 700 bp) using *Mwo*-I. In addition, we applied three other restriction enzymes, which also resulted in consistent and diagnostic RFLP patterns (*D. longispina—Sau96*-I, 120, 200, 220, 240, 380, 520; *Mse*-I, 100, 210, 420, 850; *Hinc*-II, 220, 320, 420, 900; and *D. umbra—Sau96*-I, 120, 220, 280, 340, 500; *Mse*-I, 80, 190, 520, 680; *Hinc*-II, 1400, 1450). Individuals of seven populations showed consistently mitochondrial 12S rDNA sequences similar to published *D. longispina* sequences and only one characteristic ITS-RFLP pattern, whereas 11 other populations showed consistently mitochondrial 12S rDNA sequences similar to published *D. umbra* sequences and the alternative ITS-RFLP pattern (Table 1). No population was observed that exhibited both cytonuclear genotypes. This consistent pattern of mitochondrial and nuclear DNA data indicates that subarctic *Hyalodaphnia,* which have previously been described as *D. longispina,* represent two genetically well-differentiated taxa, *D. umbra* and *D. longispina.*

Despite the high frequency of interspecific hybridization among *Daphnia* (*Hyalodaphnia*) species, we found no evidence for interspecific hybridization or introgression (e.g., cytonuclear disequilibria) among *D. longispina* and *D. umbra.* Since both species do not occur syntopically, but only in neighboring sympatry, ecological factors might have caused genetic differentiation and subsequent speciation, or ecological differentiation of species maintains prezygotic isolation. Hobæk et al. (in press) showed that one of the studied species, *D. longispina,* generates interspecific hybrids together with *D. galeata,* and one southern Norwegian lake has been found with both *D. umbra* and *D. longispina* (Hobæk and Wolf 1991). In addition, preliminary data from Canadian populations suggest that at least two morphotypes, one 'melanic' and one 'clear' form, of *D. umbra* cooccur (Taylor et al. 1996). Thus, further studies across the entire species range are necessary to uncover the level of genetic and reproductive isolation of *D. umbra.*

Although several morphological characters (e.g., melanin concentration, Hobæk and Wolf 1991) have been discussed to differentiate among northern European *D. longispina*/*D. hyalina* subspecies and morphotypes (for review *see* Flößner 2000), we did not identify a priori any apparent differences among taxa. Also, data from Rautio and Korhola (2002*b*) in combination with the genetic data revealed that the two species showed similar levels of pigmentation (analysis of variance [ANOVA], $F = 0.785$, df = 1; $P = 0.41$). However, after assignment of individuals to species, based on genetic markers, some subtle morphological differences emerged. *D. umbra* tended to have slightly more concave ventral margins of the rostrum, whereas *D. longispina* exhibited rather straight ventral margins of the rostrum. Although qualitative differences between species seem to be sparse, quantitative differences in carapace outlines resulted in a clear differentiation. The two species (as characterized by nuclear and mitochondrial DNA) differed significantly with regard to their carapace shape (Fig. 3; Wilk's Lambda = 0.106; $P < 0.001$). In order to verify the discriminant analysis, we performed a randomization test using a random association of the grouping variable ''taxon'' and morphological data. Permutation of shape data generated 50 randomized data sets that resulted in significantly higher Wilk's Lambda (0.5–0.8) than the observed value. Thus, the significant differentiation of species is not based on chance alone or due to any violation of the assumptions of a discriminant analysis (e.g., normal distribution within groups and similar within-group covariances).

Classification success of *D. umbra* and *D. longispina* remained high (above 85%) even if other taxa, such as *D. rosea, D. hyalina,* or *D. galeata,* were included. Since our data on the morphology of *D. umbra* and *D. longispina* cover only a limited geographical area, i.e., northern Finland and one population from Russia, we lack information from populations found across other arctic and subarctic portions of the species ranges (e.g., Nearctic). In order to assess the intraspecific variation of species and to verify fixed morpho-

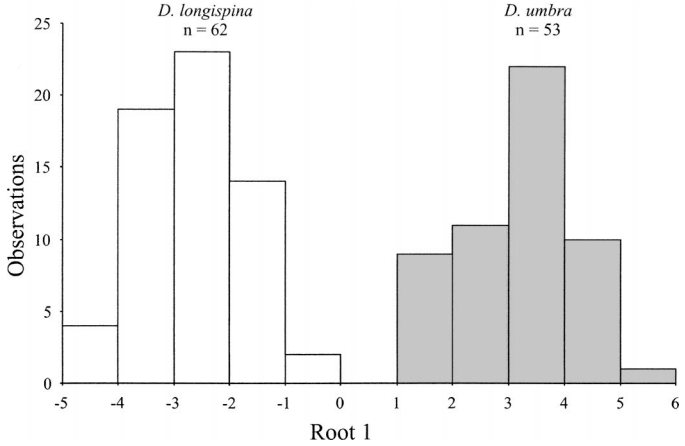

Fig. 3. Frequency distribution of 53 *D. umbra* (gray bars) and 62 *D. longispina* (white bars) individuals from Finland and Russia based on canonical discriminant analysis of Fourier transformed shape data (root1). Species, classified according to mitochondrial and nuclear DNA information, showed significant morphological differentiation (Wilk's Lambda = 0.106; $P < 0.001$).

logical differences among species, larger sample sizes are required. However, our preliminary analysis among geographically proximate populations (Fig. 1) revealed significant morphological differentiation among species. Morphological variation (shape) among species might either represent neutral morphological variation, phylogenetic constraints, or adaptation to particular environmental conditions. So far, we cannot distinguish between phylogenetic constraints, neutral variation, or adaptation, since data on functional links between fitness related traits and shape are lacking.

Although *D. longispina* and *D. umbra* are genetically highly differentiated (uncorrected genetic distance = 0.11 ± 0.0017), they are very similar phenotypically. Using a molecular clock (2.3% sequence divergence per million years, Brower 1994), we have estimated the split of the two species at about 4.7 million years ago. This discrepancy of genetic and morphological divergence explains the recent (late) discovery of *D. umbra* and the difficulty assigning diagnostic morphological characters. The morphological stasis of this species pair over long periods of evolutionary time is consistent with findings among other freshwater invertebrates such as clams (Pfenninger et al. 2002), tadpole shrimps (Suno-Uchi et al. 1997), bryozoa (Cheetham et al. 1995), cichlid fishes (Sturmbauer and Meyer 1992), and *Daphnia* (Colbourne and Hebert 1996; Schwenk et al. 2000).

D. umbra is only found in arctic and subarctic regions (A. Hobaek pers. comm.; Hebert 1995; Taylor et al. 1996; Schwenk et al. 2000), whereas *D. longispina* is described from mainly temperate and lowland regions (Flößner 2000). However, *D. longispina* was not identified in the framework of a population genetic study of several central European lakes (Schwenk unpubl. data). Genetic data revealed that many individuals, identified as *D. longispina* morphotypes, belong to either *D. galeata* or more frequently *D. rosea.* Thus, *D. longispina* sensu stricto might also represent a species that is restricted to alpine, arctic, and subarctic regions; at least our data indicate that the center of the species range is much farther north than previously anticipated. In contrast to *D. longispina,* which is only found in Europe, *D. umbra* occurs in polar regions of North America and Europe. This pattern is very similar to other *Daphnia* taxa, such as species of the *D. pulex* complex (e.g., *D. tenebrosa*), which occur mainly across arctic and subarctic regions (Weider et al. 1999*a*,*b*). Another similarity with the *D. pulex* complex is related to migration patterns: detailed studies of species of the *D. pulex* complex indicate that migration between Eurasia and Northern America is limited and that migration from Eurasia to Northern America seems more constrained than in the opposite direction (Weider et al. 1999*a*,*b*). This pattern among species of the *Daphnia longispina* sensu stricto complex might be the result of multiple colonization events (in northern Europe) from glacial refugia, such as Beringia.

Although sequence divergence within species was low (*D. longispina* = 0.005 and *D. umbra* = 0.01), haplotypes from similar geographical areas showed a lower genetic divergence than haplotypes from distant locations. For example, *D. longispina* from northern Europe clustered in one group separated from the haplotype from Poland. Similarly, Canadian haplotypes and northern European *D. umbra* formed two distinct groups (Fig. 2). These data suggest a phylogeographic structure within species and limited dispersal among and within continents.

Since *D. umbra* has not been formally described (Hebert 1995), little is known about the distributional range and ecological preferences (Hebert 1995; Taylor et al. 1996; Schwenk et al. 2000; Rautio and Korhola 2002*b*) of this species. However, previous studies (Wolf and Hobæk 1986; Hobæk and Wolf 1991) have suggested that two forms of *D. longispina* cooccurred in neighboring sympatry, one transparent and one pigmented morphotype. Taxa differed genetically (allozyme electrophoresis) and with regard to ecological preferences. Melanic forms occurred between 900- and 1600-m elevation, in ponds and large lakes up to 24-m average depth, whereas transparent forms occurred only below 1250-m elevation, in small water bodies of a maximum depth of 1 m. Only one syntopic population was detected. Our study of subarctic *Daphnia* (*Hyalodaphnia*) revealed a similar pattern: *D. longispina* was found in small water bodies below the treeline (with the exception of one site, Laassavaara, that was slightly above the treeline), and *D. umbra* was found in larger lakes above the treeline. However, all populations were similarly pigmented with melanin (total melanin content per individual), suggesting that the level of pigmentation cannot be used as a discriminatory character. On the other hand, more detailed taxonomic studies of the pigmentation of certain body parts, such as the headshield, are required to unravel a potential taxonomic differentiation.

In Finland *D. umbra* and *D. longispina* occur in neighboring sympatry and were never found in the same water body (Table 1, Fig. 1). Although both species occur in the same geographical area, they show a clear differentiation in their habitat preferences (Fig. 4). Lakes in which only *D. longispina* occur are small (0.0004–0.003 km^2), shallow (0.6–2-m average depth), 485–720 m (median = 515 m)

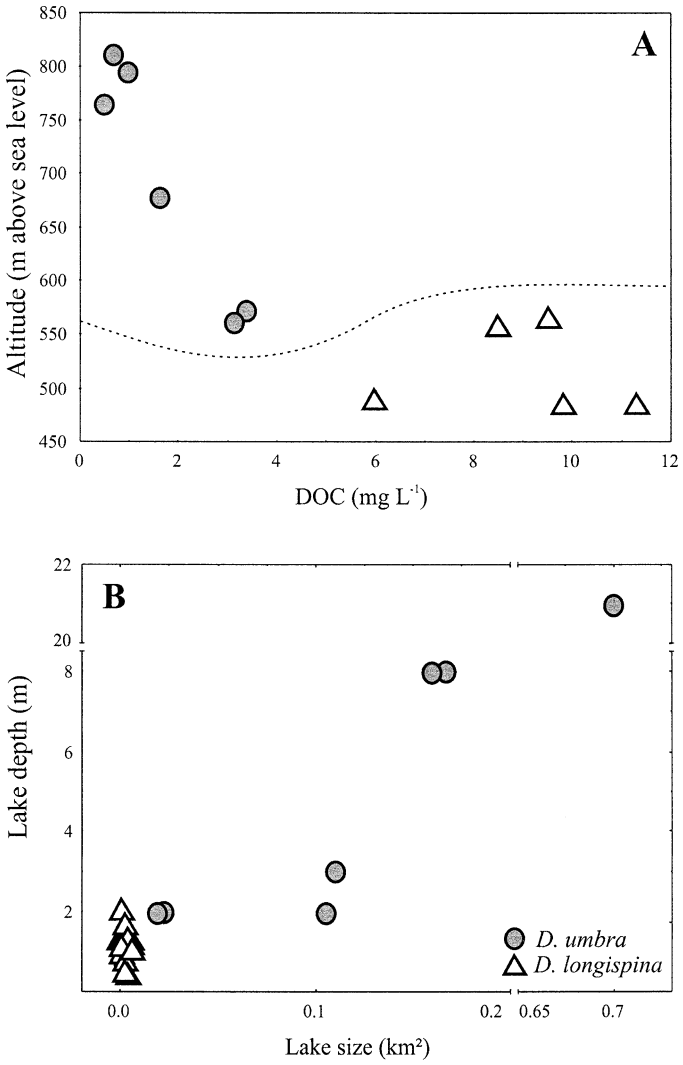

Fig. 4. Ecological preferences of *D. umbra* and *D. longispina* across 18 Finnish populations. (A) Scatter plot of dissolved organic carbon versus altitude. Dotted line represents the treeline; populations above the line are found in areas lacking trees. (B) Scatter plot of lake size versus average lake depth. *D. longispina* and *D. umbra* differed significantly (ANOVA, $P < 0.05$) in all parameters.

above sea level, and fishless, whereas lakes in which *D. umbra* occur are relatively large (0.02–0.7 km^2), deeper (2–21 m), 560–810 m (median = 479) above sea level, and often harbor fish. The shallow depth of *D. longispina* sites makes them susceptible to drying, which was detected for many of the ponds at the end of the season. They also freeze to the bottom during winter, which greatly affects the population structure (sexual reproduction and generation of resting eggs) and may control species composition. Different concentrations of DOC between *D. umbra* (0.5–3.4 mg L^{-1}) and *D. longispina* (6–11.3 mg L^{-1}) sites indicate differences in the food availability between the sites. Humic substances such as DOC are known to serve as a food source for *Daphnia* (Salonen and Hammar 1986); therefore, *D. longispina* and *D. umbra* might exhibit different food preferences related to DOC.

In summary, distributional, ecological, and phylogenetic patterns imply that speciation of *D. longispina* and *D. umbra* has been initiated by ecological differentiation, or species have maintained reproductive isolation by ecological differentiation. Both species occur in neighboring sympatry, in sharp ecological differentiation, and with no evidence of interspecific hybridization. Although we cannot completely rule out other mechanisms of speciation, such as drift-based differentiation (e.g., Boileau and Taylor 1994), a number of observations support mechanisms of ecological or neighboring sympatric speciation (Grant 1977; Schluter 2001). Differentiation of lineages in allopatry would require a significant period of time for spatial isolation and subsequent secondary contact with no exchange of genetic material. One allopatric speciation scenario could be that *D. umbra* originally occurred only in North America and *D. longispina* in Europe and *D. umbra* recently invaded the European subarctic, similar to the arctic *D. pulex* complex (Weider et al. 1999*a*,*b*). A recent invasion, however, would result in low differentiation among North American and European *D. umbra* populations. In contrast, we found that Canadian haplotypes and northern European *D. umbra* formed two distinct groups (Fig. 2). A recent invasion would most likely result in the cooccurrence of sister taxa (syntopy) and usually the indigenous species is subsequently threatened with extinction (Rahel 2002). In addition, invasive species show on average a lower genetic diversity (due to founder effects) than indigenous species (Lee 2002). However, *D. umbra* and *D. longispina* do not occur in the same habitat (no interspecific competition), and both species show similar levels of intraspecific differentiation in Europe. Genetic differentiation among *D. longispina* haplotypes was 0.005 ($\pm$0.003) and among *D. umbra* haplotypes was 0.004 ($\pm$0.002); these values were not significantly different ($P = 0.47$). The observed genetic patterns in combination with the strong ecological differentiation between lineages are in conflict with predictions from drift-based speciation models (Lynch 1985).

On the other hand, a number of indications suggest that ecological differentiation caused reproductive isolation or at least maintains reproductive isolation. *D. longispina* and *D. umbra* are ecologically differentiated, indicating that no transition zone or intermediate habitat in which coexistence would be feasible is available. This pattern might also explain the lack of interspecific hybrids since (1) spatial separation of parental species limits interspecific crosses and (2) intermediate traits of hybrids are supposed to be inferior in parental habitats (Arnold and Hodges 1995). Given the sharp distinction of ecological preferences of parental species, it seems unlikely that intermediate phenotypes could successively compete with parental taxa. Several other *Daphnia* sister species, such as *D. retrocurva–D. parvula*, *D. pulex–D. pulicaria*, and *D. catawba–D. minnehaha*, are found in neighboring sympatry, mainly in a pond–lake separation (Taylor et al. 1996). This pattern suggests that habitat shifts in concert with either founder effects or divergent selection have caused differentiation in sympatry (Grant 1977; Lynch 1985). Since *Daphnia* species generate copious amounts of resting eggs, which buffer the effects of population bottlenecks and promote local adaptation and population differentiation (De Meester et al. 2002), genetic drift and founder

effect speciation seem less important in *Daphnia.* Divergent selection, on the other hand, due to environmental variation, might cause the primary divergence of lineages that occupy neighboringly sympatric zones. Disruptive selection in neighboring zones (e.g., spatially isolated water bodies) that differ in their physical properties (e.g., ephemeral ponds due to drying/freezing versus lakes), in DOC levels or in levels of predation, might have caused the reproductive isolation of *D. umbra* and *D. longispina.* Further work on this species complex is warranted.

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