Limnol. Oceanogr., 52(6), 2007, 2507–2518
© 2007, by the American Society of Limnology and Oceanography, Inc.

Life at low temperatures: A novel breeding-system adjustment in a polar cladoceran

Paul D. N. Hebert,[1] *Chad L. Rowe, and Sarah J. Adamowicz*
Biodiversity Institute of Ontario, Department of Integrative Biology, University of Guelph, Guelph, Ontario N1G 2W1, Canada

Abstract

The typical breeding system of cladocerans, cyclic parthenogenesis, is poorly suited to polar settings because it requires one or more rounds of parthenogenesis before the production of males and sexual eggs. Past work has shown that many arctic cladocerans have secondarily made the transition to obligate, apomictic parthenogenesis. Arctic populations of *Holopedium gibberum* lack males, suggesting their possible adoption of this breeding system. However, this study shows that these lineages instead possess genotypic characteristics expected under either self-fertilization or automictic parthenogenesis, the first record of such a breeding system in the Cladocera. As populations of *Holopedium* from southerly areas reproduce by cyclic parthenogenesis, this breeding system transition appears to represent an adaptive response to living at low temperatures.

The elevated incidence of asexual reproduction in populations that inhabit extreme environments—a pattern termed geographic parthenogenesis—has been widely recognized for both plant (e.g., Bierzychudek 1985) and animal groups (e.g., Glesener and Tilman 1978). The varied explanations for this pattern, which may often act synergistically, have included the demographic benefits of unisexuality in low-productivity environments, relaxation of biotic selection pressures for the maintenance of sex (Glesener and Tilman 1978), the advantages of polyploidy in cold conditions (Grime and Mowforth 1982; Stebbins 1984; Dufresne and Hebert 1998), and the benefits of genetic diversity linked to the role of hybridization in generating many asexuals (Kearney 2005). However, in some cases the breeding-system transition may have a simpler selective basis; it may be a necessary precondition for colonization of the extreme environment.

The typical breeding system of cladocerans, cyclic parthenogenesis, aids both the founding of populations from single individuals and their rapid numerical increase where conditions allow multiple generations of parthenogenesis. However, in the north, this breeding system can lead to a fatal delay in the production of diapausing eggs and to the exclusion of species because slowed developmental rates at low temperatures prevent completion of a life cycle (Hebert and Hann 1986). In fact, the paucity of cladocerans in the arctic has been linked to this penalty of cyclic parthenogenesis (Hebert and Hann 1986). Interestingly, past studies have revealed that breeding-system adjustments are common in the few cladocerans that have colonized polar habitats. For example, the sole *Bosmina* species, as well as several species in the genus *Daphnia,* have abandoned sexual reproduction in arctic settings (Little et al. 1997; Weider et al. 1999). Obligate parthenogenesis is indeed far more prevalent among arctic *Daphnia* than among their temperate counterparts. Of the 34 species of *Daphnia* documented for North America (Hebert 1995), 30 of which occur in temperate regions, only two exhibit obligate parthenogenesis in southern locales (Černý and Hebert 1993; Hebert et al. 1993; Hebert and Finston 2001). On the other hand, of the 10 species with distributions that include habitats north of the Arctic Circle (*see* Hebert 1995), at least four species are known to reproduce parthenogenetically there (*see* Weider et al. 1999). In the particularly harsh eastern North American arctic, at least 3 of the 5 species that occur there are obligates. In fact, among the Cladocera, just one definitively known cyclical parthenogen is known from the high arctic of North America—*Chydorus sphaericus*, a diminutive species with rapid development times (Boileau et al. 1992). Several species of confirmed cyclical parthenogens have been studied in northern Europe at around 60°N latitude (e.g., Ebert et al. 2002; Haag et al. 2002, 2006), but genetic diagnoses in other areas are necessary because climatic conditions in Europe are generally milder than at North American sites of similar latitude. The present study extends investigations to a new taxon, *Holopedium gibberum* Zaddach 1855, which is dominant in many arctic lakes.

H. gibberum s.l. occurs widely in the Northern Hemisphere, typically in lakes with low solute concentrations (Rowe 2000). It has been established that populations in the temperate regions of North America reproduce by cyclic parthenogenesis (Hebert and Finston 1997), but genetic diagnoses of breeding systems have not been carried out in other areas. However, ecological studies have revealed that populations in European lakes are reestablished each spring from resting eggs produced in the fall (Hrbáček pers. comm.). This observation implies that polar populations of *Holopedium* confront a serious challenge—they must complete their life cycle each year despite slowed

[1] Corresponding author (phebert@uoguelph.ca; phone: 1-519-824-4120, ext. 56668; fax: 1-519-767-1656).

Acknowledgments

We thank Anders Hobæk, Martin Černý, and Miroslaw Slusarczyk for the European specimens of *Holopedium*, and Ian Smith for assistance with figures. Teri Crease, Jonathan Witt, and two anonymous reviewers provided helpful comments on the manuscript.

This research was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) grant to P.D.N.H., by Ontario Graduate and NSERC scholarships to C.L.R., and by a NSERC postdoctoral fellowship to S.J.A.

developmental rates at low temperatures. Interestingly, observations on Alaskan *H. gibberum* have provided tentative evidence for a breeding-system shift because female-only populations produce diapausing eggs (Hillard and Tash 1966), a situation which mirrors that seen in cladoceran populations that reproduce by obligate parthenogenesis. In addition, males were not observed among Greenland populations of a recently described *Holopedium* species (Korovchinsky 2005).

Although female-dominated populations often reproduce parthenogenetically, they may alternatively reproduce by self-fertilization. The latter breeding system is unknown in cladocerans but is common in two allied groups of branchiopod crustaceans, the notostracans and conchostracans (Sassaman 1995; Sassaman et al. 1997; Weeks et al. 2000). Because of this fact, a firm diagnosis of reproductive system for female-only populations requires either formal breeding studies or the survey of genotypic diversity in natural populations. For taxa such as *Holopedium*, which have not been successfully maintained in the laboratory (reviewed in Rowe 2000), only the latter approach is feasible. However, and fortunately, prior studies combining allozyme surveys with breeding experiments have shown that both apomictic parthenogenesis (Hebert and Crease 1983) and self-fertilization (Hebert and Beaton 1990) can be readily diagnosed when genetic variation is present. While genotypic frequencies in populations that reproduce sexually or by cyclic parthenogenesis ordinarily conform to Hardy-Weinberg (HW) expectations, obligately asexual lineages show large HW deviations. In the case of the cladocerans, obligate parthenogenesis is usually associated with an excess of heterozygotes (e.g., Hebert et al. 1993; Little et al. 1997; but *see* Dufresne and Hebert 1994). This breeding system generally arises via interspecific hybridization (and often polyploidization) (e.g., Beaton and Hebert 1988; Hebert and Wilson 1994; Little et al. 1997) or, for members of the *Daphnia pulex* species complex, via a meiosis-suppressing mutation that is spread among interbreeding lineages (Innes and Hebert 1988; Hebert et al. 1989). Apomixis ensures that offspring are genetically identical to the parent, and both of the aforementioned origination mechanisms ensure ample genetic variation, which is then observed as fixed or excessive heterozygosity. By contrast, taxa that reproduce by self-fertilization regularly consist of homozygous lineages for alternate alleles, when genetic variation is present, since heterozygosity introduced by mutation or migration decays by 50% each generation.

This study examines both allozyme and mitochondrial deoxyribonucleic acid (MtDNA) variation to probe the extent and patterning of genetic diversity in populations of *H. gibberum* from sites across the Canadian arctic. The results provide clear evidence that these lineages have made the transition to self-fertilization, or perhaps automixis, and supply the first record of such a breeding system in the Cladocera.

Materials and methods

Taxonomy employed—This study focuses on populations of *H. gibberum* s.s. from the North American arctic. We note that, following genetic and morphological analysis, Rowe (2000) concluded that *H. gibberum* s.l. actually constitutes two separate species in North America, one with a predominantly arctic distribution and one with a primarily temperate distribution. A taxonomic revision is in preparation, and thus *Holopedium* sp. nov. here refers to the soon-to-be-described sister species of *H. gibberum* s.s. This undescribed species is used here as an outgroup.

Collections—Zooplankton were collected from 290 lakes in the Canadian arctic during the summers of 1993–1997 using tow nets deployed from float-equipped helicopters. These sampling efforts, which focused on two widely separated areas, led to the collection of 53 populations of *H. gibberum* (Table 1; Fig. 1). Thirty-three of these, hereafter termed the western arctic populations, were obtained from lakes in the Northwest Territories or the Yukon at sites ranging from 67.15°N to 69.44°N and 130.65°W to 139.65°W. Twenty others, termed the eastern arctic populations, were obtained from lakes in Nunavut, at sites ranging from 67.58°N to 71.37°N and 76.56°W to 85.00°W. Hence, the lakes within each region ranged across approximately 2–4° latitude and 10° longitude, while the eastern and western regions were separated by an average of about 55° longitude, or ~2,000 km.

Allozyme analysis—Cryogenically preserved animals from 49 populations were thawed, and their jelly coat was removed by dissection before maceration. Whenever available, at least 20 individuals from each population were separately homogenized in 4–8 μL of distilled water. Allozyme variation was examined using cellulose acetate electrophoresis with a Tris Glycine (pH 8.5) buffer and standard protocols (Hebert and Beaton 1993). Preliminary screening of several *Holopedium* species revealed seven loci that both stained reliably and exhibited polymorphisms within the genus: amino aspartate transferase, supernatant form (sAat, EC [Enzyme Commission code] 2.6.1.1); arginine phosphate kinase (Apk, EC 2.7.3.3); glucose-6-phosphate isomerase (Gpi, EC 5.3.1.9); malate dehydrogenase, supernatant form (sMdh, EC 1.1.1.37) and mitochondrial form (mMdh, EC 1.1.1.37); mannose-6-phosphate isomerase (Mpi, EC 5.3.1.8); and phosphoglucomutase (Pgm, EC 5.4.2.2). Four individuals from a reference population of *H. gibberum* s.l. from Ontario were included in each assay as a mobility standard. Allelic variants were discriminated by their mobility values (Rf) relative to these standards.

Allozyme data were analyzed using the Genetic Distance Analysis (GDA) program, version 1.0 d12 (Lewis and Zaykin 1999). Genotypic frequencies in each population were tested against HW expectations using Fisher's exact test. Fixation indices were calculated from the observed and expected heterozygosities.

MtDNA analysis—Eleven populations from the eastern arctic and 12 from the west were haphazardly selected for analysis (Table 1). In most cases, only a single individual was analyzed from each population. However, multiple individuals were sequenced from a few populations with

elevated diversity at allozyme loci, bringing the number of DNA sequences for arctic populations up to 31. In addition, a single individual was examined from each of three European populations from the vicinities of Fláje (Czech Republic), Bergen (Norway), and Zakopane (Poland). Most of the DNA samples were extracted by aliquoting 1–2 μL (out of a total of 8 μL) of body homogenate into 30 μL of 6% Chelex-100 (BioRad Inc.). The remainder of this homogenate was used immediately for electrophoresis in cases where joint allozyme and DNA data were gathered. The Chelex solution was incubated at 55°C for 12 h, boiled at 100°C for 10 min, centrifuged at 14,000 rpm (revolutions per minute) for 1 min, and then incubated at 4°C overnight before the supernatant was used for polymerase chain reaction (PCR). When only ethanol-preserved animals were available, total DNA was extracted using the methods of Shiozawa et al. (1992).

A 710 base pair (bp) fragment of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene was PCR amplified using the primer pair LCO1490 and HCO2198 (Folmer et al. 1994). Each 50-μL PCR reaction contained 3–5 μL of DNA template, 4.5 μL of 10× PCR buffer (Palumbi 1996), 0.2 μmol L^{-1} of each primer, 2.2 mmol L^{-1} $MgCl_2$, 0.2 mmol L^{-1} of each dNTP, and 1 unit of Taq polymerase. Amplifications consisted of an initial denaturation step at 94°C for 1 min, annealing at 45°C for 1.5 min, and extension at 72°C for 1.5 min. The annealing temperature was then raised to 50°C for 35 cycles, followed by a final extension at 72°C for 5 min. Two replicate PCR amplifications were performed on each individual, and PCR products were separately electrophoresed and excised from a 2% agarose gel and then combined for purification using Qiaex II reagents (Qiagen). From 100 to 200 ng of purified DNA were subsequently sequenced with the LCO1490 primer on an ABI Prism 377 automated sequencer using the Taq FS dye rhodamine kit. All unique sequences were deposited in GenBank (*see* Table 1 for accession numbers).

Sequences were aligned in a Sequence Navigator (Perkin Elmer), and Kimura's (1980) two-parameter model (K2P) was used to construct a matrix of pairwise nucleotide divergences for all sequences. Distinct haplotypes were identified and numbered with consecutive digits added onto a starting code of "2-" to represent the species *H. gibberum* s.s. This system follows the codes of Rowe (2000) to maintain consistency with a taxonomic revision of *Holopedium* that is in progress (Rowe et al. unpubl. data). The neighbor-joining (NJ) method (Saitou and Nei 1987) was then used to construct a phenogram of all unique haplotypes based upon K2P distances in the program MEGA, version 3.1 (Kumar et al. 2001). As described above, several sequences from an undescribed *Holopedium* species from temperate North America were used as an outgroup (coded as haplotypes beginning with "1-"). Relationships among *H. gibberum* s.s. haplotypes were also examined through a haplotype network using the method of Templeton et al. (1992), generated with the program TCS, version 1.21 (Clement et al. 2000) using the 95% connection limit and default settings.

Joint genotypes—Both allozyme genotype and COI sequences were obtained from 23 individuals spanning 13 arctic populations. The possibility of association between particular haplotypes and allozyme genotypes was investigated using a chi-square test. However, due to the small sample size, numbers of expected occurrences were low in most data cells, which is a problematic situation for the chi-square test. Therefore, Monte Carlo simulation (with 100,000 replicates) was carried out in the program R, version 2.2.1 (R Development Core Team 2005) to estimate the *p*-value.

Results

Allozymes—Allozyme diversity was low, with diallelic polymorphisms at just 2 of the 7 loci (sMdh and sAat). Four different genotypes (G1–G4) were detected among the 1,018 individuals screened, and this diversity reflected the four possible combinations of homozygotes at these two loci (G1—sMdh 85, sAat 100; G2—sMdh 100, sAat 100; G3—sMdh 100, sAat 107; G4—sMdh 85, sAat 107). Because of the complete lack of heterozygotes, the inbreeding coefficient was 1.00 in all variable populations. These heterozygote deficits were significantly different from Hardy-Weinberg expectations (based upon Fisher's exact tests) for all polymorphic populations, except for one with a small sample size ($n = 3$) (Table 1).

Genotypic diversity was particularly impoverished in the east, with 11 of 16 populations fixed for G1 and 3 fixed for G2. These same genotypes co-occurred at the other two eastern sites, where G1 was dominant in both lakes. Genotypic diversity was higher in the west; 4 genotypes were detected and 12 of 33 populations were variable. Genotypic frequencies varied among lakes, and each of the four genotypes was dominant in at least one habitat. Twenty-one lakes contained just a single genotype, and G2 was the clear dominant in these settings, occupying 18 (i.e., 86%) of them. Six of the other 12 western populations possessed 2 genotypes, while the remainder contained 3 or 4 genotypes. At the 10 sites where it co-occurred with other genotypes, G2 made up just 34.2% of the total individuals screened. By contrast, G3 represented 53.3% of the total individuals from the eight habitats where it occurred with other genotypes.

MtDNA diversity—Eight COI haplotypes were detected among the 31 arctic individuals sequenced (Table 2), while the three European individuals contributed three additional haplotypes. Just two of the arctic haplotypes (2-1 and 2-4) were detected in populations from the eastern region, where 2-1 was the clear dominant, comprising 91% (10 of 11) of the isolates from this region. The other six haplotypes were only observed in the west, with 2-2 representing 55% of all the isolates from this region. The European haplotypes were distinct from their North American counterparts, but pairwise sequence distances (K2P) among all 11 haplotypes were small, with an average divergence of 0.7% and maximum of 1.9%. Bootstrap support for nodes among *H. gibberum* haplotypes in the NJ phenogram was generally very low (Fig. 2). Haplotype network analysis also revealed

Table 1. Collection localities of 56 populations of *Holopedium gibberum*, 53 from the Canadian arctic, divided into western and eastern regions, and 3 from Europe. COI haplotypes are indicated, along with the associated allozyme genotype (in parentheses) for those individuals for which both kinds of data were collected. The sample size for allozymes (*n*) refers to the number of individuals diagnosed for the four multilocus genotypes (G1–G4), which are described in the text. Canadian territory abbreviations are: NT, Northwest Territories; NU, Nunavut; YK, Yukon. GenBank accession numbers for COI haplotypes are: 2-1 (AF245354), 2-2 (EF025714), 2-3 (EF025715), 2-4 (EF025716), 2-5 (EF025717), 2-6 (EF025718), 2-8 (EF025720), 2-9 (EF025721), 2-10 (EF025722), 2-11 (EF025723), and 2-12 (EF025724).

Locality (site code*)	Latitude (°N)	Longitude (°W)	Collection date	COI haplotypes (allozyme genotype)	*n* for allozymes	Proportions of the 4 allozyme genotypes			
						G1	G2	G3	G4
Western Canadian arctic									
Arctic Red River 1, NT (20)	67.682	131.879	19 Aug 97	2-2 (G2)	2	–	1.00	–	–
Arctic Red River 5, NT (21)	67.746	132.762	19 Aug 97	–	7	–	1.00	–	–
Arctic Red River 7, NT (22)	67.742	132.828	21 Aug 97	–	42	–	1.00	–	–
Arctic Red River 8, NT (23)	67.840	132.621	20 Aug 97	–	42	–	1.00	–	–
Crossley Lk 1, NT (25)	68.088	130.659	12 Aug 93	–	5	–	1.00	–	–
Crossley Lk 2, NT (26)	68.050	130.646	12 Aug 93	–	11	–	1.00	–	–
Crossley Lk 3, NT (27)	68.013	130.922	12 Aug 93	–	3	–	1.00	–	–
Crossley Lk 4, NT (28)	68.055	130.960	12 Aug 93	–	2	–	1.00	–	–
Crossley Lk 12, NT (29)	67.952	132.236	19 Aug 97	–	12	–	1.00	–	–
Eskimo Lk 2, NT (40)	68.511	133.623	18 Aug 97	–	5	–	1.00	–	–
Eskimo Lk 5, NT (41)	68.554	133.746	18 Aug 97	–	12	–	1.00	–	–
Eskimo Lk 6, NT (42)	68.679	133.817	20 Aug 97	2-8	34	–	1.00	–	–
Eskimo Lk 8, NT (43)	68.759	134.126	09 Aug 93	–	22	–	–	1.00	–
Inuvik 1, NT (47)	68.127	132.430	19 Aug 97	2-2 (G1); 2-2 (G2); 2-9	20†	0.05	0.10	0.85	–
Inuvik 5, NT (48)	68.118	132.846	19 Aug 97	–	16	–	1.00	–	–
MacKenzie Delta 13, NT (49)	69.237	134.704	21 Aug 97	2-2 (G2); 2-11 (G1)	20†	0.50	0.50	–	–
MacKenzie Delta 16, NT (50)	69.438	133.063	22 Aug 97	–	10	–	1.00	–	–
MacKenzie Delta 23, NT (51)	69.359	134.180	22 Aug 97	–	11	–	1.00	–	–
Small Frog, NT (59)	67.382	134.152	12 Aug 97	–	5	–	1.00	–	–
Stony Pond, NT (62)	67.148	135.963	12 Aug 97	2-2 (G2)	40	–	1.00	–	–
Herschel Island 7, YK (105)	69.138	138.195	21 Aug 97	2-2 (G2); 2-2 (G4)	3	–	0.33	–	0.67
Herschel Island 8, YK (106)	69.092	138.173	21 Aug 97	–	13†	0.08	0.15	0.54	0.23
Old Crow 3, YK (107)	68.197	138.673	15 Aug 97	–	37	1.00	–	–	–
Old Crow 10, YK (108)	67.923	139.647	15 Aug 9	2-11 (G1)	17	1.00	–	–	–
Shingle Pt 4, YK (109)	68.823	137.303	21 Aug 97	2-3	21†	0.43	0.52	0.05	–
Shingle Pt 7, YK (110)	68.769	137.858	06 Aug 93	–	40†	0.10	–	0.90	–
Shingle Pt 8, YK (111)	68.790	138.045	06 Aug 93	–	22†	–	–	0.95	0.05
Shingle Pt 11, YK (112)	68.737	137.938	06 Aug 93	–	11	–	1.00	–	–
Shingle Pt 12, YK (113)	68.755	137.855	06 Aug 93	–	23†	–	0.96	0.04	–
Shingle Pt 13, YK (114)	68.715	137.385	06 Aug 93	–	22†	0.14	0.86	–	–
Shingle Pt 16, YK (115)	69.030	137.160	21 Aug 97	2-2 (G1)	38†	0.47	0.03	–	0.50
Shingle Pt 17, YK (116)	68.942	137.576	21 Aug 97	2-2 (G3); 2-10 (G1); 2-10 (G2); 2-10 (G4)	35†	0.06	0.14	0.49	0.31
Shingle Pt 18, YK (117)	69.030	137.874	21 Aug 97	2-2 (G3); 2-2 (G4); 2-10 (G1)	32†	0.19	0.13	0.34	0.34
Eastern Canadian arctic									
Brodeur Peninsula 3, NU (24)	71.367	84.998	24 Aug 96	2-1	22	1.00	–	–	–
Eqe Bay 4, NU (31)	69.570	76.557	11 Aug 94	2-1	–				
Erichsen Lks 1, NU (32)	70.451	82.364	27 Aug 96	2-1	22	1.00	–	–	–
Erichsen Lks 2, NU (33)	70.447	81.773	28 Aug 96	–	42	1.00	–	–	–
Erichsen Lks 4, NU (34)	70.472	81.388	28 Aug 96	–	22	1.00	–	–	–
Erichsen Lks 5, NU (35)	70.622	80.872	28 Aug 96	–	22	1.00	–	–	–
Erichsen Lks 6, NU (36)	70.666	81.138	28 Aug 96	–	22	1.00	–	–	–
Erichsen Lks 8, NU (37)	70.763	81.629	28 Aug 96	–	22	1.00	–	–	–
Erichsen Lks 9, NU (38)	70.687	81.836	28 Aug 96	–	22	1.00	–	–	–
Erichsen Lks 10, NU (39)	70.561	81.944	28 Aug 96	–	41	1.00	–	–	–
Gifford Fiord 9, NU (44)	70.286	83.401	22 Aug 96	2-1	22	1.00	–	–	–
Gifford Fiord 11, NU (45)	70.305	83.432	27 Aug 96	–	22	1.00	–	–	–
Nagvaak Lk 1, NU (52)	67.794	84.498	16 Aug 94	2-1	–				
Parry Bay 2, NU (53)‡	68.322	82.607	22 Aug 96	2-1	34	–	1.00	–	–
Quartzite Lk 7, NU (54)	67.582	81.526	23 Aug 96	–	22†	0.68	0.32	–	–
Sarcpa Lk 8, NU (55)‡	68.535	83.339	23 Aug 96	2-1 (G1)	19†	0.89	0.11	–	–

Table 1. Continued.

Locality (site code*)	Latitude (°N)	Longitude (°W)	Collection date	COI haplotypes (allozyme genotype)	*n* for allozymes	Proportions of the 4 allozyme genotypes G1	G2	G3	G4
Sarcpa Lk 13, NU (56)‡	68.341	83.329	23 Aug 96	2-4	5	–	1.00	–	–
Sarcpa Lk 15, NU (58)	68.289	82.824	23 Aug 96	2-1	22	–	1.00	–	–
Steensby 2, NU (60)	69.891	77.422	11 Aug 94	2-1	–				
Steensby 3, NU (61)	69.851	77.505	11 Aug 94	2-1	–				
Europe									
Fláje, Czech Republic (118)	~50.7	~13.6°E	03 Jun 94	2-6	–				
Bergen P2, Norway (119)	~60.3	~5.3°E	15 Jun 95	2-5	–				
Zakopane (lake: Nizny Toporowy Staw), Poland (120)	~49.3	~19.9°E	28 Sep 95	2-12	–				

* Site codes are as in Rowe (2000) to ensure consistency with other research (Rowe et al. unpubl.).
† Indicates that at least one locus is significantly out of Hardy-Weinberg equilibrium ($p<0.05$ using Fisher's exact test).
‡ *H. gibberum* at these three sites coexisted with an undescribed species of *Holopedium*. The characterization and differentiation of this species is treated elsewhere (Rowe 2000; Rowe et al. in review).

a lack of phylogenetic resolution, with close links between many sequences (Fig. 3). However, one of the European sequences emerged as being particularly divergent.

Joint genotypes—The joint determination of mtDNA haplotypes and allozyme genotypes in 23 individuals from 13 arctic populations revealed that single allozyme genotypes were always associated with more than one haplotype (Table 3). In fact, four haplotypes were associated with both dominant allozyme genotypes (G1, G2), and two haplotypes were associated with each of the less common genotypes (G3, G4). Although some combinations were never observed, a chi-square test (with Monte Carlo simulation to estimate the *p*-value in light of the small sample size) indicated no significant association ($X^2 = 19.85$; $p = 0.164$).

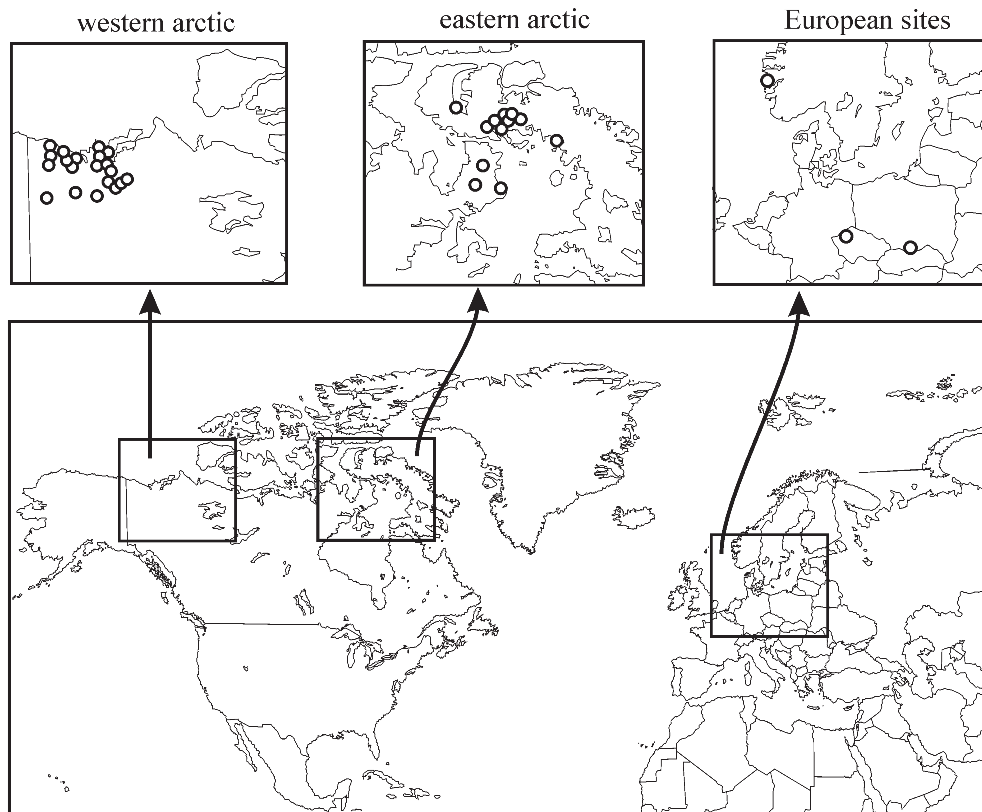

Fig. 1. Map showing the collection localities (circles) of *Holopedium gibberum* s.s. in two regions of arctic Canada, where some dots represent multiple geographically proximate collections. Three localities from Europe are also shown.

Discussion

This study has provided genetic characterization of arctic populations of *Holopedium* across a broad geographic range in North America. Allelic diversity was found at two allozyme loci, but no heterozygotes were detected among the more than 1,000 individuals screened. The presence of four homozygous genotypes, coexisting at many sites, indicate that these populations do not reproduce by either cyclic parthenogenesis, as in temperate-zone *Holopedium* (Hebert and Finston 1997; Rowe et al. unpubl. data), or by obligate, apomictic parthenogenesis, as in some other polar cladocerans (Beaton and Hebert 1988; Little et al. 1997). Instead, the genotypic arrays of arctic *H. gibberum* reflect those expected under self-fertilization or perhaps automictic (meiotic) parthenogenesis, which are the only breeding systems that would inherently enforce homozygosity in the face of widespread polymorphism. After elaborating upon our reasons for concluding such a shift, the balance of the discussion considers the importance of breeding systems for polar populations, as well as the potential evolutionary pathway to self-fertilization in *H. gibberum* in light of breeding-system variation within the branchiopod crustaceans. We further suggest that there may be more fluidity in cladoceran breeding systems than is currently recognized and that this may aid their occupancy of extreme environments.

Breeding-system diagnosis in arctic Holopedium—Since success has never been reliably achieved with rearing *Holopedium* in the laboratory (reviewed in Rowe 2000), indirect breeding-system diagnosis via genetic surveys was necessary for this study. However, due to the exclusive detection of homozygous individuals among the arctic populations, isolating individuals in a laboratory setting would not have provided additional insight in this case, beyond confirming unisexual reproduction. Thus, we consider three lines of evidence that support our primary conclusion about the type of breeding system in arctic *H. gibberum*.

Table 2. Summary of the distribution of genetic variation within *Holopedium gibberum* between eastern and western arctic Canada. Haplotypes 2-5, 2-6, and 2-12 were found only in Europe.

	East	West
COI haplotypes (in numbers of sequences; total n = 31)		
2-1	10	–
2-2	–	11
2-3	–	1
2-4	1	–
2-8	–	1
2-9	–	1
2-10	–	4
2-11	–	2
Allozyme genotypes (in frequencies; total n=1,018)		
G1	0.817	0.170
G2	0.183	0.547
G3	–	0.209
G4	–	0.074

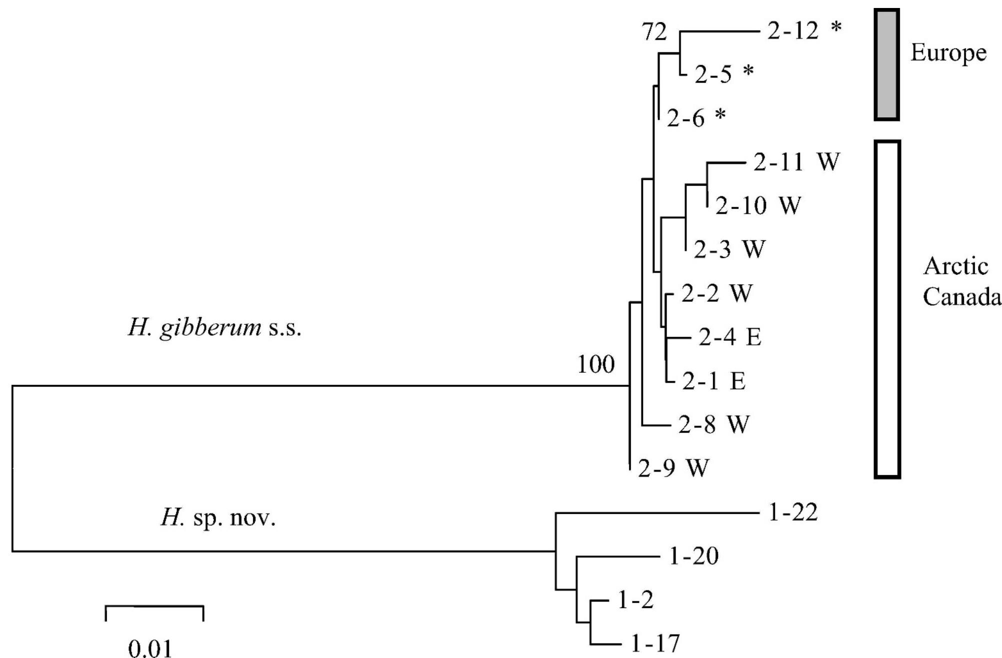

Fig. 2. NJ phenogram based upon K2P distances for 11 COI haplotypes of *Holopedium gibberum* s.s. from arctic Canada and Europe. Numerals indicate the haplotypes (following Rowe 2000); those from the eastern (E) and western (W) Canadian arctic are designated so. Sequences from European individuals are identified with an asterisk. Populations of an undescribed species that is sister to *H. gibberum* s.s. (*see* text) are included as an outgroup. Bootstrap values were generally low, with those >70% shown here.

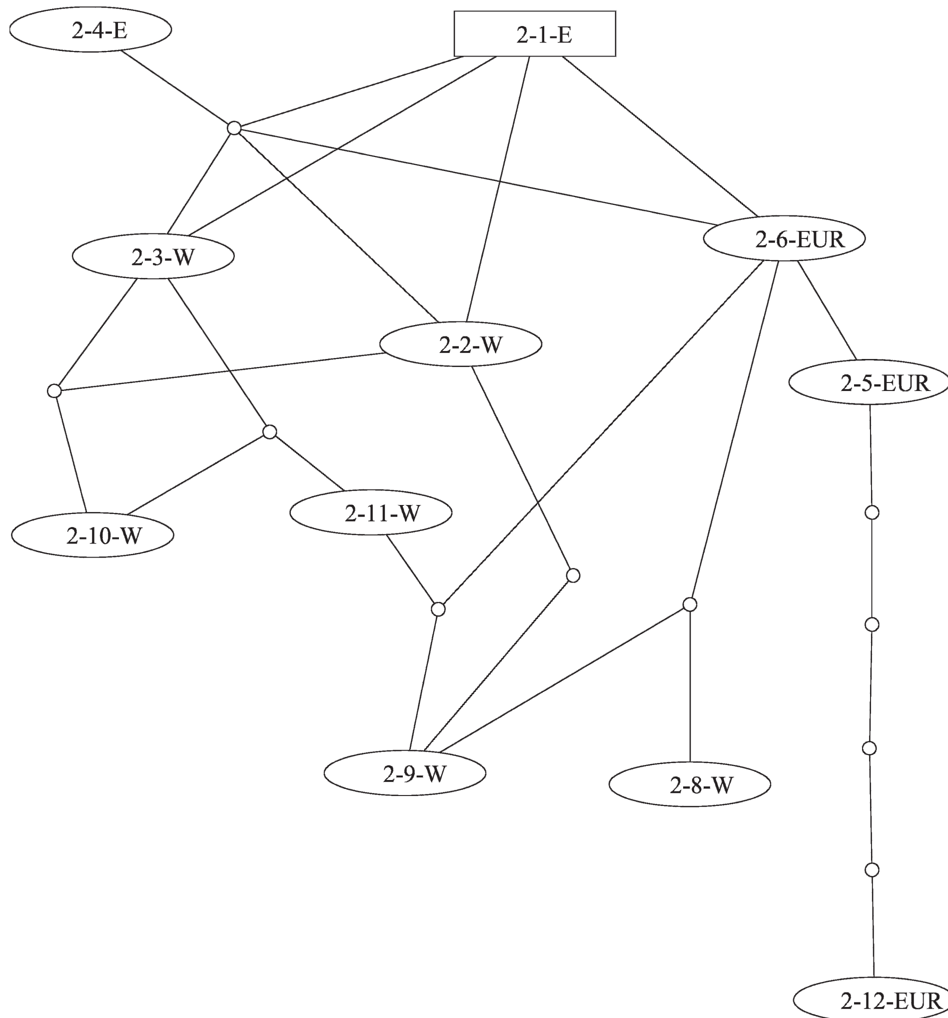

Fig. 3. Relationships among the same *H. gibberum* s.s. haplotypes as in Fig. 2, presented as a haplotype network. Branch lengths have no meaning but show the number of substitutions present between haplotypes. Localities of haplotypes are designated as EUR (Europe), E (eastern Canadian arctic), or W (western Canadian arctic).

First, the severe deviation from Hardy-Weinberg (HW) expectations provided strong evidence that the arctic *Holopedium* populations are not reproducing by the same mechanism as the temperate populations. The general conformance of temperate North American *Holopedium* populations to HW (Hebert and Finston 1997; Rowe 2000; Rowe et al. unpubl. data) is indicative of their reproduction by cyclical parthenogenesis. This is the typical breeding mode of cladocerans, whereby one or more rounds of parthenogenesis are followed by environmentally-induced male production, typically at the end of the growing season when densities are highest, which is then followed by sexual reproduction and diapausing egg formation occur. When deviations were detected among temperate *Holopedium* populations (Rowe 2000; Rowe et al. unpubl. data), cases of both heterozygote excess and deficits were found, but with a tendency toward the latter. Thus, the genotype frequencies of the arctic populations are completely different from temperate profiles in exhibiting such extreme HW deviations.

Table 3. Test for association between allozyme genotypes and COI haplotypes for 23 individuals of *H. gibberum*, sampled from 13 populations from two regions of the Canadian arctic. The association was nonsignificant ($X^2 = 19.85$; $p = 0.164$, calculated using Monte Carlo simulation due to the small sample size). None of the individuals possessing haplotypes 2-4 or 2-8 was profiled for allozyme genotypes.

Allozyme genotype	COI haplotype					
	2-1	2-2	2-3	2-9	2-10	2-11
G1	1	2	–	–	2	2
G2	2	5	1	–	1	–
G3	–	2	–	2	–	–
G4	–	2	–	–	1	–

Second, the nature of the arctic *H. gibberum* HW deviations differs from any genetic patterns yet observed among any cyclically parthenogenetic cladocerans, even though HW disturbances are not uncommon among such taxa. Because populations can be founded by one or a few

individuals, and because interclonal selection can occur during the parthenogenetic phase of reproduction, founder effects and loss of multilocus genotypes can contribute to HW deviations even within cyclics (reviewed in De Meester et al. 2006). Low genetic diversity or genetic monomorphism are often observed among cladocerans (e.g., Ebert et al. 2002), especially in small habitats or permanent pond environments (De Meester et al. 2006). However, 100% homozygosity *and* within-site polymorphism seldom occur jointly. Even though interclonal selection is expected to occur, the highly seasonal nature of arctic habitats ensures yearly production of diapausing eggs, which occurs sexually in cyclics. This process would be expected to restore heterozygosity in polymorphic *H. gibberum* populations were they cyclic parthenogens.

Thirdly, genotypic patterns in arctic *Holopedium* also contrast with those observed in other cladocerans that exhibit shifts in breeding mode. Typically, complete deviation from HW equilibrium in cladocerans is associated with a transition to obligate, apomictic parthenogenesis, in which each offspring is genetically identical to its parent. In most cases, this shift manifests itself in the form of excessive and fixed heterozygosity, due to the nonrecombining nature of apomictic parthenogenesis and to the major role of hybridization in generating apomictic polyploids (Beaton and Hebert 1988; Hebert and Wilson 1994; Little et al. 1997). Diploid apomicts can also arise, via a meiosis-suppressing mutation that is transmitted among lineages (Innes and Hebert 1988; Hebert et al. 1989), and these also exhibit excessive heterozygosity. In just one previous case, excessive (but not complete) homozygosity was observed in an apomict, due to the apparent silencing of one parental genome after hybridization (Dufresne and Hebert 1994). However, the hybrid origin of these populations was still signaled in their allelic profiles and mtDNA sequences. Thus, the complete homozygosity observed here, combined with population-level polymorphism, differs sharply from patterns observed in other cladocerans. Moreover, extensive comparisons of allelic identities and mtDNA sequences revealed no evidence for interspecific hybridization in *Holopedium* (Rowe 2000; Rowe et al. unpubl. data). Thus, although apomictic parthenogenesis cannot be completely ruled out, it seems improbable for arctic *H. gibberum*.

Therefore, we conclude that arctic *H. gibberum* populations most likely exhibit a unique breeding system for the Cladocera. In contrast to the other known cladoceran breeding systems discussed already, strict self-fertilization is a breeding system that would perfectly explain the observed results. Under self-fertilization, any initial heterozogysity in the founding individuals is eroded by 50% per generation, leading to rapid segregation of genetic diversity into homozygous genotypes. Some forms of automixis also rapidly impose homozygosity (Suomalainen et al. 1987), and thus distinguishing self-fertilization from automixis would require histological and cytological study. Nevertheless, the population genetic consequences of these breeding types are similar.

Only habitats in the Canadian arctic were investigated here, but, interestingly, this breeding-system shift may be widespread among arctic populations of *Holopedium*. In particular, males were not detected in either Alaskan populations of *H. gibberum* (Hillard and Tash 1966) or in Greenland populations of a recently described species, *H. groenlandicum* (Korovchinsky 2005). Genetic surveys and breeding-system diagnosis across its Eurasian range would be most informative for furthering our understanding of geographic variation in *Holopedium* breeding systems.

Unique attributes of polar colonizers—Despite the high density of aquatic habitats, the zooplankton fauna of the arctic is impoverished, particularly in regions remote from the Beringian refugium. In part, this impoverishment reflects the slow pace of range expansion, as evidenced by the fact that both species diversity (Hebert and Hann 1986) and genetic diversity (e.g., Weider and Hobæk 2003) are higher in Beringia than in climatically similar regions far from it. The species that have colonized arctic regions remote from this major glacial refugium are taxonomically eclectic, but they all possess distinctive biological attributes.

The only two calanoid copepods, *Limnocalanus macrurus* and *Eurytemora affinis*, found in the central North American arctic are euryhaline taxa that have employed marine waters as dispersal corridors. By contrast, the two polar anostracans, *Artemiopsis stefanssoni* and *Branchinecta paludosa*, appear to have persisted throughout the Pleistocene in microrefugia scattered along the margins of the ice, as evidenced by the presence of phylogroups restricted to the glacial heartlands (Cox 2001). These species occur in very oligotrophic rock pools along the margins of glaciers, suggesting that they may have persisted in similar settings throughout the Pleistocene.

Other species have breeding systems that likely aided their dispersal following the retreat of the ice sheets. For example, because the sole polar notostracan, *Lepidurus arcticus*, regularly reproduces by self-fertilization, its populations can be established by a single female. Prior work on polar cladocerans has revealed that the daphniid fauna is dominated by members of the *D. pulex* complex, which are very genetically diverse, but which generally reproduce by obligate parthenogenesis (Weider et al. 1999). Multiple origins of this breeding system in the north, via hybridization and polyploidization, are well established (Dufresne and Hebert 1995, 1997). The same breeding-system transition has occurred in *Bosmina longirostris* s.l. from polar lakes (Little et al. 1997). Although most sites harbor a single clone of this species, diversity is high on a regional scale, and individuals are highly heterozygous, reflecting the probable origins of these bosminid lineages through hybridization and polyploidization as well (Little et al. 1997). Despite allozyme study, the breeding systems of several other cladocerans found in the North American high arctic—*Daphnia longiremis, D. umbra*, and *Eurycercus glacialis*—could not be diagnosed because of their lack of allozyme diversity (P. Hebert unpubl. data). Although northern European populations of several species (e.g., *D. longispina* and *D. magna*) are known to reproduce by cyclic parthenogenesis (e.g., *see* Ebert et al. 2002; Haag et al. 2002, 2006), diagnosis of populations inhabiting the harsh North American arctic would be desirable because breeding

systems can vary within cladoceran species. Although further studies of breeding systems throughout the circumpolar region are necessary, breeding-system shifts seem to be a common feature of branchiopods inhabiting arctic environments.

Breeding-system shifts: Adaptation or coincidence?—Although we cannot assume on a case-by-case basis that breeding-system transitions represent adaptations to arctic life, as the selective regimes at the time of the shifts cannot be known, the argument for an adaptive significance becomes compelling when considering both the prevalence and number of independent transitions in the arctic. For example, in the case of the cladoceran genus *Daphnia*, asexual members of one species complex (*D. pulex*) do occur in temperate settings, but asexuals only become dominant in the arctic, where they apparently have multiple origins (Dufresne and Hebert 1995, 1997). In *Bosmina*, 10 species occur in North America (DeMelo and Hebert 1994*a*,*b*), but asexuals are only known from the arctic (Little et al. 1997). Similarly for *Holopedium*, with four species known from North America and extensively characterized for allozyme variation (Rowe 2000; Rowe et al. unpubl. data), unisexual breeding is found exclusively in the north. Although a few cyclic parthenogens are known to live in the arctic (such as *D. magna*), there is no doubt that the prevalence of unisexual reproducers is much higher in arctic than in temperate settings, relative to total diversity. Moreover, the phenomenon of "geographical parthenogenesis" is well documented for environmentally harsh settings, particularly arctic, alpine, and desert environments (*see* Kearney 2003), in other groups of both plants and animals (e.g., Glesener and Tilman 1978; Bierzychudek 1985; Kearney 2005).

Despite the numerical case that can be made for elevated rates of breeding-system shifts in polar regions, coincident, rather than adaptive, causes must be considered as well. For example, selection could favor heterozygosity (*see* Kearney 2005) or polyploidy (Grime and Mowforth 1982; Stebbins 1984; Dufresne and Hebert 1998), rather than unisexual reproduction itself. In the case of *Holopedium*, the invariant homozygosity indicates that within-individual genetic diversity is not the trait being favored in the north. However, selection for polyploidy remains a possibility, and future diagnosis of ploidy levels would be desirable. For example, a latitudinal cline in both ploidy levels and breeding systems has been documented among members of the *D. pulex* complex in Europe (Ward et al. 1994), and in North America, a ploidy cline is observed even amongst asexual individuals of this group (Beaton and Hebert 1988). Moreover, experimental evidence indicates thermal-related advantages to polyploidy (Dufresne and Hebert 1998). Unfortunately, the genome sizes of polar *Holopedium* have not been compared with southern locales, so the possibility of polyploidy remains uninvestigated.

However, even if there is a selective advantage of polyploidy, that does not discount the importance of unisexuality, since explanations for biological patterns may often be multidimensional. Transitions to unisexual reproduction are favored when population densities are so low that some females fail to encounter a mate. Under this scenario, selection pressures for breeding-system transitions are likely to be most intense during population establishment. Moreover, in cladocerans, breeding-system transitions also permit an escape from a complex life cycle, involving one or more rounds of parthenogenesis to produce males, the durations of which may otherwise impede the occupancy of low-temperature settings. In the arctic, both factors conspire to favor a switch from cyclic parthenogenesis. Indeed, given the extremely short growing season faced by arctic zooplankton, the demographic advantage of direct resting egg production is striking. While adoption of apomictic parthenogenesis was previously known for several cladocerans, the case of *Holopedium* indicates a different evolutionary solution to the same problems of living in the arctic.

Self-fertilization in the branchiopods—Although this is the first report of self-fertilization in cladocerans, this breeding-system transition is already known within the class Branchiopoda and should be considered in future genetic screening studies within the Cladocera. If self-fertilization, rather than automixis, indeed occurs in *Holopedium* or other cladocerans, it could simply reflect the recovery of a trait that was common in ancestral branchiopod lineages. Certainly, self-fertilization is prevalent in both notostracans (Sassaman et al. 1997) and conchostracans (Sassaman 1995). In particular, breeding systems appear to be very labile among members of the conchostracan order Spinicaudata. Species reproduction methods in this group (and sometimes even populations within species) range from pure gonochoristic sexuality through androdioecy (with various frequencies of males coexisting with hermaphrodites) to unisexuality. In these latter cases, breeding appears to be entirely by self-fertilization (Sassaman 1995; Weeks et al. 2000, 2005). Given the morphological and molecular evidence for the close relationship between cladocerans (together with *Cyclestheria*) and spinicaudatans (Olesen 2000; deWaard et al. 2006; 6-gene topology), it is possible that the adoption of self-fertilization in cladocerans could represent a resurrection of this breeding mode acquired from a common ancestor.

However, there appear to be key differences between the breeding modes of *Holopedium* and those of notostracans and spinicaudatans. Hermaphroditic individuals of the latter two groups invariably possess both ovarian and testicular tissues (e.g., Sassaman 1995; Weeks et al. 2000), but gonadal chimaerism is unknown for cladocerans. Moreover, there may be alternate ways for self-fertilization to occur. Although little is known of oogenesis in *Holopedium*, cytogenetic studies on other cladocerans have shown that their diapausing eggs are released into the brood chamber in prophase I. As a result, diploidy might be restored via fusion between the maturing egg and one of its polar bodies. The restitution of diploidy through this mechanism would lead to the loss of 50% of heterozygosity each generation, thus ensuring the rapid emergence of homozygous lines. Therefore, the breeding-system similarities between the large branchiopods and arctic *H.*

gibberum, despite having similar population genetic consequences, seem more likely to represent convergence and analogy than atavism.

The history of Holopedium in the arctic—Adoption of a unisexual mode of reproduction may have been critically important in enabling *Holopedium* to invade arctic environments. Moreover, the biogeographic history of this invasion appears to be reflected in its patterns of genetic diversity. Our allozyme analyses revealed four genotypes in populations from the western arctic, but just two in the east. Similarly, mtDNA diversity was higher in the west, with six haplotypes detected, versus just two in the east. The higher genotypic diversity in the west suggests that this species persisted in the Beringian refugium during the Pleistocene and subsequently diffused across the arctic, showing the loss of variation expected to accompany this process (Boileau and Hebert 1991; Weider and Hobæk 2003).

The lack of shared haplotypes between these regions suggests that populations in both areas of the arctic, as well as those in Europe, do have a history of isolation. However, because the divergences among populations from these areas were low (typically 0.6–1.0%), and phylogenetic relationships among haplotypes could not be resolved, this isolation is likely recent. Application of commonly applied calibrations for the rate of COI evolution (2% divergence per million years, Brown et al. 1979; 2.3%, Brower 1994; 1.4%, Knowlton and Weigt 1998) suggests divergence times in the range of half a million years. However, given the evidence for rapid accumulation of small levels of sequence change (Howell et al. 1996; Denver et al. 2000; Ho et al. 2005), the isolation may be much more recent, perhaps just a few thousand years.

Thus, the distribution of genetic variation, combined with the shallow divergences, is indicative of a recent North American range expansion out of Beringia, and recent exchange also between North America and Europe. Although breeding systems have not been critically diagnosed, temperate European populations of *H. gibberum* likely reproduce by cyclic parthenogenesis because they produce males (Korovchinsky 1992). The close haplotype affinities therefore suggest the recent adoption of self-fertilization by the Canadian arctic populations. The lack of association between mtDNA haplotypes and allozyme genotypes is suggestive of multiple independent breeding shifts, at least in the Canadian arctic. However, further sequencing and breeding-system diagnoses for *H. gibberum* populations from across its Eurasian range would shed greater light upon both the relationships among lineages and the number of breeding-system transitions.

The present study has provided yet another example of the way in which extreme environments can induce shifts in breeding systems (e.g., Glesener and Tilman 1978; Bierzychudek 1985; Kearney 2003). The wide diversity of breeding systems in the branchiopods, along with their phylogenetic distribution, is suggestive of multiple independent transitions to unisexual reproduction, with a high prevalence of such modes in polar settings. This pattern suggests that breeding shifts represent predictable, likely adaptive, responses to extreme conditions. The success of branchiopods in colonizing extreme habitats, compared with other small crustaceans, inidcates that their breeding-system fluidity may represent a key pre-adaptation to their penetration of polar settings.

References

Beaton, M. J., and P. D. N. Hebert. 1988. Geographical parthenogenesis and polyploidy in *Daphnia pulex*. Am. Nat. **132:** 837–845.

Bierzychudek, P. 1985. Patterns in plant parthenogenesis. Experientia **41:** 1255–1264.

Boileau, M. G., and P. D. N. Hebert. 1991. Genetic consequences of passive dispersal in pond-dwelling copepods. Evolution **45:** 721–733.

———, ———, and S. S. Schwartz. 1992. Non-equilibrium gene frequency divergence: Persistent founder effects in natural populations. J. Evol. Biol. **4:** 25–39.

Brower, A. V. Z. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. P. Natl. Acad. Sci. USA **91:** 6491–6495.

Brown, W. M., M. George Jr., and A. C. Wilson. 1979. Rapid evolution of animal mitochondrial DNA. P. Natl. Acad. Sci. USA **76:** 1967–1971.

Černý, M., and P. D. N. Hebert. 1993. Genetic diversity and breeding system variation in *Daphnia pulicaria* from North American lakes. Heredity **71:** 497–507.

Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. **9:** 1657–1659.

Cox, A. J. 2001. Freshwater phylogeography: The impact of life history traits on the post-glacial dispersal of zooplankton in North America. M.Sc. thesis, University of Guelph.

De Meester, L., J. Vanoverbeke, K. De Gelas, R. Ortells, and P. Spaak. 2006. Genetic structure of cyclic parthenogenetic zooplankton populations—a conceptual framework. Arch. Hydrobiol. **167:** 217–244.

Demelo, R., and P. D. N. Hebert. 1994*a*. Allozymic variation and species diversity in North American Bosminidae. Can. J. Fish. Aquat. Sci. **51:** 873–880.

———, and ———. 1994*b*. A taxonomic reevaluation of North American Bosminidae. Can. J. Zool. **72:** 1808–1825.

Denver, D. R., K. Morris, M. Lynch, L. L. Vassilieva, and W. K. Thomas. 2000. High direct estimate of the mutation rate in the mitochondrial genome of *Caenorhabditis elegans*. Science **289:** 2342–2344.

de Waard, J. R., V. Sacherová, M. E. A. Cristescu, E. A. Remigio, T. J. Crease, and P. D. N. Hebert. 2006. Probing the origins of the branchiopod crustaceans. Mol. Phylogenet. Evol. **39:** 491–502.

Dufresne, F., and P. D. N. Hebert. 1994. Hybridization and origins of polyploidy. P. Roy. Soc. B–Biol. Sci. **258:** 141–146.

———, and ———. 1995. Polyploidy and clonal diversity in an arctic cladoceran. Heredity **75:** 45–53.

———, and ———. 1997. Pleistocene glaciations and polyphyletic origins of polyploidy in an arctic cladoceran. P. Roy. Soc. B–Biol. Sci. **264:** 201–206.

———, and ———. 1998. Temperature-related differences in life-history characteristics between diploid and polyploid clones of the *Daphnia pulex* complex. Écoscience **5:** 433–437.

Ebert, D., C. Haag, M. Kirkpatrick, M. Riek, J. W. Hottinger, and V. I. Pajunen. 2002. A selective advantage to immigrant genes in a *Daphnia* metapopulation. Nature **295:** 485–488.

FOLMER, O., M. BLACK, W. HOEH, R. LUTZ, AND R. VRIJENHOEK. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. **3:** 294–299.

GLESENER, R. R., AND D. TILMAN. 1978. Sexuality and the components of environmental uncertainty: Clues from geographic parthenogenesis in terrestrial animals. Am. Nat. **112:** 659–673.

GRIME, J. P., AND M. A. MOWFORTH. 1982. Variation in genome size—an ecological interpretation. Nature **299:** 151–153.

HAAG, C. R., J. W. HOTTINGER, M. RIEK, AND D. EBERT. 2002. Strong inbreeding depression in a *Daphnia* metapopulation. Evolution **56:** 518–526.

———, M. RIEK, J. W. HOTTINGER, V. I. PAJUNEN, AND D. EBERT. 2006. Founder effects as determinants of within-island and among-island genetic structure of *Daphnia* metapopulations. Heredity **96:** 150–158.

HEBERT, P. D. N. 1995. The *Daphnia* of North America: An illustrated fauna.CD-ROM. Distributed by author. Department of Integrative Biology, University of Guelph.

———, AND M. J. BEATON. 1990. Breeding system and genome size of the rhabdocoel turbellarian *Mesostoma ehrengergii*. Genome **33:** 719–724.

———, AND ———. 1993. Methodologies for allozyme analysis using cellulose acetate electrophoresis. Helena Laboratories.

———, ———, S. S. SCHWARTZ, AND D. J. STANTON. 1989. Polyphyletic origins of asexuality in *Daphnia pulex*. I. Breeding-system variation and levels of clonal diversity. Evolution **43:** 1004–1015.

———, AND T. CREASE. 1983. Clonal diversity in populations of *Daphnia pulex* reproducing by obligate parthenogenesis. Heredity **51:** 353–369.

———, AND T. L. FINSTON. 1997. Taxon diversity in the genus *Holopedium* (Crustacea: Cladocera) from the lakes of eastern North America. Can. J. Fish. Aquat. Sci. **54:** 1928–1936.

———, AND ———. 2001. Macrogeographic patterns of breeding system diversity in the *Daphnia pulex* group from the United States and Mexico. Heredity **86:** 1–9.

———, AND B. J. HANN. 1986. Patterns in the diversity of arctic zooplankton communities. Can. J. Fish. Aquat. Sci. **43:** 1416–1425.

———, S. S. SCHWARTZ, R. D. WARD, AND T. L. FINSTON. 1993. Macrogeographic patterns of breeding system diversity in the *Daphnia pulex* group. I. Breeding systems of Canadian populations. Heredity **70:** 148–161.

———, AND C. C. WILSON. 1994. Provincialism in plankton: Endemism and allopatric speciation in Australian *Daphnia*. Evolution **48:** 1333–1349.

HILLARD, D. K., AND J. C. TASH. 1966. Freshwater algae and zooplankton, p. 363–413. *In* N. J. Wilimovsky and J. N. Wolfe [eds.], Environment of the Cape Thompson Region, Alaska. Vol. 16. United States Atomic Energy Commission, Division of Technical Information.

HO, S. Y. W., M. J. PHILLIPS, A. COOPER, AND A. J. DRUMMOND. 2005. Time dependency of molecular rate estimates and systematic overestimation of recent divergence times. Mol. Biol. Evol. **22:** 1561–1568.

HOWELL, N., I. KUBACKA, AND D. A. MACKEY. 1996. How rapidly does the human mitochondrial genome evolve? Am. J. Hum. Genet. **59:** 501–509.

INNES, D. J., AND P. D. N. HEBERT. 1988. The origin and genetic basis of obligate parthenogenesis in *Daphnia pulex*. Evolution **42:** 1024–1035.

KEARNEY, M. R. 2003. Why is sex so unpopular in the Australian desert? Trends Ecol. Evol. **18:** 605–607.

KEARNEY, M. 2005. Hybridization, glaciation and geographical parthenogenesis. Trends Ecol. Evol. **20:** 495–502.

KIMURA, M. 1980. A simple method for estimating the evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. **16:** 111–120.

KNOWLTON, N., AND L. A. WEIGT. 1998. New dates and new rates for divergence across the Isthmus of Panama. P. Roy. Soc. B–Biol. Sci. **265:** 2257–2263.

KOROVCHINSKY, N. M. 1992. Sididae and Holopediidae (Crustacea: Daphniiformes). Guides to the identification of the microinvertebrates of the continental waters of the world. Vol. 3. SPB Academic Publishing.

———. 2005. New species of *Holopedium* Zaddach, 1855 (Crustacea: Cladocera: Ctenopoda) from Greenland. J. Limnol. **64:** 103–112.

KUMAR, S., K. TAMURA, I. B. JAKOBSEN, AND M. NEI. 2001. MEGA2: Molecular evolutionary genetic analysis software. Version 2.1. Distributed at http://www.megasoftware.net/.

LEWIS, P. O., AND D. ZAYKIN. 2001. Genetic distance analysis: computer program for the analysis of allelic data.Version 1.0. Distributed at http://hydrodictyon.eeb.uconn.edu/people/plewis/software.php.

LITTLE, T. J., R. DEMELO, D. J. TAYLOR, AND P. D. N. HEBERT. 1997. Genetic characterization of an arctic zooplankter: Insights into geographic polyploidy. P. Roy. Soc. B–Biol. Sci. **264:** 1363–1370.

OLESEN, J. 2000. An updated phylogeny of the Conchostraca-Cladocera clade (Branchiopoda, Diplostraca). Crustaceana **73:** 869–886.

PALUMBI, S. R. 1996. Nucleic acids II: The polymerase chain reaction, p. 205–247. *In* D. M. Hillis, C. Moritz and B. K. Mable [eds.], Molecular systematics, 2nd ed. Sinauer Associates, Inc.

R Development Core Team. 2005. Distributed at http://www.r-project.org.

ROWE, C. L. 2000. Global distribution, phylogeny and taxonomy of the freshwater zooplankton genus *Holopedium*. M.Sc. thesis, University of Guelph.

SAITOU, N., AND M. NEI. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4:** 406–425.

SASSAMAN, C. 1995. Sex determination and evolution of unisexuality in the Conchostraca. Hydrobiology **298:** 45–65.

———, M. A. SIMOVICH, AND M. FUGATE M. 1997. Reproductive isolation and genetic differentiation in North American species of Triops (Crustacea: Branchiopoda: Notostraca). Hydrobiology **359:** 125–147.

SHIOZAWA, D. K., J. KUDO, R. P. EVANS, S. R. WOODWARD, AND R. N. WILLIAMS. 1992. DNA extraction from preserved trout tissues. Great Basin Nat. **52:** 29–34.

STEBBINS, G. L. 1984. Polyploidy and distribution of arctic-alpine flora: New evidence and a new approach. Bot. Helv. **94:** 1–13.

SUOMALAINEN, E., A. SAURA, AND J. LOKKI. 1987. Cytology and evolution in parthenogenesis. CRC Press, Inc.

TEMPLETON, A. R., K. A. CRANDALL, AND C. F. SING. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics **132:** 619–633.

WARD, R. D., M. A. BICKERTON, T. FINSTON, AND P. D. N. HEBERT. 1994. Geographical cline in breeding systems and ploidy levels in European populations of *Daphnia pulex*. Heredity **73:** 532–543.

WEEKS, S. C., B. R. CROSSER, R. BENNETT, M. GRAY, AND N. ZUCKER. 2000. Maintenance of androdioecy in the freshwater shrimp, Eulimnadia texana: Estimates of inbreeding depression in two populations. Evolution **54:** 878–887.

———, R. T. Posgai, M. Cesari, and F. Scanabissi. 2005. Androdioecy inferred in the clam shrimp *Eulimnadia agassizii* (Spinicaudata: Limnadiidae). J. Crust. Biol. **25:** 323–328.

Weider, L. J., and A. Hobæk. 2003. Glacial refugia, haplotype distributions, and clonal richness of the *Daphnia pulex* complex in arctic Canada. Mol. Ecol. **12:** 463–473.

———, ———, P. D. N. Hebert, and T. J. Crease. 1999. Holarctic phylogeography of an asexual species complex: II. Allozymic variation and clonal structure in arctic *Daphnia*. Mol. Ecol. **8:** 1–13.

Received: 20 March 2006
Accepted: 2 May 2007
Amended: 29 May 2007