Pleistocene glaciations and polyphyletic origins of polyploidy in an arctic cladoceran

FRANCE DUFRESNE* AND PAUL D. N. HEBERT

Department of Zoology, University of Guelph, Guelph, Ontario, Canada N1G 2W1

SUMMARY

RFLP analysis of the ND4–ND5 genes of the mtDNA genome in *Daphnia middendorffiana* and three closely allied species was used to investigate its origin and age. Populations of *D. middendorffiana* from arctic Canada were found to possess three distinct mtDNA lineages, only one of which appears unique to this species. The other two mtDNA lineages are either closely allied or identical to haplotypes in *D. pulicaria*, suggesting that it is the maternal parent of many clones of *D. middendorffiana*. Within *D. pulicaria*, mtDNA lineages have largely disjunct distributions, suggesting that populations of this species persisted in three glacial refugia (arctic, western, eastern) during the Pleistocene. Hybridizations between these refugial stocks and other species such as *D. melanica* and *D. pulex* likely generated many of the polyploid lineages of *D. middendorffiana* following the Wisconsinan glaciation. The presence of one unique mtDNA lineage in *D. middendorffiana* suggests that at least some of its clones are more ancient, but further studies are needed to rule out the possibility of their recent derivation from an as yet undetected sexual species. As a general result, this study suggests that polyploid cladocerans are unlikely to predate the Pleistocene.

1. INTRODUCTION

Species restricted to the arctic experienced cycles of population contraction and expansion during the Pleistocene. Ice advances led to the isolation of populations in refugia, diminishing effective population sizes and setting the stage for chance fixation of alleles. As a result, species restricted to a single refugium might be expected to show impoverished levels of genetic diversity (Avice 1994). By contrast, when species persisted in several refugia, local gene pool divergence sets the stage for secondary contacts following ice retreat that enhanced genotypic diversity. In some cases hybridization between these divergent lineages also appears to have provoked transitions to polyploidy.

Although the abundance of polyploid lineages in the arctic is well documented, the timing of transitions to polyploidy is often uncertain. Some polyploids clearly predate the Pleistocene. For example, salmonid fishes, which dominate arctic lakes, made their transition to polyploidy between 25 and 100 million years ago (Ohno 1970). In other groups, polyploidy has a more recent genesis, as evidenced by the presence of closely allied diploid taxa. However, because the precise age of the latter polyploids is uncertain, it is unclear if successive glaciations produced a gradual enrichment in the incidence of polyploids in the arctic or if most polyploids have a recent origin. Resolution of this uncertainty is now possible, as the age of a polyploid

lineage can be determined by examining its divergence from its closest diploid relative. If, for example, a polyploid owes its origin to a recent hybridization event, then its mitochondrial genome will be closely similar or identical to that of its diploid maternal parent, while ancient polyploids will show haplotypes distinct from those possessed by any sexual relatives.

This study investigates the origin and age of polyploid lineages of *D. middendorffiana*, a cladoceran crustacean which is a dominant component of the zooplankton communities in arctic ponds (see fig. 1 in Dufresne & Hebert (1995) for recent taxonomic reassignments). Polyploidy is so far known in only two genera of cladocerans, *Bosmina* (Little *et al.* personal observation) and *Daphnia* (Beaton & Hebert 1988; Ward *et al.* 1994), and polyploid members of both genera are restricted to the arctic and appear to have arisen through hybridization. In the genus *Daphnia*, polyploidy has been detected in two species: *D. middendorffiana* and *D. tenebrosa*. The latter species includes both diploid and polyploid lineages, suggesting its recent adoption of polyploidy (Dufresne & Hebert 1995). By contrast, *D. middendorffiana* consists solely of polyploids reproducing by obligate asexuality and includes a large number of allozymically distinct clones showing ecological and morphological divergence (Weider & Hebert 1987). Its high genetic diversity and lack of close diploid allies provide *prima facie* evidence that polyploid lineages of *D. middendorffiana* are ancient. However, morphological and DNA sequencing studies have shown that the species is one of a closely allied tetrad of species, whose other members are *D. melanica*, *D. pulex* and *D. pulicaria* (Colbourne & Hebert 1996). The first of these species is endemic to Oregon, while the latter taxa are broadly

* Corresponding author.

* Present address: Département de biologie, Université du Québec à Rimouski, 300 Allée des ursulines, Rimouski, Québec, Canada G5L 3A1.
(France-dufresne@uqar.quebec.ca)

distributed in North America (Hebert 1995). Diploid hybrids between *D. pulex* and *D. pulicaria* are common in the temperate regions of North America, and invariably have the former species as a maternal parent (Crease *et al.* 1989). Allozyme analyses have suggested that *D. middendorffiana* is also of hybrid origin, and past work has shown that its mitochondrial lineages are separable into two major groups (Dufresne & Hebert 1994; Van Raay & Crease 1995). Van Raay & Crease (1995) argued that both of these lineages were unique to *D. middendorffiana*, providing further evidence that the species had persisted as a polyploid for a substantial period of time.

The present study had the goal of surveying mtDNA diversity in populations of *D. middendorffiana* at sites across the eastern Canadian arctic. The study also aimed to ascertain the genetic similarity between mitochondrial DNA lineages of *D. middendorffiana* and its three closest allies. Particular emphasis was placed on analysis of *D. pulicaria* because prior work has suggested its potential involvement as a maternal parent of *D. middendorffiana* (Dufresne & Hebert 1994; Van Raay & Crease 1995).

2. MATERIALS AND METHODS

(a) Sampling

Sites in the eastern arctic of North America were sampled during July and August of 1992–1994 (figure 1). Eighty-nine allozymically distinct clones were obtained from ponds or lakes on the Melville Peninsula, as well as on Igloodik and Baffin Islands. Fourteen populations of *D. pulicaria* were also collected from eastern, central, and western temperate North America. In addition, one population of *D. pulex* from temperate North America, and one of *D. melanica*, were added to the analysis.

(b) Allozyme analyses

Cellulose acetate electrophoresis (Hebert & Beaton 1993) was used for clonal identification (see Dufresne & Hebert 1994). Both morphological and allozymic characteristics were employed to assign clones to a particular taxon. The Lactate Dehydrogenase (LDH) locus is particularly useful in taxon recognition—*D. pulicaria* and *D. pulex* are fixed for the F and the S alleles respectively, while *D. pulex-pulicaria* hybrids from temperate areas are SF heterozygotes. Individuals of *D. middendorffiana* possess either an SS or SF phenotype at LDH, but they are generally morphologically distinct from individuals of both *D. pulex* and *D. pulex-pulicaria* hybrids because of their cuticular melanization. It must be noted however that some *middendorffiana* clones are very weakly melanized and may be mistaken for *pulex* clones.

(c) MtDNA analyses

A variable region of the mitochondrial genome (ND4–ND5 genes) was PCR-amplified to examine intraspecific mtDNA diversity, as well as to provide a finer resolution of relationships among closely related taxa. The primers' sequences for the ND4 gene (5'-TATTCCTTCAGTAGCTC-ATAT) and the ND5 gene (5'-AGGAAGAAACCATAT-TAAAC) were designed by D. Stanton based on sequence information from *D. pulex*. DNA extraction, PCR protocols, and enzyme digests were carried out as described in Dufresne

& Hebert (1994). The analysis of the ND4–ND5 PCR products initially focused on 89 clones from the eastern arctic. Allozyme and morphological analyses of these clones classified 40 as *D. pulicaria*, 48 as *D. middendorffiana*, and one as *D. pulex*. Ten of the *D. middendorffiana* clones and nine of the *D. pulicaria* clones from the eastern high arctic were screened for variation at the ND4–ND5 region with seven restriction enzymes (*Cfo*I, *Hae*III, *Hinf*I, *Hpa*II, *Mbo*I, *Rsa*I, and *Taq*I). This analysis revealed the occurrence of four major mtDNA groups and showed that analysis of three enzymes (*Cfo*I, *Mbo*I, and *Rsa*I) was sufficient to assign clones to one of these lineages. Thirty-eight additional clones of *D. middendorffiana* and 31 clones of *D. pulicaria* from the eastern high arctic were subsequently examined for variation using these three restriction enzymes. Fourteen clones of *D. pulicaria* from the temperate zone and one clone each of *D. melanica* and *D. pulex* were also added to the RFLP analysis using the full set of seven enzymes. Finally, ten *D. middendorffiana* and six *D. pulex* clones from Churchill, Manitoba (Dufresne & Hebert 1994), as well as one *D. pulicaria*, one *D. middendorffiana*, and one *D. pulex* from Tuktoyaktuk, NWT, were analysed for the same set of seven enzymes.

(d) Data analysis

Overall percentage distances were calculated based on shared fragment patterns according to Nei & Li (1979) using the REAP program (McElroy *et al.* 1991). This approach is used when gel profiles are too complex to be resolved as sites (Gill *et al.* 1993; Dufresne & Hebert 1994; Weider *et al.* 1996). The matrix of estimated distances was clustered by neighbour-joining (NJ) analysis using the PHYLIP package, version 3.5c (Felsenstein 1993). The banding profiles are available from the authors upon request.

3. RESULTS

(a) Allozymes and habitat distribution of arctic clones

Unmelanized or weakly melanized *Daphnia* with a LDH-FF phenotype made up 60% of the daphniid fauna in lakes in the eastern arctic. Their relative lack of pigmentation, LDH phenotype, and general morphology led to their identification as *D. pulicaria*. By contrast, 70% of the *Daphnia* clones from pond habitats in the same area were strongly melanized and possessed LDH-SS or SF phenotypes, and were therefore identified as *D. middendorffiana*.

(b) RFLP analyses

Thirty-four mtDNA haplotypes were detected following digestion of the DNA from 54 different clones with seven restriction enzymes. Clustering of genetic distances by NJ revealed the presence of four major mtDNA clusters each separated by more than 5% sequence divergence (figure 2). The unweighted pair group method with arithmetic averages (UPGMA) analysis (not shown) was congruent with the NJ analysis. Furthermore, sequence analysis of representative clones from these four mtDNA clusters has confirmed the validity of the four groups (Colbourne *et al.* in preparation).

Clones of *D. middendorffiana* possessed mtDNA genomes that could be assigned to three of these four major groups. Group I clones had haplotypes that were unique to *D. middendorffiana*. By contrast, group II and

Figure 1. Sampling locations for members of the *Daphnia pulex* complex in North America. Symbols represent the five mtDNA groups found with the RFLP analyses (■ = group I, □ = group IIA, ○ = group IIB, ● = group III, and △ = *pulex* group. Numbers refer to clones in figure 2. The lower box represents Churchill, Manitoba, whereas the upper one includes the Melville Peninsula, Igloolik and Baffin Islands.

group III clones possessed haplotypes that were very similar to lineages present in *D. pulicaria*. Group II clones could be further divided into two subgroups (A and B), each associated with a particular lineage of *D. pulicaria*. Subgroup A had mtDNA haplotypes that

were identical to those of arctic *D. pulicaria*, whereas subgroup B clones clustered with the western lineage of *D. pulicaria*. Finally, a single clone (Mi42) of *D. middendorffiana*, assigned to group III, was closely related to eastern populations of *D. pulicaria*.

Figure 2. Neighbour-joining phenogram of 38 arctic clones of *D. middendorffiana* (Mi), *D. pulicaria* (Pc), and *D. pulex* (Px), and 16 temperate zone clones of *D. pulex*, *D. pulicaria*, and *D. melanica* (Me), based on RFLP analysis of variation at the ND4-ND5 mtDNA region. The first two letters designate the species to which each clone belongs. The location of each clone is indicated on figure 1.

D. pulicaria also showed a large amount of mtDNA diversity, being represented in three of the four major mtDNA groups. There was clear evidence of phylogeographic structuring in this species, with populations clustered into arctic (2A), temperate western (2B), and temperate eastern (3) groups. The arctic group consisted of seven *D. pulicaria* clones from the eastern high arctic and one *D. pulicaria* clone from the western arctic, each sharing a single distinct haplotype (Pc14-20 and Pc21 in cluster 2A). The eastern group (cluster 3) was found in populations from Ontario, Maine, and New Brunswick, while the western group (cluster 2B) was detected in populations from Ontario, New York, Saskatchewan, and Washington. Other clones of *D. pulicaria* had haplotypes which showed close affinities with haplotypes detected in other sexual species, suggesting cases of introgression. For example, four clones of *D. pulicaria* from temperate western areas (Pc45, Pc52, Pc53, Pc54 in cluster 4) had mtDNA haplotypes identical to those of *D. pulex*. Also, one clone from Yellowstone lake (Pc41 in cluster 3) had a mtDNA haplotype that was closely related to that of *D. melanica* (Me40). Finally, two clones of *D. pulicaria* from the eastern arctic with LDH-FF or FF' phenotypes

(Pc36, Pc38 in cluster 3) had mtDNA haplotypes that were closely related to those of eastern populations of *D. pulicaria*. Clones of *D. pulex* possessed mtDNA genomes that were distinct from those of both *D. pulicaria* (as discussed above) and *D. middendorffiana*. Only one clade, within which diversity was low relative to *D. pulicaria*, was found in this species. A sexual population of *D. pulex* from Tuktoyaktuk (Px50) showed very close affinities to asexual clones of the same species from Churchill and Ontario.

4. DISCUSSION

The analysis of mtDNA diversity in *D. middendorffiana* revealed the presence of three divergent groups, two of which (I and II) have been previously detected in the Canadian arctic (Dufresne & Hebert 1994; Van Raay & Crease 1995). Prior work suggested that *D. middendorffiana* was of hybrid origin (Stanton 1988; Dufresne & Hebert 1994; Van Raay & Crease 1995) and possessed high mtDNA diversity as a result of hybridization between *D. pulicaria* or *D. melanica* with *D. pulex* (Dufresne & Hebert 1994). The present survey has confirmed the close association between *D. middendorffiana* and *D. pulicaria*. It is now apparent that *D. middendorffiana* has 'captured' *D. pulicaria* mtDNA through hybridization, as two of its three mtDNA lineages are closely related to those in this species. Hence group III *middendorffiana* have mtDNA haplotypes that are closely related to those of populations of *D. pulicaria* from eastern Canada, while group IIA clones have haplotypes identical to those of arctic populations of *D. pulicaria*, and group IIB clones are closely allied to the western group of *D. pulicaria*. Allozyme markers suggest that either *D. pulex* or *D. melanica* could have served as paternal ancestors for these groups, since both species possess a slow allele at LDH. The presence of pigmentation in *D. melanica* further suggests that it could have served as a paternal ancestor. However, an examination of additional nuclear markers is needed to gain more definite information concerning the paternal origin of these clones.

It is now clear that the bulk of extant asexual lineages have recently diverged from closely related sexual species (Crease *et al.* 1989; Avise *et al.* 1992). *D. middendorffiana* provides no exception to this pattern as two of its three mtDNA groups are closely related to haplotypes detected in sexually reproducing populations of *D. pulicaria*. The sole exception to this pattern of recent transitions to asexuality are the group I clones for which no closely related sexual ancestor has yet been found. While an old age for this group cannot be ruled out, there is a need for a broader survey of populations at sites throughout the Holarctic.

The present analysis has revealed a high level of mtDNA divergence in *D. pulicaria*, with four different mtDNA groups discovered in this species. Prior work on its temperate zone populations revealed the occurrence of two mtDNA lineages, one of which was very similar to that of *D. pulex* (Crease *et al.* 1989). The present study has confirmed the broader occurrence in western North America of *D. pulicaria*-carrying *pulex*-

like haplotypes. These two species are known to hybridize widely in the temperate zone with *D. pulex* invariably serving as the maternal parent (Crease *et al.* 1989). It seems likely that backcrossing between these hybrids and *D. pulicaria* has resulted in mitochondrial introgression. This leakage explains why a recent study, which examined only mtDNA markers, failed to recognize these two species as distinct (Lehman *et al.* 1995). However, analysis of nuclear markers has shown that the two species are genetically distinctive (Hebert *et al.* 1993).

The other three lineages of *D. pulicaria* showed largely allopatric distributions. Populations of *D. pulicaria* in both the eastern and western arctic shared mtDNA haplotypes that were not detected in temperate zone populations. Temperate zone populations themselves possessed two mtDNA groups but they only co-occurred regionally in the central portion of the continent. One of these groups was fixed in eastern populations (Maine and New Brunswick), while the other was the sole group detected in western populations (Saskatchewan, Washington). Interestingly the sole isolate of *D. melanica*, analysed in the present study, possessed an mtDNA genome similar to that of eastern populations of *D. pulicaria*, suggesting the possibility of introgression between these taxa. Two *D. pulicaria* clones from the eastern arctic, which showed allozymic signs of polyploidy, possessed mtDNA haplotypes similar to those of the eastern *pulicaria* lineage, suggesting their possible origin through hybridization between eastern and arctic populations of *D. pulicaria*.

The disjunct distributions of the three major mtDNA lineages restricted to *D. pulicaria* suggest that individuals with these haplotypes dispersed from separate refugia following the last glaciation. While the present analysis cannot provide an accurate estimate of their divergence time, a recent RFLP study on the entire mtDNA of *D. middendorffiana* reports a 2.4% sequence divergence between group I and IIA (Van Raay & Crease 1995). The application of the *Drosophila* clock (Monnerot *et al.* 1990) to this result suggests that these groups diverged 1.2 million years ago, corresponding to the middle of the Pleistocene. Two of the *D. pulicaria* groups may have been restricted to refugia south of the ice sheets, with eastern mtDNA haplotypes dispersing from an Atlantic refuge, and western haplotypes persisting in a Mississippian refuge. By contrast, the northern mtDNA haplotypes of *D. pulicaria* probably dispersed from a Beringian or an eastern high arctic refuge (Dyke & Prest 1987) such as that occupied by other fish and aquatic invertebrates (Bailey & Smith 1981; Hebert & Hann 1986; Väinölä *et al.* 1994).

There is now increasing evidence that Pleistocene glaciations have played a significant role in the process of divergence and speciation in many polar taxa. The secondary contact of divergent refugial races following range expansion has led to the formation of hybrid zones in a wide variety of organisms (Hewitt 1993). For example, the higher frequency of hybridization in freshwater than marine fish species is thought to be due to the secondary contacts among races which evolved during the Pleistocene (Billington & Hebert 1991). Since hybridization is a frequent route to polyploidy,

we suggest that polyploidy evolved in *D. middendorffiana* following hybridization between divergent refugial races of *D. pulicaria*, and closely allied species such as *D. pulex* and *D. melanica*. Numerous polyploid plants and the few known polyploid insects are also widely distributed in once-glaciated areas, while their diploid relatives are restricted to small areas in proximity to glacial refugia (Ehrendorfer 1980; Lokki & Saura 1980; Stebbins 1984). The presence of similar distributional patterns in these different organisms suggest that Pleistocene glaciations have played a key role in the origin of polyploidy by promoting isolation and population divergence, which was followed by secondary contact among divergent refugial populations following glacial retreat. Thus, rather than reducing genetic diversity, glacial events have apparently indirectly served to enhance genetic novelty in hybridizing organisms. This secondary contact hypothesis was invoked by Stebbins (1984) to explain the prevalence of polyploid plants in glaciated areas. A somewhat similar hypothesis has also been proposed to explain the origin of apomixis in weevils (Vepsäläinen & Järvinen 1979). However, our study is the first to show that the same processes have been important in generating polyploid animals in arctic environments.

This research was supported by an FCAR scholarship to F.D. and an NSERC grant to P.D.N.H. We thank the Polar Continental Shelf Project for logistics support that made the sampling program possible and the Nunavut Science Institute in Igloolik for access to their laboratory facilities. Liz Strazynski provided help with the molecular analyses. We are grateful to Ian Smith for his help with the figures. We thank T.J. Crease and three anonymous referees for helpful comments on the manuscript.

REFERENCES

- Awise, J. C. 1994 *Molecular markers, Natural History, and Evolution*. New York: Chapman & Hall.
- Awise, J. C., Quattro, J. M. & Vrijenhoek, R. C. 1992 Molecular clones with organismal clones: mitochondrial DNA phylogenies and the evolutionary histories of unisexual vertebrates. *Evol. Biol.* **26**, 225–246.
- Bailey, R. M. & Smith, G. R. 1981 Origin and geography of the fish fauna of the Laurentian Great Lakes. *Can. J. Fish. Aquatic Sci.* **38**, 1539–1561.
- Beaton, M. J. & Hebert, P. D. N. 1988 Geographical parthenogenesis and polyploidy in *Daphnia pulex*. *Am. Nat.* **132**, 837–845.
- Billington, N. & Hebert, P. D. N. 1991 Mitochondrial diversity in fishes and its implication for introductions. *Can. J. Fish. Aquatic Sci.* **48** (Suppl. 1), 80–94.
- Colbourne, J. K. & Hebert, P. D. N. 1996 The systematics of North American *Daphnia* (Crustacea: Anomopoda): a molecular phylogenetic approach. *Phil. Trans. R. Soc. Lond. B* **351**, 349–360.
- Crease, T. J., Stanton, D. J. & Hebert, P. D. N. 1989 Polyphyletic origins of asexuality in *Daphnia pulex*. II. Mitochondrial DNA variation. *Evolution* **43**, 1016–1026.
- Dufresne, F. & Hebert, P. D. N. 1994 Hybridization and origins of polyploidy. *Proc. R. Soc. Lond. B* **258**, 141–146.
- Dufresne, F. & Hebert, P. D. N. 1995 Polyploidy and clonal diversity in an arctic cladoceran. *Heredity* **75**, 45–53.

- Dyke, A. S. & Prest, V. K. 1987 Paleogeography of northern North America. 18000–5000 years ago. Geological survey of Canada, Map 1703A, scale 1:12 500 000.
- Ehrendorfer, F. 1980 Polyploidy and distribution. In *Polyploidy: Biological relevance* (ed. W. H. Lewis) pp. 45–60. New York, Plenum Press.
- Felsenstein, J. 1993 *PHYLIP (phylogeny inference package)*. Version 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle.
- Gill, F. B., Mostrom, A. M. & Mack, A. L. 1993 Speciation in North American chickadees. I. Patterns of mtDNA genetic divergence. *Evolution* **47**, 195–212.
- Hebert, P. D. N. 1995 *The Daphnia of North America: an illustrated fauna*. CD-ROM, University of Guelph, Ontario.
- Hebert, P. D. N. & Beaton, M. J. 1993 *Methodologies for allozyme analysis using cellulose acetate electrophoresis*. Helena Laboratories, Beaumont, Texas.
- Hebert, P. D. N., Schwartz, S. S., Ward, R. D. & Finston, T. J. 1993 Macrogeographic patterns of breeding system diversity in the *Daphnia pulex* group. I. Breeding systems of Canadian populations. *Heredity* **70**, 148–161.
- Hebert, P. D. N. & Hann, B. J. 1986 Patterns in the composition of arctic tundra pond microcrustacean communities. *Can. J. Zool.* **43**, 1416–1425.
- Hewitt, G. W. 1993 Postglacial distribution and species substructure: lessons from pollen, insects, and hybrid zones. In *Evolutionary patterns and processes* (ed. D. R. Lees & D. Edwards), pp. 97–123. San Diego: Academic Press.
- Lehman, N., Pfreder, M. W., Morin, P. A., Crease, T. J. & Lynch, M. 1995 A hierarchical molecular phylogeny within the genus *Daphnia*. *Molec. Phylog. Evol.* **4**, 395–407.
- Lokki, J. & Saura, A. 1980 Polyploidy in insect evolution. In *Polyploidy: Biological relevance* (ed. W. H. Lewis), pp. 277–312. New York: Plenum Press.
- McElroy, D., Moran, P., Bermingham, E. & Kornfield, I. 1991 *REAP, the restriction enzyme analysis package*. Version 4. University of Maine, Orono.
- Monnerot, M., Solignac, M. & Wolstenholme, D. R. 1990 Discrepancy in divergence of the mitochondrial and nuclear genomes of *Drosophila teissieri* and *Drosophila yakuba*. *J. Molec. Evol.* **30**, 500–508.
- Nei, M. & Li, W. H. 1979 Mathematical models for studying genetic variation in terms of restriction endonucleases. *Proc. Natn. Acad. Sci. USA* **76**, 5269–5273.
- Ohno, S. 1970 *Evolution by gene duplication*. New York: Springer-Verlag.
- Stanton, D. J. 1988 Evolution of asexuality in *Daphnia pulex*: implications from mitochondrial DNA analysis. Ph.D. thesis. University of Windsor, Ontario.
- Stebbins, G. L. 1984 Polyploidy and distribution of arctic-alpine flora: new evidence and a new approach. *Bot. Helvetica* **94**, 1–13.
- Väinölä, R., Riddoch, B. J., Ward, R. D. & Jones, R. I. 1994 Genetic zoogeography of the *Mysis relicta* species group (Crustacea: Mysidacea) in northern Europe and North America. *Can. J. Fish. Aquat. Sci.* **51**, 1490–1504.
- Van Raay, T. J. & Crease, T. J. 1995 Mitochondrial DNA diversity in an apomictic *Daphnia* complex from the Canadian high arctic. *Molec. Ecol.* **4**, 149–161.
- Vepsäläinen, K. & Järvinen, O. 1979 Apomictic parthenogenesis and the pattern of the environment. *Amer. Zool.* **19**, 739–751.
- Ward, R. D., Bickerton, M. A., Finston, T. & Hebert, P. D. N. 1994 Geographic cline in breeding systems and ploidy levels in European populations of *Daphnia pulex*. *Heredity* **73**, 532–543.
- Weider, L. J. & Hebert, P. D. N. 1987 Microgeographic genetic heterogeneity of melanistic *Daphnia pulex* at a low arctic site. *Heredity* **58**, 391–399.
- Weider, L. J., Hobaek, A., Crease, T. J. & Stibor, H. 1996 Molecular characterization of clonal population structure and biogeography of arctic apomictic *Daphnia* from Greenland and Iceland. *Molec. Ecol.* **5**, 107–118.

Received 22 July 1996; accepted 5 September 1996