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Glacial refugia, haplotype distributions, and clonal richness of the *Daphnia pulex* complex in arctic Canada

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Abstract

As part of a large international Arctic biodiversity expedition (Tundra Northwest '99), we examined the distribution of members of the arctic *Daphnia pulex* complex (Cladocera, Anomopoda) from 121 tundra ponds, spread across 16 sites spanning a large portion of arctic Canada (i.e. from 62°22′ N to 79°01′ N; 66°45′ W to 139°37′ W). Using allozyme electrophoresis and mitochondrial (mt)DNA analyses, we examined the population genetic (clonal) structure of these populations. The following taxa were detected in this complex: *Daphnia pulicaria*, *D. middendorffiana* and *D. tenebrosa*. Clear geographical differences in mean clonal richness and diversity were observed, with most western sites exhibiting higher clonal richness and diversity, than sites in the eastern Canadian Arctic. For both the pulicaria group (i.e. *D. pulicaria* and *D. middendorffiana*) and *D. tenebrosa*, the highest mean regional clonal richness was detected from the southern section of Banks Island, an unglaciated site situated on the edge or directly in the eastern fringe of the Beringian glacial refuge. A significant negative correlation was found between geographical distance from the Beringian edge, and overall regional clonal richness (i.e. sites closer to the edge harboured greater clonal richness). These results clearly indicate that more recently deglaciated regions (i.e. eastern Canadian Arctic) harbour lower levels of clonal richness than western regions nearer Beringia. We discuss the role that glacial refugia have played in influencing both biotic and genetic diversity in arctic taxa.

Keywords: arctic phylogeography, clonal distributions, glacial refugia, species complex

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Introduction

A recent series of studies (Colbourne *et al*. 1998; Hobæk & Weider 1999; Weider *et al*. 1999a,b) has examined the phylogeography of the arctic *Daphnia pulex* species complex, circumarctically. As noted by Colbourne *et al*. (1998), the slow rate of morphological evolution in the Daphniidae, when compared with underlying molecular markers, has caused considerable taxonomic confusion in this complex leading to the presence of morphologically similar taxa that show high levels of sequence divergence [e.g. 17% sequence divergence for the mitochondrial (mt)DNA *ND5* gene between '*D. pulex*' populations from Europe and North America]. These authors have found that two major groups exist within arctic members of this complex. The first group, termed the tenebrosa group, consists of two clades; European *D. pulicaria* (EuroPC), which is restricted to sites west of the Ural Mountains (Weider *et al*. 1999a,b) and *D. tenebrosa*, which exhibits a much broader distribution with many lineages scattered across the northern fringe of Eurasia across the Bering Sea into Alaska and northwestern Canada. The second group, termed the pulicaria group, is made up of three distinct North American lineages of *D. pulicaria* (i.e. eastern — EPC; western — WPC; polar — PPC), Panarctic *D. pulex* (PanPX), and the hybrid taxon, *D. middendorffiana* (MIDD). These two major groups overlap in northern Fennoscandia into western Russia, as well as in western arctic Canada (Weider *et al*. 1999a,b). Although large expanses of the Arctic have been surveyed for genetic and taxonomic variation in this complex, there are still large gaps in our knowledge related to the distribution of genetic and biotic variation in this complex across a good portion of the Nearctic.

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As part of a large Swedish–Canadian Arctic biodiversity expedition (Tundra North-west '99, abbreviated to TNW99; Grönlund 1999), we were able to sample tundra pond habitats across very broad latitudinal and longitudinal ranges in the Canadian Arctic, to better characterize population genetic (clonal) variation in this complex, and determine whether there is any clear association between present-day levels of genetic variation and distribution of haplotypes/genotypes in relation to putative Pleistocene glacial refugia. Here we provide the first results from the genetic survey from this expedition. We compare and contrast our results with previous work on this complex, and place our findings into the much broader context of arctic phylogeography.

Specifically, we ask the following questions:

1 If Beringia served as a major source of extant clones in the Canadian Arctic (as suggested by previous studies, Weider *et al*. 1999a,b), do we see a significant decline in genetic (clonal) diversity with distance from Beringia?
2 Do we see any differential distribution of haplotypes across the Canadian Arctic in relation to Beringia and/or other (putative) glacial refugia?

Materials and methods

In total 173 tundra ponds from 19 sites across the breadth of arctic Canada (Fig. 1) were sampled. Three sites with a total of 38 ponds (Bathurst Island North, 10 ponds; Ellef Ringnes Island, 25 ponds; Cape Hooper, Baffin Island, 3 ponds; Fig. 1) yielded no *Daphnia*. Of the remaining 135 ponds from the other 16 sites, 121 contained *Daphnia*. Animals were sampled by either wading into the ponds with hand-held plankton nets (100–153 µm mesh) or sampling from shore using plankton throw nets. Animals were returned to the laboratory, where they were sorted, frozen immediately in cryovials, and held at –80 °C, until they could be analysed genetically. No attempt was made to quantify population densities.

Cellulose acetate electrophoresis (Hebert & Beaton 1993) was conducted, as described previously (Weider *et al*. 1999b) using the following eight enzyme systems: aldehyde oxidase (AO, EC 1.2.3.1), arginine phosphokinase (APK, EC 2.7.3.3), fumarase (FUM, EC 4.2.1.2), glutamate-oxaloacetate transaminase (GOT, EC 2.6.1.1., also known as AAT), lactate dehydrogenase (LDH, EC 1.1.1.27), mannose phosphate isomerase (MPI, EC 5.3.1.8), phosphoglucose isomerase (PGI, EC 5.3.1.9, also known as GPI) and phosphoglucomutase (PGM, EC 5.4.2.1).

Previous studies (Weider & Hebert 1987; Weider *et al*. 1987; Beaton & Hebert 1988) have indicated the presence of unbalanced heterozygous electromorph patterns (i.e. unequal staining intensity differences among alleles) at certain loci (e.g. *MPI* and *PGM*), which indicate the presence of polyploid clones in this arctic complex (Beaton & Hebert 1988). We also detected unbalanced heterozygous electromorph patterns at several loci (i.e. *PGM*, *MPI* and *APK*), indicating the presence of polyploid clones across the breadth of our survey.

mtDNA analyses of samples followed the methods of Weider *et al*. (1999b), with the following exception. We used previously designed primers (Colbourne *et al*. 1998), labelled DpuND5a (5′-ATAAAACTCCAATCAACCTTG-3′) and DpuND5b (5′-GGGGTGTATCTATTAATTCG-3′)

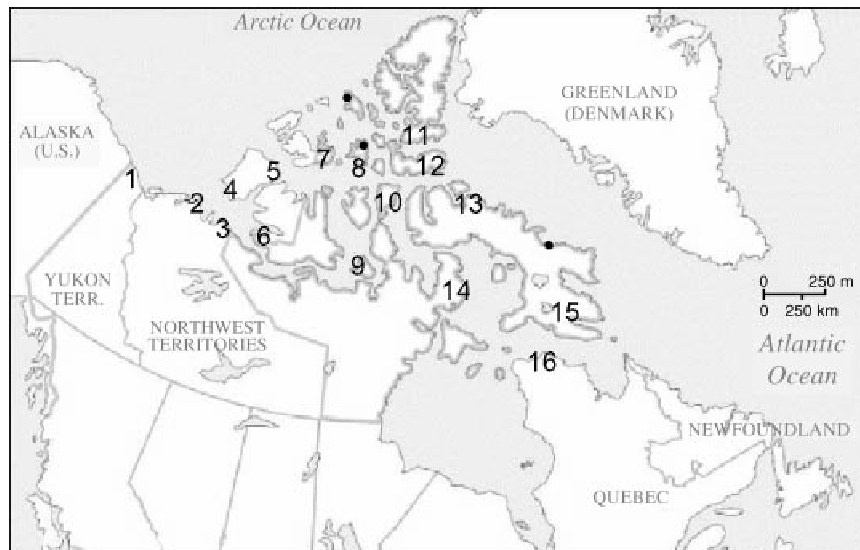

Fig. 1 Map showing the location of the sampling regions during the Tundra Northwest (TNW) '99 expedition. See Table 1 for region designations. Three regions represented by black dots were sampled, but no *Daphnia* were detected.

with the following polymerase chain reaction (PCR) thermal cycling parameters: one cycle of initial denaturation at 94 °C for 1 min, followed by 35 cycles of 94 °C for 1 min, annealing at 50 °C for 1 min, and an extension of 1 min at 72 °C. These ≈ 950 bp PCR products were screened for restriction site variation using a suite of endonucleases, which included *Dpn*II, *Hae*III, *Hha*I, *Hpa*II, *Sau*96I and *Taq*I (New England BioLabs). By screening control isolates representing the major clades in the complex (Weider *et al*. 1999b), we determined that diagnostic restriction sites/fragments at *Dpn*II and *Hae*III allowed us to categorize TNW99 animals into the different clades.

Digested PCR products were subjected to agarose gel electrophoresis (2.5% 3:1 Amresco agarose, run at 3–4 cm/h), stained with ethidium bromide (4 μg/ml) and visualized using a Kodak Electrophoresis Documentation and Analysis System (EDAS) 290 digital camera and KODAK 1D Image Analysis Software. The DNA fragment profiles were measured along side molecular mass standards, and composite haplotypes were identified. (Note: our ability to detect fragment sizes smaller than 100 bp was limited. Therefore, values for total length of PCR products may not add up to 950 bp.)

Statistical analyses

Allozyme data were analysed using the TFPGA program (Miller 1997). Intrapopulational clonal richness and clonal diversity estimates based on the allozyme data, as well as regional clonal richness estimates, were calculated following the methods of Weider *et al*. (1999b). More specifically, we used Chao's S* nonparametric method (Chao 1987; Chao & Lee 1992) to calculate clonal richness estimates from each region. [Note: we consider the sampling effort and geographic coverage applied at each region to be comparable, so no compensation was applied to Chao's S* estimates, in contrast to the earlier circumarctic analyses of Weider *et al*. (1999b)]. As mentioned by Weider *et al*. (1999b), we calculated a more conservative estimate of Chao's S* statistic, which has been used previously for species richness estimates (Colwell & Coddington 1994). We calculated 'singletons' (i.e. clones found only in single populations) and 'multipletons' (i.e. clones found in more than one population) rather than 'doubletons' (i.e. clones found in no more than two populations), and calculations were made separately for the pulicaria and tenebrosa groups in each region. 'Mixed' populations (i.e. those suspected of harbouring members of both groups) were omitted from the analysis. This excluded five populations from region 4 (Fig. 1, Table 1) and single populations each from regions 1, 2 and 15 (Fig. 1, Table 1).

Results

Allozymic variation

Six alleles at the *Pgm* locus, four alleles each at the *Ao*, *Ldh*, *Mpi* and *Pgi* loci, three alleles each at the *Fum* and *Got* loci, and two alleles at the *Apk* locus were detected across the entire sampling area (Tables 3 and 4). The relative distribution of alleles between regions was not homogeneous

Table 1 Summary of intrapopulational clonal richness and clonal diversity estimates, along with Chao's nonparametric estimator of regional clonal richness for members of the pulicaria group. *N* = number of animals (number of populations)

Region no.	Region name	Latitude	Longitude	*N* (popn)	Mean (± 1 SE) clonal richness per popn	Mean (± 1 SE) clonal diversity per popn	Chao's nonparametric estimator of regional clonal richness (S*)	Var S*/S *(%)
1	Ivvavik Natl Park	69°26′ N	139°36′ W	60 (2)	1.00 (0.00)	1.00 (0.00)	6.0	—
2	Cape Bathurst	70°29′ N	127°50′ W	150 (5)	3.80 (1.85)	1.86 (0.81)	127.0	27.6
3	Amundsen Gulf	69°46′ N	122°05′ W	58 (2)	5.00 (5.70)	2.68 (2.38)	42.0	17.8
4	Banks Isle S.	71°43′ N	123°43′ W	158 (5)	4.20 (1.43)	1.86 (0.82)	462.0	—
5	Banks Isle N.	73°37′ N	115°50′ W	247 (10)	2.40 (0.50)	1.60 (0.18)	58.1	5.8
6	Wollaston Peninsula	69°23′ N	114°47′ W	256 (9)	5.11 (1.10)	2.44 (0.57)	218.5	5.1
8	Bathurst Isle S.	75°04′ N	98°34′ W	107 (6)	2.00 (0.71)	1.55 (0.38)	62.0	4.6
9	King William Isle	69°06′ N	98°56′ W	328 (13)	8.08 (1.55)	4.55 (1.11)	329.7	1.6
10	Somerset Isle	72°54′ N	93°30′ W	403 (13)	1.84 (0.40)	1.42 (0.19)	32.1	12.1
11	Ellesmere Isle	76°28′ N	86°55′ W	210 (7)	1.00 (0.00)	1.00 (0.00)	2.0	9.1
12	Devon Isle	74°33′ N	82°48′ W	323 (11)	1.55 (0.16)	1.38 (0.14)	5.2	0.1
13	Pond Inlet	72°41′ N	77°59′ W	89 (3)	3.33 (1.08)	2.32 (0.91)	22.8	29.5
14	Melville Peninsula	67°33′ N	81°41′ W	134 (5)	1.40 (0.45)	1.08 (0.09)	8.0	42.1
15	Iqaluit	63°44′ N	68°33′ W	69 (2)	1.50 (0.71)	1.55 (0.28)	3.2	0.9
16	Ungava	62°22′ N	73°42′ W	70 (2)	1.50 (0.70)	1.36 (0.51)	12.0	—

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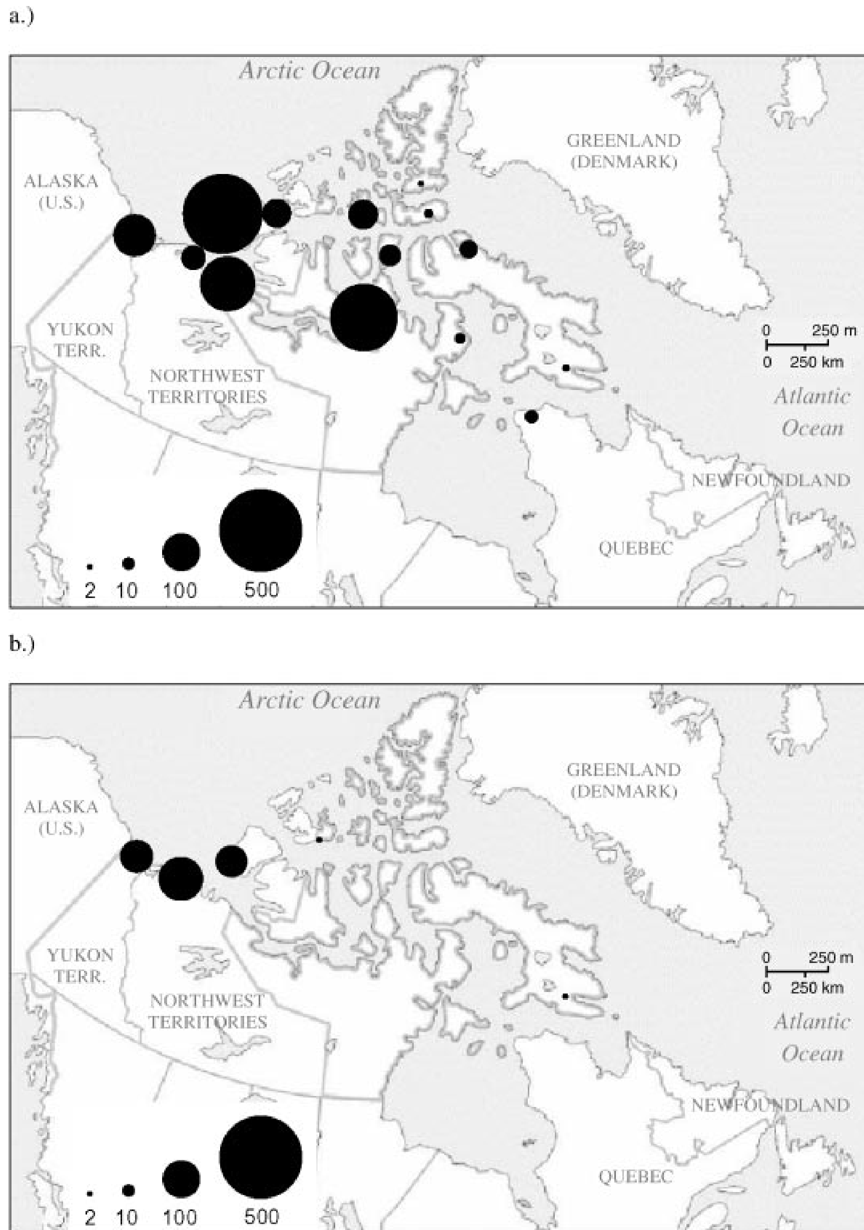

Fig. 2 Map depicting values of regional clonal richness (Chao's S* statistic). The size of the pie is proportional to clonal richness; (a) pulicaria group, (b) tenebrosa group.

(Tables 3 and 4). For example, for the tenebrosa group (Table 4) at the *Got* locus, allele 1 was detected only at regions 1 and 2 on the mainland (Fig. 1), and not on the islands (i.e. regions 4 and 7, Fig. 1). Likewise for the pulicaria group (Table 3) at *Mpi*, alleles 1 and 5 were restricted to regions 1 and regions 2 and 5, respectively (Table 3).

Estimates of regional clonal richness (Chao's S*) for the pulicaria group also showed a nonrandom distribution (Fig. 2a), with regions located west of the 98 °W parallel (sites 1–9, Fig. 1, Table 1) harbouring significantly greater levels of clonal richness (Mann–Whitney *U*-test, $U_{8,7} = 52$, $P < 0.005$) than regions east (i.e. sites 10–16, Fig. 1, Table 1) of this longitude. Owing to the lack of tenebrosa group clones east of the 98 °W parallel (Fig. 2b), a comparable analysis could not be conducted.

When an analysis is carried out of the relationship between interregional genetic distance (based on the entire allozyme data set, using Nei 1978 unbiased estimator) and geographic distance (km), for each group, separately, one can see that there is some clustering of neighboring regions (Fig. 3a, e.g. regions 2 and 4 and regions 12 and 13; Fig. 3b, regions 1 and 3), but no overall isolation-by-distance was

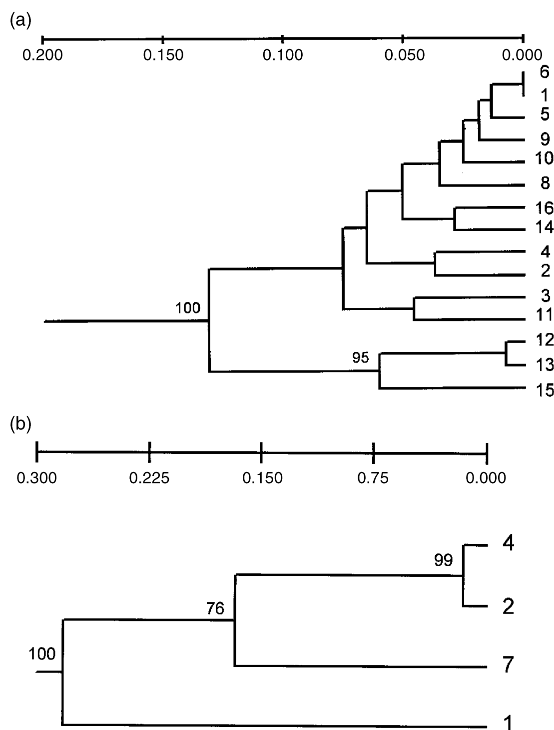

Fig. 3 UPGMA phenogram depicting the relationship of interregional genetic distance (Nei 1978 unbiased estimator) based on all eight allozyme loci, and site locations (see Table 1 and Fig. 1 for site information and location). Bootstrap estimates (based on 1000 random permutations) that were > 50% are given; (a) pulicaria group, (b) tenebrosa group.

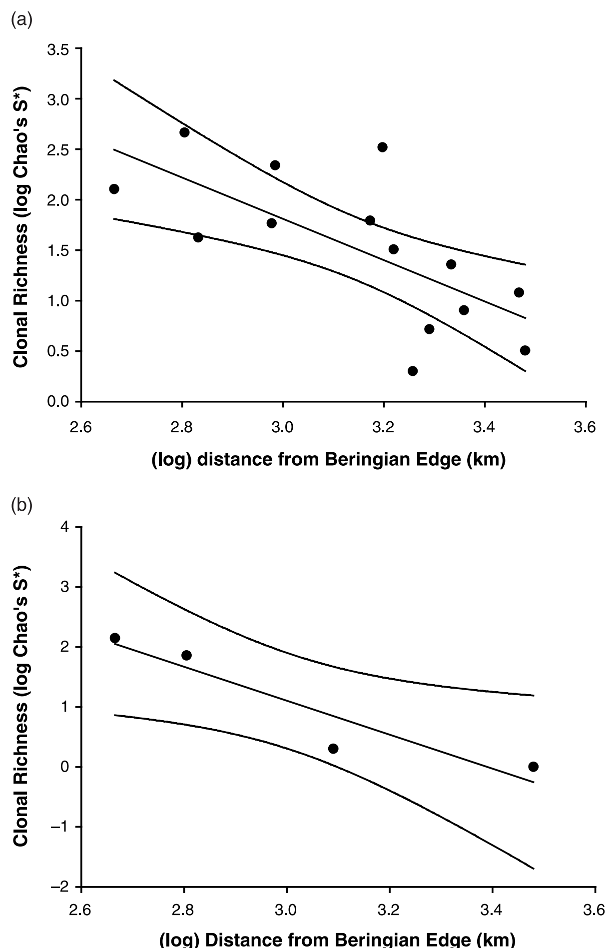

Fig. 4 Regression analysis of mean regional clonal richness (log Chao's S*) and geographic distance (log km) from the Beringian edge (defined as distance from region 1, Table 1); (a) pulicaria group, (b) tenebrosa group.

detected across the entire sampling area (i.e. pulicaria group, Mantel Z statistic = 9.88×10^3; $r = 0.061$, 1000 random permutations, $P = 0.34$; tenebrosa group, Mantel Z statistics = 8.22×10^2; $r = 0.705$, 1000 random permutations, $P = 0.16$; TFPGA program, Miller 1997).

For the pulicaria group, however, a significant negative relationship between similarity of regional clonal arrays (i.e. based on the eight allozyme loci, using Jaccard's coefficient, Pielou 1984) and geographic distance (km) was found to exist for the entire allozyme (clone) data set across the whole sampling area (Mantel $Z = 777.87$, $r = -0.200$, 1000 random permutations, $P < 0.019$; TFPGA program, Miller 1997). These results indicate that for the pulicaria group, clonal arrays were more similar in composition between regions that were in closer proximity than those arrays found at greater distances from each other. This suggests some differential movement/dispersion of clones, as noted previously by Weider & Hobæk (1997) in the arctic *Daphnia pulex* complex from Siberia/Russia. Owing to the small number of regions where tenebrosa group clones were detected and the complete absence of any shared clones between regions, a similar analysis could not be conducted.

If one compares the relationship between mean regional clonal richness (i.e. log Chao's S*) and (log) distance (km) from the putative edge of the Beringian glacial refuge (defined here as the distance from region 1, Fig. 1), a significant negative correlation ($r = -0.696$, df = 13, $P < 0.01$) is found for the pulicaria group (i.e. clonal richness is higher the closer one is to the Beringian edge, Fig. 4a). Likewise, a significant negative correlation ($r = -0.939$, df = 3, $P < 0.02$) was found for the tenebrosa group. No significant correlations were found for either group when comparing mean regional clonal richness (log Chao's S*) with latitude.

Average regional heterozygosities ranged from 0.095 (region 5, Fig. 1) to 0.511 (region 3, Fig. 1) for the pulicaria group (Table 3) and from 0.250 (region 7, Fig. 1) to 0.316 (region 1, Fig. 1) for the tenebrosa group (Table 4). Exact tests for deviations from Hardy–Weinberg equilibrium (HWE) were performed on 25 populations, specifically from regions exhibiting high clonal richness (e.g. regions 1, 2, 4, 6 and 9). Two of these 25 populations (i.e. from region

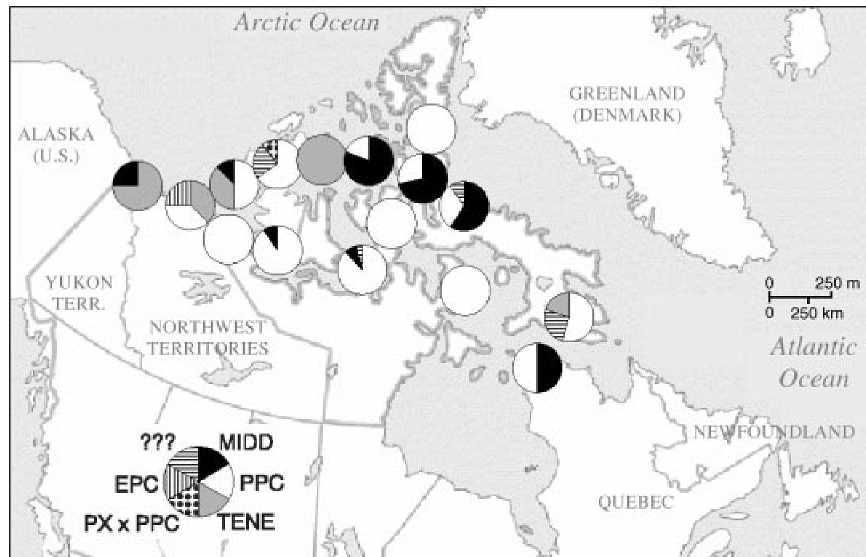

Fig. 5 Pie diagram showing the relative distribution of different arctic *Daphnia pulex* complex mtDNA clades across the TNW'99 sampling area, based on the mitochondrial (mt)DNA DpuND5a and DpuND5b primers. The size of each pie fraction is proportional to the number of haplotypes from each clade. EPC, eastern *D. pulicaria*; MIDD, *D. middendorffiana*; PPC, polar *D. pulicaria*; PX × PPC, potential hybrids between panarctic *D. pulex* (PanPX) and PPC; TENE, *D. tenebrosa*; ??, unclear to clade designation.

4, Fig. 1) were found to be 'mixed' clonal assemblages of pulicaria and tenebrosa group clones, and were omitted from further analysis. Two of the remaining 23 populations were tenebrosa group populations from region 1 (Fig. 1). Of the possible comparisons of individual loci (i.e. total of 12 comparisons, missing data at *Mpi* and *Apk* for one population and *Ldh* for both populations), 5 of the 12 showed significant deviations from HWE expectations, with 4 being heterozygote excesses and 1 being a heterozygote deficiency. For the remaining 21 pulicaria group populations, there were significant deviations from HWE expections in the majority of cases (i.e. 65 of 125 comparisons), with heterozygote excesses out-numbering heterozygote deficiencies nearly 4:1. However, there were a couple of populations (i.e. one each from regions 6 and 9; Fig. 1), which exhibited high intrapopulational clonal richness, and which did not exhibit any significant deviations from HWE expected genotype frequencies. Although no males were detected in these populations, these data suggest that some sporadic sexual reproduction may be occurring in certain regions (i.e. in or near Beringia, regions 1, 2, and 4), as suggested by previous arctic studies of this species complex (Weider & Hobæk 1997; Weider *et al*. 1999b), as well as sporadic sexual populations in the central Canadian Low Arctic (regions 6 and 9, Fig. 1). However, the genetic (allozyme) data for the vast majority of populations surveyed in this study indicate that the primary mode of reproduction is via obligate parthenogenesis across much of the Canadian Arctic, as shown previously (Weider *et al*. 1987, 1999b).

mtDNA variation

Data using the DpuNDa and DpuNDb primers were obtained from 66 of the 121 populations (total sample size = 269 animals screened) collected from the 16 regions found to contain *Daphnia* (Fig. 5). The distributional patterns of the different clades indicate that western regions (e.g. regions 1, 2 and 4, Fig. 5) harbour a large number of haplotypes belonging to the *D. tenebrosa* (TENE) clade, which is known to dominate Eurasia, particularly Siberia and Beringia (Weider *et al*. 1999a). With the lone exception of region 15 (Iqaluit, Fig. 1, where one *D. tenebrosa* haplotype was detected in a 'mixed' population with pulicaria group clones), no TENE haplotypes were detected in the eastern half of our sampling area. Instead, this area was dominated by haplotypes belonging to the *D. pulicaria* (PPC) and closely allied hybrid taxon, *D. middendorffiana* (MIDD) (Weider *et al*. 1999a,b). In fact, the MIDD distribution appears to be concentrated in the high east-central Canadian Arctic, specifically in regions 8 (Bathurst Island S), 12 (Devon Island) and 13 (Pond Inlet, Fig. 5).

In addition, we detected what appears to be a number of, as yet, undefined lineages (??, Fig. 5), and found evidence for potential mtDNA introgression between Panarctic *D. pulex* (PX) and PPC in region 5 (Banks Island, N). Likewise, we found evidence of a single haplotype of eastern *D. pulicaria* (EPC) in region 2 (Cape Bathurst, Fig. 1). EPC is found primarily in the eastern half of arctic/subarctic and temperate Canada, as well as on Greenland, Iceland and

Table 2 Summary table showing intrapopulational clonal richness and clonal diversity estimates, along with Chao's nonparametric estimator of regional clonal richness for members of the tenebrosa group. *N* = number of animals (number of populations)

Region no.	Region name	Latitude	Longitude	*N* (popn)	Mean (± 1 SE) clonal richness per popn	Mean (± 1 SE) clonal diversity per popn	Chao's nonparametric estimator of regional clonal richness (S*)	Var S*/S *(%)
1	Ivvavik Natl Park	69°26′ N	139°36′ W	62 (3)	8.33 (2.86)	5.18 (1.01)	76.8	20.8
2	Cape Bathurst	70°29′ N	127°50′ W	149 (5)	4.00 (0.79)	2.30 (0.63)	140.3	13.2
4	Banks Isle S.	71°43′ N	123°43′ W	50 (2)	4.00 (2.83)	2.71 (2.32)	72.0	—
7	Melville Isle	75°06′ N	107°40′ W	211 (7)	1.00 (0.00)	1.00 (0.00)	2.0	9.1

Table 3 Relative distribution of alleles detected in each of the regions sampled during the TNW'99 expedition for the pulicaria group. Alleles in bold were detected at a frequency of > 10%. ND, no data. Region numbers follow those given in Table 1. Average heterozygosity values (across all loci) and average number of alleles per locus are given

Region	*Pgm*	*Pgi*	*Ao*	*Got*	*Ldh*	*Mpi*	*Fum*	*Apk*	Av. no. alleles/locus	Av. heterozygosity
1	**2,3**	**4,5**	**1,2**	**2**	**1,3**	**1,3**	**3**	**3,4**	1.8	0.312
2	0,1,**2,3,4**	1,**4,5**	**0**,1,**2**	**2**	**1,3**	**3,4**,5	**3,4**	**3,4**	2.6	0.291
3	0,**2,4**	**1,4**,5	**1,2**	**2,3**	**1,3**	**3,4**	ND	ND	2.3	0.511
4	**1,2,3**	1,**4,5**	0,**1,2**	**2,3**	**1,3**	**3,4**,5	1,**3**,4	3,**4**	2.6	0.364
5	**2,3,4**	**4**	**1,2**	**2,3**	**1,3**	**3,4**	**1,3**	3,**4**	2.0	0.095
6	0,**1,2,3**,4	0,**1,4**,5	**1,2**	**2,3**	**1,3**	**3,4**	**1,3**	**3,4**	2.6	0.406
8	**1,2**,3	**1,4,5**	**1**	**2**	**1,3**	**3,4**	**1,3**	**3,4**	2.0	0.314
9	0,1,**2,3**,4	0,**1,4**,5	0,**1,2**,3	**2,3**	0,**1,3**	**3,4**	**1,3**	**3,4**	3.0	0.454
10	0,1,**2**,3	1,**4**	**1,2**	**2,3**	**1,3**	**3**,4	**1,3**	**4**	2.1	0.333
11	**0**,1,**2**	**1,4**	**2**	**2,3**	**1,3**	**3,4**	**1,3**	**4**	1.9	0.408
12	0,1,**2,3**	**1,4**	**3**	**2,3**	**0,1,3**	**3,4**	**1,3**	**4**	2.6	0.286
13	**0,1,2**,2½,**3**	**1,4,5**	1,**2,3**	**2,3**	**1,3**	**3**	**1,3**	**4**	2.3	0.309
14	**2**	**4**	**2**	**2**	**1,3**	**3,4**	**3**	**3,4**	1.4	0.200
15	**0**,1,**2**	**1,4**	**2,3**	**2,3**	**1,3**	**3**	**3**	**3,4**	1.9	0.458
16	**2,3**	**4,5**	**1,2**	**2**	**1,3,4**	**3**	**3**	**3,4**	1.8	0.358

Svalbard (Weider *et al*. 1999a), so its presence in western arctic Canada, extends its known range by several thousand kilometres.

Discussion

Clonal richness

Clonal richness (based on eight allozyme loci) as estimated by Chao's S* varied from 2 to ≈ 460 clones per region (Table 1) for the pulicaria group, and from 2 to ≈ 140 clones per region (Table 2) for the tenebrosa group. The highest values for the pulicaria group exceed our previous high estimates (i.e. 227 clones based on 6 allozyme loci) per site in eastern Siberia (Weider *et al*. 1999b) for the tenebrosa group. However, our previous lowest estimate at any site was 16 clones, similarly based on 6 loci. Hence, the gradients in clonal richness within this complex in arctic Canada are extremely steep. The general tendencies are for attenuation of clonal richness to the north and east (Fig. 2a,b), suggesting that clonal richness may be related to climatic factors (i.e. shorter growing seasons, colder mean annual temperatures, Bradley & England 1979), as well as long-standing effects of dispersal from glacial refugia. It should be noted that of the three sites from which we did not find *Daphnia*, two (i.e. Ellef Ringnes Island, northern Bathurst Island) are located to the north and east of the Beringian edge, whereas the third site (Cape Hooper) was located on far eastern Baffin Island (Fig. 1).

In addition, the allozyme data indicate that allelic arrays vary across the study area (Tables 3 and 4). For example, within the pulicaria group (Table 3), allele 3 at *Ao* was restricted to regions 9, 12, 13 and 15, whereas allele 0 was restricted to regions 2, 4 and 9 (Fig. 1). Likewise for this group, allele 5 at *Mpi* was restricted to regions 2 and 4 (Fig. 1). Within the tenebrosa group (Table 4), allelic arrays

Table 4 Relative distribution of alleles detected in each of the regions sampled during the TNW'99 expedition for the tenebrosa group. Alleles in bold were detected at a frequency of > 10%. Region numbers follow those given in Table 2. Average heterozygosity values (across all loci) and average number of alleles per locus are given

Region	*Pgm*	*Pgi*	*Ao*	*Got*	*Ldh*	*Mpi*	*Fum*	*Apk*	Av. no. alleles/locus	Av. heterozygosity
1	1,**2**,3	**1,4,5**	**0,1**,2	**1,2**	**1**	**1**,3	1,**3**,4	**3,4**	2.4	0.316
2	**0,1,2,3,4**	1,**4,5**	**0,1,2**	1,**2**,3	**1,3**	**1,3,4,5**	1,**3,4**	**3,4**	3.1	0.296
4	**1,2,3**,4	**4,5**	**0,1,2**	**2**	**1,3**	1,**3,4**	**3**,4	**4**	2.3	0.266
7	**2**	**4**	**2**	**2,3**	**1**	**3,4**	**3**	**4**	1.4	0.250

also differed between regions (e.g. allele 1 at *Fum* was restricted to the western regions 1 and 2, Fig. 1). Interestingly, region 9 (King William Island) in the middle of our study area (Fig. 1), harbours pulicaria group clones that possess alleles found in both western and eastern regions (Table 3). Further, the average number of alleles per locus (3.0) found in populations sampled at King William Island was the highest value of any region (Table 3). These data taken together suggest that the King William Island region represents a contact zone between pulicaria group clones/lineages from both western and eastern arctic Canada. Somewhat surprisingly, however, there was no evidence of the presence of TENE lineages at King William Island; instead, populations from this region are composed primarily of either PPC or MIDD haplotypes (Fig. 5).

Table 5 Diagnostic restriction fragment length polymorphisms (RFLPs) for the different clades in the arctic *Daphnia pulex* complex

Clade	*Dpn*II (bp)	*Hae*III (bp)
PPC	500, **150**	350, **200**
MIDD	500, **150**	320, **125**
EPC	500 or 500, 300	350, **130**
PanPX	630, **150**	270, **200**
TENE	470, **100**	320, 280, 250 or 320, 225
PanPX × PPC	630, **150**	350, **200**
??	290, 270, 210	400, 200, 190

Clade abbreviations/designations as follows: EPC, eastern *D. pulicaria*; MIDD, *D. middendorffiana*; PPC, polar *D. pulicaria*; PanPX, panarctic *D. pulex*; PanPX × PPC, potential hybrids between PanPX and PPC; TENE, *D. tenebrosa*; ??, unclear to clade designation. Fragment sizes smaller than ≈ 100 bp could not be scored accurately. Putative doublets are indicated in bold.

Haplotype distributions

The distribution patterns of mitochondrial haplotypes presented here is preliminary, being based on a few isolates from each site. Nonetheless, the mtDNA data (Fig. 5) indicate that members of the tenebrosa clade (TENE) are primarily restricted to western regions (e.g. regions 1, 2, 4, 7; Fig. 5), the lone exception being the aforementioned single *Daphnia tenebrosa* haplotype detected in one 'mixed' population from region 15 (Fig. 5). These data are consistent with earlier reports (Weider *et al*. 1999a,b) showing that TENE lineages were very rare in eastern arctic Canada, and suggest a connection between the TENE dominated lineages of northern Eurasia (i.e. Siberia) and those detected along the eastern fringe of Beringia (i.e. Alaska, northwestern arctic Canada; Weider *et al*. 1999a,b). Our data indicate that TENE lineages are indeed dominant in the vicinity of the Beringian refuge, as TENE haplotypes were detected in regions 1, 2 and 4 (Fig. 5). MIDD and PPC are also common in the west. In addition, we detected the presence of one EPC haplotype. Although TENE haplotypes are rare in the east, they do occur with moderate clonal richness along the western shore of Hudson Bay (Weider *et al*. 1999a,b). Assuming that these haplotypes have dispersed from the west (i.e. Beringia), it is surprising that none were detected at sites along the Northwest Passage (i.e. the area between regions 6 and 9, northwards to regions 12 and 13, Figs 1 and 5). If this pattern stands up to further analyses, it may suggest an inland dispersal corridor towards Hudson Bay during the meltdown of the Laurentide ice sheet (Dyke & Prest 1987).

One somewhat puzzling aspect of our study was the inability to clearly differentiate the group/clade affinity for a small number of haplotypes detected across the study area (specifically, one from region 9, and several each from regions 5, 13 and 15, Figs 1 and 5). The mtDNA restriction patterns were inconclusive in assigning clade affinities to these haplotypes. Specifically, we detected, what could very well be intergroup/interspecific hybrids between tenebrosa and pulicaria group haplotypes (Table 5). Furthermore, we detected the possible presence of within-group hybridization between PanPX and PPC (Table 5, Fig. 5), which appears to be localized in region 5 (Banks Isle N, Fig. 5). Evidence for such hybrids has come from previous investigations (Dufresne & Hebert 1994, 1997; Weider *et al*. 1999b). For example, Weider *et al*. (1999b) in their circumarctic survey of this complex found that ≈ 20% of pulicaria group genotypes possess nuclear genes from the tenebrosa group, whereas roughly 10% of tenebrosa group genotypes expressed pulicaria group

nuclear genes. Thus, intra- and intergroup hybrids are not uncommon in this complex.

Our results are concordant with earlier studies from the Canadian Arctic (Weider *et al*. 1987, 1999b), which showed the presence of large numbers of clones within the arctic *Daphnia pulex* complex. Furthermore, our results have added valuable data to the overall picture of arctic phylogeography in this species complex, particularly from the central Canadian Arctic (i.e. regions 3–9, Figs 1 and 2), which prior to this study, had not been surveyed.

Phylogeography of Holarctic taxa

Results from our study clearly indicate that there is a nonrandom distribution of clonal richness (Fig. 2a,b), with regions west of the 98° W meridian exhibiting significantly higher levels of richness, than regions east of this longitude, as well as a significant negative correlation between distance from the putative edge of the Beringian refuge, and mean regional clonal richness (Fig. 4a,b). Glaciological studies (Andrews 1970; Dyke & Prest 1987) have shown that the Laurentide ice sheet covered most sites east of this longitude up until 10 000 years before present (YBP). This indicates that all of these eastern sites are glacialogically, very young, although the evidence for nunataks (i.e. ice-free localized regions) in parts of eastern arctic Canada, remains contentious (Löken 1966; Steig *et al*. 1998).

When one examines the levels of regional clonal richness/diversity in the pulicaria group (Fig. 2a), it is clear that high levels of clonal diversity are present a fair distance (i.e. region 9, King Williams Isle) from the putative edge of the Beringian refuge; this is suggestive of a zone of secondary contact within the pulicaria group, where disparate clonal assemblages from western and eastern arctic Canada have come together in a contact zone. Previous molecular studies (Dufresne & Hebert 1994, 1997; Weider *et al*. 1999a,b) using a combination of mtDNA and nuclear (allozyme) markers have detected other contact zones in this species complex, which have centred on the area just to the east (i.e. Melville Peninsula/Foxe Basin). Interestingly, the King William Island and Melville Peninsula regions are located very close to an area known as the Keewatin Ice Divide (Andrews *et al*. 1986), a region where glacial ice flows are believed to have diverged in two opposing directions (i.e. southeastwards towards Hudson Bay and northwestwards toward Victoria Island), thus serving as a natural dividing line between eastern and western movements of fauna/flora.

Beringia and other glacial refugia

One main objective of this study was to compare/contrast levels of genetic (clonal) diversity between regions that have experienced distinct glacial histories, i.e. unglaciated vs. formerly glaciated regions. Based on glacialogical data (Dyke & Prest 1987), regions 1 and 4 (Fig. 1) clearly represent regions right on the eastern fringe of Beringia, and were unglaciated for good portions of the late Pleistocene (Vincent 1982, 1984). Region 2 (Cape Bathurst, Fig. 1) lies directly to the east of the Beringian edge, and, along with all sites further to the east, it was heavily glaciated during the late Pleistocene (Dyke & Prest 1987).

An increasing number of investigators has examined whether genetic/molecular markers can help unravel the role of Pleistocene glacial cycles on influencing the phylogeography of a variety of Holarctic taxa, possibly leading to speciation events, as well as having influenced present-day levels of genetic variation in Holarctic organisms (see Weider & Hobæk 2000 for review). For example. Abbott *et al*. (2000) examined the phylogeography and population genetic structure of the arctic plant, *Saxifraga oppositifolia*, across northern Alaska and the Canadian High Arctic. The authors found significant differences in the level of molecular genetic variation (i.e. chloroplast DNA variation) in this taxon with populations sampled in the western regions (i.e. Beringia) harbouring high levels of genetic variation, a result strikingly similar to our results. Examples of elevated levels of genetic variation in taxa found in the Beringian glacial refuge (Quinn 1992; Bernatchez & Dodson 1994; Bernatchez & Wilson 1998; Ehrich *et al*. 2000), including primarily clonal (asexual) organisms (Weider & Hobæk 1997; Weider *et al*. 1999a,b; Stenström *et al*. 2001), are not uncommon, when compared with populations outside the refugium, but such a relationship is not universal (Fedorov *et al*. 1999; Ehrich *et al*. 2000).

The studies of Weider & Hobæk (1997) and Weider *et al*. (1999a,b) have shown evidence for higher levels of genetic (clonal variation) in populations of the *Daphnia pulex* complex inhabiting the Beringian refuge. Furthermore, Stenström *et al*. (2001) sampled populations of the facultatively clonal arctic sedge complex, *Carex* spp., from many of the same northern Eurasian and Beringian sites that Weider & Hobæk (1997) sampled for *D. pulex*, and found greater genetic variation at unglaciated sites (i.e. eastern Siberia, Beringia) than glaciated sites in northwestern Russia and Fennoscandia. These concordant patterns lend further support to the notion that Beringia has played a major role as a glacial refuge for a wide variety of present-day arctic organisms.

Beringia was the largest and is believed to have been the most important Holarctic glacial refugium (see Sanmartin *et al*. 2001 for a recent review), but it appears to have not been the sole refugium to play a role in the postglacial colonization of the Nearctic. Evidence exists for the presence of multiple refugia in the Nearctic (Holder *et al*. 1999; Tremblay & Schoen 1999; Weider *et al*. 1999a,b; Abbott *et al*. 2000). For example, Tremblay & Schoen (1999) examined

chloroplast (cp)DNA in the arctic perennial plant, *Dryas integrifolia*. They found evidence for elevated levels of cpDNA diversity in areas in or near the putative Beringian refuge, but they also found evidence for the existence of at least four other refugia: high arctic (Canadian archipelago and Greenland), southwestern (Rockies), southeastern (New Jersey to Quebec) and eastern coastal (east coast of Canada and west coast of Greenland). Their study concluded that multiple refugia have played a role in the postglacial recolonization of arctic North America, and that the central Canadian High Arctic appears to be a zone of secondary contact between haplotypes from eastern and western refugia. These results are very similar to our results, where we detected a zone of secondary contact in the central Canadian High Arctic (i.e. King William Island, region 9, Fig. 1, Table 1).

Similarly, Abbott *et al*. (2000), in their circumarctic study of the arctic/alpine perennial purple saxifrage, *Saxifraga oppositifolia*, found evidence for elevated cpDNA diversity in Alaska, western Canada/US, and the Canadian Arctic, as well as northern Greenland and the Taymyr Peninsula of Siberia, suggesting the existence of multiple refugia beyond Beringia. These authors also detected a zone of secondary contact for the two main cpDNA lineages of *S. oppositifolia* in northern Greenland.

Still further, Holder *et al*. (1999) examined mitochondrial and nuclear DNA sequence variation in the rock ptarmigan, *Lagopus mutus*, from across the Nearctic. They determined that previously recognized subspecies were clearly differentiated into distinct refugial lineages, with divergence times between lineages, ranging from 135 000 to 11 000 YBP. Their results clearly indicate that multiple glacial refugia can play a significant role in influencing present-day distributions of arctic organisms, as well as impacting underlying genetic variation present in these taxa.

We have shown that a number of studies of arctic biota have demonstrated that multiple glacial refugia appear to have played a role in the evolution of various species complexes (Hewitt 1996, 2000). It is also clear that Beringia has served as the major glacial refugium during the Pleistocene. But what is not clear is whether any general predictions about the phylogeography of holarctic taxa can be made solely on the basis of inferring the presence and positioning of putative glacial refugia. Earlier work (Dufresne & Hebert 1997; Weider & Hobæk 1997; Weider *et al*. 1999a,b) in the arctic *D. pulex* complex has indicated that secondary contact among lineages that have evolved in separate refugia, is not uncommon; this holds for taxa other than *Daphnia* as well (Bernatchez & Dodson 1994; Bernatchez & Wilson 1998; Holder *et al*. 1999; Tremblay & Schoen 1999; Weider & Hobæk 2000). Our study has added to our knowledge of how genetic variation in Nearctic taxa has been influenced by Pleistocene glaciations. The next step should be a comparative approach, which would involve comparing and contrasting the phylogeographies of a wide range of taxa, collected from the same sites/locations. Such a comparative study would greatly increase our understanding of how Pleistocene glacial cycles have sculpted the genetic variation of Holarctic taxa that currently inhabit the arctic region.

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